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Molecular Aspects of Plant–Pathogen Interactions in Relation to Novel Strategies of Breeding for Disease Resistance

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Knowledge of plant–pathogen interactions is expanding rapidly. A growing body of information has now been available on the molecular genetic background of recognition, pathogenicity and host plant resistance. Novel strategies of resistance breeding based on transgenic plants obtained by introducing resistance genes of plant or microbial origin have recently been developed. The first part of this review gives a survey of genes encoding pathogenicity determinants of the microorganisms, the second part summarizes our current understanding of molecular aspects of plant resistance. Special emphasis is placed on attempts to improve plant resistance by genetic engineering.

Basic terms in resistance biology

Terminological questions are not discussed in this article and in no way would we intervene in the existing controversies over the subject. However, the terms related to pathogenicity and resistance are briefly summarized below in order to make clear our wording for breeders.

Pathogenicity is a qualitative term referring to the potentiality of particular strains of a microorganism to induce disease in certain individuals of a plant species. For example, *Magnaporthe grisea* is able to maintain itself in a parasitic mode on rice and is therefore a pathogen of this plant, while *Phytophthora infestans* is unable to do this. Hence, it is a *non-pathogenic* microbe on *Oryza sativa*. From the other side, rice is a *non-host* plant of the latter fungus but may serve as a *host* for the former. Genetic determinants of pathogenicity are collectively designated as *pathogenicity genes*. These genes provide the possibility of a parasitic way of living. As this way of living requires many attributes, pathogenicity is always under polygenic (possibly oligogenic) control. *Virulence* is a quantitative trait referring to the degree of pathogenicity. *Virulent* pathogens are able to cause disease on an otherwise resistant plant. For example, race 1 of *P. infestans* is virulent on patato lines that carry the *R1* resistance gene but *avirulent* (i.e. unable to incite disease) on potato plants containing the *R2* resistance gene. In the majority of cases, avirulence is controlled by a single gene but data on oligogenic regulation of this trait are also available. According to another definition, virulence is a measure of the microbial reproductive rate in a given host. *Aggressive* ('highly virulent') strains of a particu-

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lar microorganism cause more serious symptoms on the same host than less aggressive virulent strains. *Aggressiveness* is a complex property controlled by polygenes.

Considering the other partner of the host-pathogen interaction, a plant is *susceptible* to a particular pathogen if the latter is able to cause disease on that plant while the same pathogen is unable to incite disease on a *resistant* plant. In the case of *absolute susceptibility*, the microbial infection results in symptoms and disease development while in a *tolerant* plant, pathogens replicate without visible symptoms. According to another definition, symptoms may develop in tolerant plants following infection but symptom development is not parallelled with yield loss or the losses are practically negligible. *Local resistance* is restriced to special organs or tissues while *systemic resistance* prevails throughout the plant. *Vertical (race-specific) resistance* exists between cultivars (or lines) of a given plant species and physiological races (or pathovars) of a microorganism. This type of resistance is generally controlled by a single gene and manifested in the hypersensitive reaction and maintained until genetic changes occur in populations of the host or the pathogen. *Horizontal ('field') resistance*, controlled by polygenes, is efficient against many races and never appears as an absolute resistance. A few or more individuals in a plant population are always infected and the infection results in mild or moderate symptoms. *Non-host resistance*, a special but the most frequent form of the plant/pathogen interactions, refers to those cases when the plant could by no means support the propagation of an otherwise pathogenic microbe. The biochemical and physiological backgrounds of non-host resistance are largely unknown. One hypothesis suggests that this type of resistance results from a milieu that is simply unsuitable for the survival and propagation of most fungi and bacteria either because of the lack of proper nutrients or due to the presence of antimicrobial plant compounds. Another theory indicates that non-host resistance is due to the recognition of ubiquitous elicitors, products of avirulence genes present in most pathogens, and that these elicitors trigger a general defence reaction.

The proper interpretation of virulence, avirulence, susceptibility, resistance, and the other above-mentioned terms always requires the addition of the other partner, i.e. a susceptible or resistant plant and a virulent or avirulent pathogen. Our efforts are to focus on the interrelationships between plants and microbes and use the terms *compatibility*, which refers to a parasitic mode of microbial reproduction on a plant that consequently becomes diseased, and *incompatibility*, which means the absence of parasitic reproduction and disease development.

The gene-for-gene theory

A hundred years ago, it was recognized that resistance of plants to pathogens, more exactly, the yellow rust resistance in wheat, was an inheritable trait. From that time, plant breeders consciously made efforts to develop disease resistance in new cultivars. However, this resistance had always 'broken down' during the large-scale production of these cultivars. Later studies showed that the plants never lose their resistance but the

resistance could be overcome by more virulent individuals of the pathogen that had emerged in the huge, rapidly propagating microbial population.

In a series of simple but impressive experiments, Flor (1956) was the first to demonstrate that a genetically determined, dynamic interrelationship exists between host plants and pathogenic microorganisms. Based on these experiments, he elaborated the gene-for-gene system which was published in its final version in a subsequent review article (Flor, 1971). Flor pointed out that flax cultivars that harbour a dominant resistance gene (*R*) are resistant to rust (*Melampsora lini*) races that carry a dominant avirulence gene (*AVR* or *V*). Flax cultivars that contain a (generally recessive) gene encoding susceptibility (*r*) are attacked by rust races that harbour a corresponding virulence gene (*v*). However, if a flax cultivar with an *r* gene meets a *M. lini* race lacking the proper *v* gene, no disease develops. The gene-for-gene relationship is valid not only for plant/fungus interactions but can also be detected in other plant/parasite systems including viruses, bacteria, nematodes, and insects. (On the other hand, this principle is nonexistent in diseases caused by secondary plant pathogens or toxins.)

The gene-for-gene system, in its narrow sense, refers to the most simple interaction when a single resistance gene faces a single avirulence gene and the plant (line) harbouring this resistance gene shows no phenotypic difference from other, near-isogenic lines lacking this resistance gene. Moreover, no differences can be detected between the two lines by using traditional biochemical methods and the two microorganisms are also almost identical except for the presence or absence of a particular virulence/avirulence gene. In an earlier work, Gabriel et al. (1982) have already demonstrated the uniformity of these microbe strains when they identified some 600 polypeptides in *Erysiphe graminis* f. sp. *tritici* by means of two-dimensional gel electrophoresis. No differences were found between the polypeptide patterns of the parental strain (avirulent to wheat lines carrying the *Pm* resistance gene) and the progenies of this strain that became virulent on the same line after induced mutagenesis. In these experiments, minor mutations not detectable by protein mapping proved to be sufficient to turn an incompatible interaction into a compatible one. In such a strict interaction, mutations most probably affected the pathogen's avirulence gene product which could be an elicitor molecule suitable to incite a plant response. From the plant side, point mutations may cause changes in the resistance gene products which are postulated to be receptor molecules able to recognize the elicitors and transfer the signal towards the defence system. Previously, these minor genetic changes could only be detected by evaluating the phenotypic interaction of the two partners that may result in disease or lack of disease. However, there are now a growing number of studies, discussed below, where the molecular basis of the plant/pathogen specificity have exactly been determined.

The gene-for-gene principle is sometimes used in a broader sense for cases when the host-pathogen interaction is under polygenic control and/or the gene products are well-characterized enzymes or other molecules. A plant capable of synthesizing a thick cutin layer may exert improved resistance. Genes coding for the key cutin biosynthesis enzymes could be described as *R* genes while cutinase encoding genes of a pathogen may be classified as *v* genes. Furthermore, *v* genes are responsible for microbial toxin produc-

tion, and the synthesis of detoxifying enzymes of plants are controlled by *R* genes. As there are appropriate methods for the purification of large enzyme molecules, it is easier to clone genes coding for various enzymes than to detect point mutations. This is the reason why the majority of the tentative pathogenicity or resistance genes were found to code for enzymes.

In the present review, we prefer to use the gene-for-gene principle in a narrow sense and postulate that the outcome of the plant-pathogen relationship is determined by the elicitor-receptor interaction. Plant receptors encoded by *R* genes are able to recognize the pathogen-derived elicitors encoded by *V* genes. This interaction triggers major biochemical changes in the plant cell resulting in a generalized host defence called the hypersensitive reaction (HR). The HR is manifested in necrotic lesions, restricted to a single cell, or expanded to larger regions. This reponse arrests the pathogen but is probably not a direct cause of its inhibition or destruction.

Hypersensitive reaction – The basic defence mechanism of plants

The physiological and pathological aspects of the hypersensitive reaction are discussed in early comprehensive studies (*e.g.* Klement and Goodman, 1967; Király et al., 1972; Klement, 1982) while the biochemical events of this response became clear more recently (Király, 1980; Goodman and Novacky, 1994). The first, most likely consequence of the elicitor-receptor interaction is the generation of reactive oxygen species, a cascade event called oxidative stress. Superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen cause lipid peroxidation and destroy the membrane structures which results in uncontrolled ion leakage, rapid host cell death, and kills the pathogen as well. Recognition of the pathogen or its elicitor gives rise to transport of signals towards the neighboring cells. The consequence of this signal transduction is a general defence response including cell-wall reinforcement, phytoalexin synthesis, and accumulation of pathogenesis-related proteins, some of which are extracellular hydrolytic enzymes (various chitinases or glucanases).

Plant cell death is probably not a direct cause of inhibition or destruction of the pathogen. This would be nonsense in the case of necrotrophs as these pathogens prefer to colonize dead, necrotic tissues. Another dilemma is that physiological changes typical of the hypersensitive response could also be detected in a compatible interaction. All the biochemical steps preceding HR take place in a susceptible plant too but this reaction is strongly delayed. Furthermore, a susceptible tissue is also able to retard disease development to a certain extent. Enlargement of necrotic lesions is somehow restricted and propagation of the pathogen is inhibited. Altogether, some kind of defence reaction could be observed even in compatible interactions but this reaction is too slow and not efficient.

The gene-for-gene theory stimulated genetic studies on plant-pathogen interactions. Breeders made efforts to indentify *R* genes and use them to improve plant resistance to pathogens. Plant pathologists interested in the gene-flow within populations of pathogenic microbes have attempted to find avirulence genes. For a long time, however,

only indirect evidence supported the existence of these genes. Their presence or absence was deduced from phenotypic interactions observed between selected plant cultivars (lines) and pathogen races (pathotypes). Purified gene products or cloned genes have not been available until quite recently, with the exception of *Agrobacterium* which causes tumors on a wide range of dicotyledonous plants by transferring tumor- of hairy-root-inducing genes (located on the Ti or Ri plasmid, respectively) into the host genome. The molecular background of tumor induction and the regulation of the plasmid-located virulence genes in *Agrobacterium* have been summarized in full details (Charles et al., 1992; Ream, 1989).

Since the early nineties, a growing number of papers have been presented on cloning and sequencing genes involved in plant-pathogen interactions. The first part of this review attempts to survey characterized microbial genes encoding pathogenicity, virulence, avirulence, or aggressiveness. Due to ample informations available in other works, *Agrobacterium* genes are omitted from our lists and only selected examples of viral genes will be mentioned because of the excessive number of viral sequences known to be involved in viral infection. The second part of this article focuses on resistance genes followed by an overview of novel breeding strategies to produce resistant plants through transformation of *R* genes from other plants, avirulence genes or other sequences from other plant pathogens, and alien sequences cloned from non-pathogen microorganisms or higher animals.

Virulence/avirulence genes of plant viruses

According to early observations, many plant viruses are able to replicate in protoplasts of non-host plants. Hence, the cellular host range of plant viruses exceeds their natural host range. Although viruses are specialized to a few plant species, they may find an appropriate milieu for their replication in isolated cells (protoplasts) of other plants. They are still not true pathogens of the latter being incapable of cell-to-cell movement. Researchers define the host range of a virus strain as all those plants that are infected and systemically invaded by the given virus either in nature or under experimental conditions (Dawson and Hilf, 1992). We follow this definition and focus on the natural compatible interactions.

Viruses are obligate intracellular parasites possessing a rather simplified genome. Their replication is absolutely dependent upon the host cell's biosynthetic capacity. As replication and parasitism are simultaneous categories in the case of viruses, all viral gene sequences encoding various enzymes like proteinases, RNA-polymerases, and reverse transcriptases (in DNA viruses) as well as other parts of the genome containing the genetic determinants of coat protein, replicase, movement protein, and NTP-binding sequences that support pathogenicity are, in fact, pathogenicity genes. A more detailed discussion on these general viral genes far exceeds our original purpose. For further in-

formation, the reader is referred to handbooks on virology (e.g. Matthews, 1991). However, we are intending to deal with the virulence genes of plant viruses. Recall that certain well-characterized viral sequences are known to determine compatibility and host rage according to the gene-for-gene principle. For example, Schoelz et al. (1986) determined that the gene VI region of cauliflower mosaic caulimovirus (CaMV) encodes such a host range determinant. Another well undestood concept in plant virology is the hypersensitive reaction elicited by viral coat proteins (CPs) in incompatible interactions. *Nicotiana sylvestris* plants possessing the N' resistance gene are systemically infected by wild-type strains of tobacco mosaic tobamovirus (TMV). Mutants of this virus containing a single amino acid change in the coat protein became avirulent and induced HR on these same plants (Knorr and Dawson, 1988; Saito et al., 1987). Coat proteins of plant viruses may thus act as elicitors (Culver and Dawson, 1989). The genes encoding CPs are virulence/avirulence genes as a single basepair substitution in their sequence may turn a once compatible interaction into an incompatible one, or vice versa.

Genes encoding the 126-kDa and the 183-kDa proteins in tomato mosaic tobamovirus (TMV-L) are also virulence genes. Wild-type-TMV-L is unable to replicate in tomato cultivars homozygous for the *Tm-1* resistance gene. A mutant that had two nucleotide changes in the sequences coding for these proteins caused typical disease symptoms on the *Tm-1/Tm-1* plants. A probable explanation for this increase in virulence lies in the nature of the replication complex which is composed of virus-encoded and plant-encoded proteins and is required for the replication of positive-sense RNA viruses. In homozygous (*Tm-1/Tm-1*) tomato plants, the host protein encoded by the *Tm-1* gene is unsuitable for a proper interaction with the 126-kDa and 138-kDa proteins of wild-type TMV-L. Therefore, the replication complex becomes nonfunctional. On the other hand, modified proteins of the mutant virus efficiently interact with the plant component of the replicase complex allowing the replication of the virus to a high titer. The mutation resulted in an expanded host range, i.e. an increase of virulence (Meshi et al., 1988).

Viruses move from cell-to-cell in plants through plasmodesmatal connections. This movement is not a passive event. Viruses synthesize movement proteins to promote their transport from one cell to another. These proteins allow easier movement of the viral RNA by enlarging the plasmodesmata and by unfolding the coiled viral RNA. The most extensively studied movement protein is the 30-kDa protein of TMV. A cell wall binding domain and an RNA binding domain have been identified in this protein (Berna et al., 1991; Citovsky et al., 1990). Sequences encoding movement proteins are real virulence genes as their mutation (or deletion) decreases (or destroys) movement activity and results in a more narrow host range.

Genes controlling pathogenicity in plant pathogenic bacteria

Seminal studies on the molecular background of pathogenicity of phytobacteria have been performed in *Pseudomonas solanacearum* (Boucher et al., 1992). Due to its cosmopolitan nature, wide host range, and economical significance, this bacterium proved to be an especially useful subject of such experiments.

Avirulence genes

The *P. solanacearum* strain, AW, incites HR while another strain, NC252, causes wilt disease on the same tobacco plants. When a 2.0 kb DNA fragment (named, subsequently, *avrA*) cloned from AW was used to transform NC252, the transformants lost their pathogenicity and induced HR on tobacco. A progeny of AW containing a mutation in the above-mentioned DNA fragment lost its HR-inducing ability and became virulent on tobacco (Carney and Denny, 1990). Thus, the *avrA* gene restricts the host range of this bacterium and is therefore classified as an avirulence gene. Similar *avr* genes are also known from *Pseudomonas syringae* pv. *glycinea* and *P. syringae* pv. *tomato* (Kobayashi et al., 1989). The *avrRxv* gene of *Xanthomonas campestris* pv. *vesicatoria* is also responsible for avirulence in an unusually wide range of hosts. When this gene was introduced into other pathovars of *X. campestris* (pathogenic to soybean, cowpea, alfalfa, maize, and cotton), all the transformants became avirulent and induced HR on their original host plants (Whalen et al., 1988).

Genes encoding aggressiveness

A 2.7 kb clone from strain AW of *P. solanacearum* proved to contain the genetic determinants of endoglucanase, a member of the complex cellulase system that was hereinafter named as the *eglA* gene (Roberts et al., 1988). When this structural gene was inactivated by transposon mutagenesis, the mutants lost their endoglucanase activity but still managed to cause disease on tomato. Symptoms were much less severe and the disease development was slower. In other words, the aggressiveness of these mutants was reduced. Therefor, *eglA* is an aggressivity gene. Similar genes involved in the control aggressiveness were also cloned from *P. solanacearum* like *pglA* and *pehA*, structural genes of polygalacturonase from strains AW and K60, respectively (Schell et al., 1988; Allen et al., 1991). These experiments provide molecular evidence of earlier observations on the nonessential role of cell wall degrading enzymes in pathogenicity. Many studies have shown that mutants impaired in their hydrolytic activity never became absolutely avirulent. Their disability to synthesize one or other polysaccharide degrading enzymes was somehow compensated by other, mostly unknown, pathogenicity traits. In the majority of cases, a total compensation of the reduced hydrolytic activity was not observed. The mutants had a diminished aggressiveness. Therefore, genes encoding cell wall degrading enzymes can be classified as aggressivity genes.

Similar conclusions were drawn from results obtained for the extracellular polysaccharide (EPS) components which are important pathogenicity determinants of phytobacteria. EPSs have several functions in pathogenicity such as the occlusion of vascular bundles, the prevention of recognition, the adsorption of water from plant tissues, and the inactivation of antibacterial plant compounds.

Genes encoding EPSs (*opsA-G*) have been cloned from *P. solanacearum* (Cheng Kao and Sequeira, 1992). Many EPS-deficient mutants were analyzed and none of them proved to be absolutely nonpathogenic. However, the aggressiveness of these mutants decreased as they were unable to incite typical wilt symptoms but were able to cause chlorosis and stunting (Denny et al., 1988).

Two regulatory genes affecting agressiveness from *P. solanacearum* are also known. One of these, *phcA* (Brumbley and Denny, 1990), encodes a transcriptional regulatory protein. Mutations in this gene result in sluggish motility, reduced endoglucanase activity, and deficiencies in EPS production, traits known to contribute to aggressiveness. The other regulatory gene, *epsR*, is also involved in the control of EPS production (Huang and Sequeira, 1990). Its putative gene products shows striking similarities to effector proteins demonstrated to participate in a two-component regulatory network of other bacteria.

HR inducing ability is encoded by hrp *genes*

A group of genes known as *hrp* genes, or more recently as *hrp/hrm* genes, plays a decisive role in plant-bacterium interactions. Mutants whose *hrp* genes have been altered simultaneously lost their ability to cause disease on their host plants and to induce HR on nonhost plants (Macol and Denny, 1989). However, these mutants exhibited wild-type growth on complete medium. In the strain GMI1000 of *P. solanacearum*, the *hrp* genes are located on the megaplasmid of this bacterium and form a ~ 23 kb cluster in which at least six transcriptional units could be identified (Arlat et al., 1992). Several lines of evidence suggest that *hrp* genes are activated at the earliest stages of the plant-bacterium interaction (Trigalet and Demery, 1986) with the level of their expression dependent upon the nutrient sources available for the bacterium. Plant extracts were found to stimulate transcription of the *hrp* genes (Arlat et al., 1992). One of the possible functions of these genes is the promotion of nutrient transport from the plant to the bacteria (Brisset and Paulin, 1991). Sequence analysis of the *hrp* genes revealed striking similarities to genes known from other sources (Boucher et al., 1992). Several open reading frames (ORFs) have been identified, one of which encodes a putative protein that shows significant homology with pullulanase, an enzyme that catalyzes strach decomposition (Pugsley et al., 1990). This capability might help with the parasitic nutrition of a bacterium which resides in plant tissues. Another ORF has homology with ATPase encoding genes known to control the synthesis of flagellar components (Vogler et al., 1991). These components are good immunogens in higher animals and their tertiary structure could probably be suitable to elicit specific responses in plant systems as well. Several other sequences have tentatively been identified as possible regulatory genes of the *hrp* clusters

(Inonuye et al., 1986); *hrp* gene clusters are also known from *Xanthomonas campestris* (Boucher et al., 1987), *Pseudomonas syringae* (Lindgrean et al., 1988; Het et al., 1993; Huang et al., 1995) and *Erwinia amylovora* (Barny et al., 1990; Bauer and Beer, 1991; Wei et al., 1992).

More recently, Gopalen et al. (1996) pointed out that a *hrp* gene of *P. syringae* controls the transfer of a bacterial avirulence gene (*avr*) into the host cells. They also demonstrated, for the first time, that a direct interaction of the *R* and *avr* gene products is a prerequisite of the hypersensitive response (necrosis) in the plant.

Cloned pathogenicity genes of fungi

Penetration into the host plant

Many pathogens enter the plant tissues through wounds or natural openings of the plant surface, while others directly penetrate through the cuticle by means of a non-differentiated germ tube. However, in the most advanced fungal pathogens, special structures (appressorium and penetration peg) initiate the infection process. Two differentiation-specific genes (*INF24*, *INF56*) present in polymorphic copies identified in the bean rust fungus (*Uromyces appendiculatus*), were found to be induced by sensing of the physical structures of stomata (Bhairi et al., 1989; Xuei et al., 1992). Inactivation of these genes resulted in the failure of the appressorium formation, so that the mutants lost their infectivity (and viability). *INF24* and *INF56* are pathogenicity genes encoding vital functions in an obligate pathogen, like *U. appendiculatus*.

Another appressorium-specific gene, *Mpg1*, has been isolated by differential cDNA cloning from the rice blast fungus, *Magnaporthe grisea* (Talbot et al., 1993). *Mpg1* mutants were found not to lose their viability (as *M. grisea* grows well in artificial media) with about 20% of the germinating conidia able to develop appressoria. The mutants retained their pathogenicity although their aggressiveness was greatly reduced. The whole process of appressorium formation is controlled by several genes, one of which, *Mpg1*, may code for a cysteine-rich hydrophobin that controls attachment and recognition of topographical signals of the plant surface (see below). In *Colletotrichum lagenarium* an additional gene, designated *buf*, was found to assist in penetration (Kubo et al., 1991). Buff-coloured mutants lack melanin and are defective in host penetration. The *buf* gene has therefore been postulated to control melanin biosynthesis. (Melanin accumulates in the appressorial wall and supports both the osmotic pressure and the mechanical force needed to penetrate the cuticle layer of the host.)

Extracellular hydrolytic enzymes may also be required for penetration although controversies exist among plant pathologists about the role of these enzymes. Cutinase seems to be an important but not essential factor of pathogenicity. A cutinase encoding gene has been cloned from *Fusarium solani* (Dickman et al., 1989). Promoter analysis of the gene (Bajar et al., 1991) revealed that transcription is induced by cutin monomers and

a ~ 100 kDa transcription-activating protein is involved in the regulation of gene expression. This protein, which is probably of nuclear origin, is phosphorylated in the presence of cutin monomers and activates transcription by binding to the appropriate promoter region of the cutinase gene, with binding occurring only in the phosphorylated form. The role of cutinase was directly evaluated by testing the pathogenicity of cutinase deficient *F. solani* f. sp. *pisi* (*Nectria haematococca*) mutants obtained by transformation-mediated gene disruption (Stahl and Schäfer, 1992). As the pathogenicity of the cutinase deficient mutants was similar to that of the wild type parental strain, cutinase was proven to be nonessential for infection. Genes encoding various cutinases are not, therefore, virulence determinants. Cutinase genes may, however, be involved in the genetic control of aggressiveness. When *Cochliobolus heterostrophus* (a pathogen of maize, but non-pathogenic to pea) was transformed with the cutinase gene of *N. haematococca*, no effect on the virulence of the transformants to pea was observed. A simultaneous transformation of another gene of *N. haematococca* encoding phytoalexin degradation (see below) resulted in an extended host range, i.e., these co-transformants caused distinct lesions on pea, a non-host plant of *C. heterostrophus* (Oeser and Yoder, 1994).

Genes encoding enzymes able to detoxify antimicrobial plant compounds

Genes encoding phytoalexin detoxifying enzymes are determinants of fungal aggressiveness. A pisatin demethylase gene, *pda*, has been cloned from *F. solani* (Schäfer et al., 1989) followed by successful attempts to characterize the genetic backgrounds of other phytoalexin degrading systems (Van Etten et al., 1989). Six *PDA* loci have been identified in *F. solani* with three different levels of pisatin demethylase activity found to be associated with these genes. One of these genes, *PDA1*, proved to be the most active. After induction by pisatin, this gene was highly expressed with a very short lag period preceding the expression (Straney and Van Etten, 1994). Karyotype analysis of *F. solani* showed that *pda* genes are located on a dispensable chromosome and if this chromosome is lost, the resultant isolate is nonpathogenic to pea (Miao et al., 1991). However, later studies demonstrated that disruption of the *pda* genes caused no reduction in the pathogenicity of many of these mutants. If the loss of a dispensable chromosome containing the pisatin demethylase code is parallelled with the losss of pathogenicity in *F. solani* f. sp. *pisi*, this could be explained by the presence of additional pathogenicity genes on the same chromosome (Van Etten et al., 1994) or there are other mechanisms of phytoalexin detoxification.

Pre-formed plant defence compounds may also be decomposed by certain phytopathogenic fungi. Cyanogenic plants like flax and sorghum are attacked only by pathogens capable of converting cyanide to nontoxic compounds. Cyanide hydratase is a well characterized enzyme involved in this conversion. A cyanide hydratase encoding gene has been cloned from *Gloeocercospora sorghi*. Distribution of this gene resulted in cyanide sensitive mutants that still produced symptoms on sorghum indicating that the cyanide hydratase gene is not involved in the pathogenicity of *G. sorghi*. A different conclusion was drawn from studies on the avenacinase gene of *Gaeumannomyces graminis*.

Avenacin is a preformed antifungal molecule of oats, decomposed only by strains of *G. graminis* f. sp. *avenae*. *G. graminis* f. sp. *tritici* isolates are sensitive to avenacin and are unable to infect oats. *G. graminis* f. sp. *avenae* avenacinase gene mutants lost their virulence to oats but retained their pathogenicity to wheat which is an avenacin-non-producing alternative host of the fungus (Wang and Van Etten, 1992). Avenacinase is classified as a pathogenicity gene (Schäfer, 1994) instead of a virulence gene possibly because this gene determines compatibility at the species level rather than at a race-cultivar level.

Toxin genes

Genes encoding host-specific fungal toxins are virulence determinants. Their disfunction results in a limitation of host range. A large body of molecular information is available on HC-toxin produced by *Cochliobolus (Helminthosporium) carbonum* race 1 (Walton and Pannacione, 1993). HC-toxin, a small cyclic tetrapeptide, selectively alters the plasma membrane permeability of maize plants lacking the *Hm1* resistance gene. The biosynthesis of the small tetrapeptide is catalyzed by 'HC-toxin synthetase' which is composed of at least two enzymes; HTS-1 and HTS-2. These enzymes epimerize L-proline to D-proline and L-alanine to D-alanine, respectively. Genes encoding these enzymes form a large cluster. The locus containing these genes is called *TOX2* and is at least 56 kilobases in size. At its central core is *hts-1* which contains extended sequence duplications and is flanked by sequence repeats as well (Scott-Craig et al., 1992). The *hts-1* gene contains the largest open reading frame known from any genome capable of encoding a huge polypeptide composed of more than 5200 amino acids. HTS-1 mutations result in the reduction of both HTS-1 and HTS-2 activities. This huge sequence is absent from all other *Cochiobolus* species, and from race 2 of *C. carbonum* as well, which are only weakly virulent on all maize cultivars irrespective of the presence or absence of the *Hm1* resistance gene.

On the other hand, non-selective phytotoxins are not virulence determinants but may contribute to the aggressiveness of the pathogen. A good example is cerato-ulmin which is produced by the Dutch elm disease fungus, *Ceratocystis (Ophiostoma) ulmi*. This toxin is a hydrophobin, composed of 75 amino acids and produced in high amounts by more aggressive isolates of the fungus. Cerato-ulmin is encoded by the *cu* gene which has been cloned and characterized by Bowden et al. (1994).

Fungal avirulence genes

Early studies on the elicitor compounds of plant pathogenic microorganisms have indicated that a single point mutation in the pathogen might turn an icompatible interaction to a compatible one. In race-cultivar interactions, careful mutagenic treatments almost always increased the virulence of biotrophic pathogens whereas avirulent mutants have practically never been obtained by this method. It is hypothesized that these mutations affected those avirulence genes that encode elicitor molecules. The modified elici-

Table 1

Genes encoding pathogenicity, virulence, avirulence or aggressiveness cloned from microorganisms

Gene or locus	Source	Activity	Reference
avenacinase	*G. graminis* f. sp. *avenae*	avenacin decomposition (pathogenicity)	Schäfer, 1994
arvA	*P. solanacearum*	avirulence	Carney and Denny, 1990
avr genes	*P. syringae* pv. *glycinea* and pv. *tomato*	avirulence	Kobayashi et al., 1989
avrRxv	*X. campestris* pv. *vesicatoria*	avirulence	Whalen et al., 1988
avr4	*C. fulvum*	cysteine-rich elicitor (avirulence)	Joosten et al., 1994
avr9	*C. fulvum*	cysteine-rich elicitor (avirulence)	Van Kan et al., 1991
buf	*C. lagenarium*	melanin synthesis (pathogenicity)	Kubo et al., 1991
cu	*C. ulmi*	aggressivity (cerato-ulmin)	Bowden et al., 1994
eglA	*P. solanacearum*	endoglucanase (aggressivity)	Roberts et al., 1988
hrp	*P. solanacearum*	HR-inducing ability (pathogenicity)	Boucher et al., 1987 Arlat et al., 1992
	E. amylovora	HR-inducing ability (pathogenicity)	Barny et al., 1990
	P. syringae	HR-inducing ability (pathogenicity)	Lindgren et al., 1988
INF24	*U. appendiculatus*	appressorium development (pathogenicity)	Bhairi et al., 1989
INF56	*U. appendiculatus*	appressorium development (pathogenicity)	Xuei et al., 1992
Mpg1	*M. grisea*	appressorium development (aggressivity)	Talbot et al., 1993
opsA-G	*P. solanacearum*	EPS-production (aggressivity)	Cheng Kao and Sequeira, 1992
pda	*F. solani*	pisatin-demethylase (aggressivity)	Weltring et al., 1988
pglA, pehA	*P. solanacearum*	poligalacturonase (aggressivity)	Schell et al., 1988
TOX2	*C. carbonum*	HC-toxin (virulence)	Scott-Craig et al., 1992
–	*F. solani*	cutinase (aggressivity)	Dickman et al., 1989
–	*G. sorghi*	cyanide-hydratase (?)	Wang and Van Etten, 1992
–	tomato mosaic tobamovirus	130 and 180 kDa proteins (virulence)	Meshi et al., 1988
Gene *VI*	*CaMV*	host range determinant	Schoelz et al., 1986

tors were not recognized by the plants and, consequently, the induction of HR failed and disease symptoms developed. Based on these experiments, several trustworthy models were developed for the specific recognition in plant-pathogen interactions (*cf.* Érsek and Hornok, 1985). However, molecular evidence for this basically correct hypothesis became available nearly a decade later. A-race-specific elicitor was first isolated from the

intercellular fluids of tomato leaves infected with *Cladosporium fulvum* (*Fulvia fulva*) race 9. This elicitor induced HR on tomato cultivars that carried the *Cf9* resistance gene. The cDNA of this protein has been identified and the corresponding avirulence gene, designated *avr9*, cloned and sequenced (Van Kan et al., 1991). Races of *C. fulvum* carrying the *avr9* gene were unable to cause disease on cultivars harbouring the *Cf9* gene. These plants recognized the AVR9 protein, and exerted HR. When other *C. fulvum* races, virulent on *Cf9*-tomato plants were transformed with the *avr9* gene, they became avirulent and incited HR (Van der Ackerveken et al., 1992) while *avr9* mutants of *C. fulvum* became virulent on these same plants (Marseisse et al., 1993).

AVR9 is a small cysteine-rich protein and a member of the so-called fungal hydrophobins. Hydrophobins confer hydrostatic properties on the surfaces of dry-spored fungi. They may be involved in hyphal binding and in appressorium development so that they are considered to be substantial compounds of the fungi. AVR9 may be such a functional hydrophobin and, as these proteins are located on the outermost parts of the fungal structures, they are involved in cell-to-cell contact and may therefore be the first recognized fungal compounds especially suitable to induce plant responses.

Similar cysteine-rich elicitors have been identified in other races of *C. fulvum*. One of these is encoded by the *avr4* gene and incites HR on tomato cultivars carrying the *Cf4* resistance gene. When a 510 bp segment of *avr4* was used as a probe, homologues of the *avr4* gene were identified in seven additional races of *C. fulvum* able to infect the *Cf4*-tomato plants. RFLP-analyses revealed no differences among homologues. This finding seemed to disprove the avirulence gene theory. However, sequence analysis demonstrated minor differences among the *avr4* homologues of the seven races. Every *avr4* homologue contained a single base pair substitution in one of the cysteine codons. In all cases, a cysteine codon (TGT) was changed to a tyrosine codon (TAT) (Joosten et al., 1994). As cysteine plays a major role in the formation of the tertiary structure of proteins, minor mutations in the hydrophobin encoding genes result in continuous changes of the AVR proteins enabling the pathogen to avoid recognition by plant products encoded by resistance genes. More recent studies demonstrated, that besides the cystein–tyrosine substitution, other amino acid exchanges in the *avr4* gene product could also increase the virulence of *C. fulvum*. These products, called isoforms are unstable molecules and this instability is the major cause of the absence of their recognition by the host (Joosten, 1997).

The second part of the present paper provides an overview of plant genes involved in susceptibility or resistance towards pathogens. Prior to this, Table 1 presents a selection of pathogenicity genes cloned from various pathogens.

Plant resistance genes

Genes encoding general resistance

A large group of plant genes encode enzymes that are involved in several steps of biochemical or physical defence.

Resistance in cell walls is increased by the polymerization of different wall phenol compounds. This is catalyzed by a wall-bound special *peroxidase enzyme.* Two genes have been identified in tomato (*tap*1 and *tap*2) that encode two homologous anionic peroxidases. These genes can be activated by wounding, elicitor treatments, or fungal infection (Mohan et al., 1993).

Another important enzyme is the *phenylalanine ammonia-lyase* (PAL) which is a key enzyme catalyzing the synthesis of anti-microbial aromatic compounds (including phenols). One of the related genes, *PAL-1*, was cloned many years ago (Lois et al., 1989). In the promoter of that gene, three inducible protein "footprints" have been identified. Two were induced after treatment with UV light or a fungal elicitor. One was detected only after treatment with an elicitor.

Phytoalexins could be involved in the biochemical defence system. Phytoalexins cannot determine the specific incompatibility process between host plants and their pathogens (cf. Király et al., 1972, Glazerbrook and Ausubel, 1994, Mauch-Mani and Slusarenko, 1996), their accumulation begins only after the recognition reaction between the gene products of the host and the infecting pathogen. These compounds may have important roles in reducing growth or multiplication of plant pathogens and in inhibiting secondary infections. In addition, phytoalexins can be increased by inducers of resistance well before infection (preformed resistance). Enzymes catalyzing phytoalexin synthesis can be activated by wounding, elicitors or fungal infections. The most important enzyme in these processes is the 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMGR) which catalyzes the formation of mevalonate from the HMG-coenzyme A. Several HMGR gene (*hmg1*, *hmg2*, *hmg3*) have been cloned from potato (Choi et al., 1992). Members of this gene family are activated by different control mechanisms. The *hmg1* gene is activated by wounding, while *hmg2* and *hmg3* are activated after infection. As a result of induction, the accumulation of phytoalexins has been shown. Another case is the activation of stilbene synthase genes (*Vst1*, *Vst2*) in peanut and grapes (Hain et al., 1990, 1993), which participate in the synthesis of the phytoalexin resveratrol. This subject will be treated later.

Additional elements for the biochemical defence of plants are the *lytic enzymes*, such as *chitinases* and β-*1,3-glucanases*, that appear in host tissues as a result of acquired resistance. These enzymes are able to attack cell walls of penetrating fungi. In tomato leaves infected with *Cladosporium fulvum*, several nucleic acid sequences were determined to code for chitinases (*Chi3*, *Chi9*, *Chi14*, *Chi17*) (Danhash et al., 1993). Two of these sequences carry information for the synthesis of extracellular acidic chitinase. During incompatible host-pathogen interactions, intensive transcription is initiated from these genes. In cucumber, three chitinase genes have been identified (*CHI1*, *CHI2*,

CHI3) and their coding regions were highly homologous (Lawton et al., 1994). As a result of fungal infection or treatment with an aspecific elicitor, only the *CHI2* gene was expressed. It has been generally accepted that plant chitinases are inducible enzymes. However, molecular evidence has only recently been received. The promoter of the chitinase gene derived from tobacco contains specific protein-binding motifs (Fukuda and Shinshi, 1994). It is supposed that these motifs are the elicitor-sensitive elements. However, the roles of chitinases and glucanases in plant disease resistance have recently been questioned (Joosten et al., 1995). The tomato pathogen *Cladosporium fulvum* turned out *not* to be sensitive to the chitinase and the β-1,3-glucanase of its natural host, tomato. Thus, the above-mentioned two defence proteins could indeed be regarded only as stress proteins.

A unique case of resistance is determined by the recessive allele (*mlo*) of the barley *Mlo* gene that confers race non-specific (horizontal) resistance to the fungus *Erysiphe graminis* f. sp. *hordei*. The barley *Mlo* gene has been isolated by positional cloning and characterized as a novel control element of resistance (Büschges et al., 1997). Indeed, the broad spectrum resistance to the fungus is determined by a defective *Mlo* gene causing arrest of the pathogen without tissue necrosis (HR).

Genes encoding recognition and signal transduction

Again, it is stressed that the general resistance genes encoding physical or biochemical defence systems are not the key factors determining compatible or incompatible host/pathogen relationships. Genes of general resistance could be involved in the restriction, arresting or killing of the pathogen in a resistant plant. These genes, however, cannot alone determine recognition.

Incompatibility is determined by another group of genes: those nucleic acid sequences that are involved in host resistance characterized by the gene-for-gene relationship between host and pathogen.

This type of resistance gene encodes receptor molecules that recognize pathogens or elicitors of pathogens. According to the generally accepted hypothesis, after the recognition process between host resistance gene products (receptors) and pathogenicity (avirulence) gene products (the elicitors of pathogens), signal transduction mechanisms are induced. As a consequence of signalization, the transcription of "general" resistance genes begins.

Several "specific" resistance genes that are involved in the gene-for-gene relationship have been cloned, such as the *Pto*, *Pti1* and the *Cf9* genes from tomato, the *N* gene from tobacco, the *RPS2* from *Arabidopsis thaliana*, the *L6* gene from flax, and the *Xa21* gene from *Oryza* spp. (cf. Hammond-Kosack and Jones, 1996).

It must be stressed, however, that there is no convincing evidence yet of resistance gene products binding the pathogen's elicitor molecules. Isolated gene sequences are usually compared to other sequences at computer databases and suppose where the action takes place. According to some opinions this is "computer biology", not real biology. Recently, however, Gopalan et al. (1996) have shown experimentally that gene products

of the host R and pathogen *avr* genes interact prior to development of the hypersensitive death. In addition, Leister et al. (1996) and Van den Ackerveken et al. (1996) demonstrated that *R* genes and bacterial *avr* genes were expressed and interacted within the same plant cell.

The *RPS2* gene confers resistance to strains of *Pseudomonas syringae* that express the *avrRpt2* avirulence gene. The polypeptide corresponding to the resistance gene sequence possesses leucine-rich repeats with interesting functions. One can suppose that the leucine-rich repetitive region produces receptors of elicitor molecules of pathogens. It was also supposed that the P-loop domain serves for binding nucleotide triphosphates. Thus, the product of the *RPS2* gene is probably a complex protein which recognizes the elicitor of the infecting pathogen and transfers the signal, activating other genes that are involved in the development of the hypersensitive response (HR) (Bent et al., 1994; Mindrinos et al., 1994).

The *N* gene sequence cloned from tobacco (Whitham et al., 1994) and the *RPS2* gene sequence of *Arabidopsis* are 49% similar and 24% identical. Plants possessing the N gene mediate resistance to tobacco mosaic virus (TMV). In addition, the *N* gene, when transferred to virus susceptible tobacco, confers resistance.

It was established by sequence analysis that the *N* gene encodes a protein similar to the cytoplasmic domain of the *Drosophila* Toll protein and to the human interleukin-1 receptor and contains leucine-rich repeats. On the basis of sequence similarities, it was suggested that the protein encoded by the *N* gene recognizes the product of TMV replicase gene. As a consequence, transcription factors are activated that induce expression of genes responsible for the *N*-mediated hypersensitive response. However, it is not clear whether the transcription factors are directly activated by the *N* gene or through other molecules such as kinases, phosphatases or proteases.

The HR induction in resistant plants expressing the *N* gene correlates with inhibition of the virus. However, there is no direct evidence that the hypersensitive necrosis of host plants inhibits the infecting virus. The biochemical events of inhibition of viral replication and spread are largely unknown at the moment.

The tomato *Pto* gene confers resistance to strains of *Pseudomonas syringae* pv. *tomato* carrying the gene *avrPto*. When tomato plants susceptible to the bacterium were transformed with *Pto*, they turned resistant to the above-mentioned bacterial infection. The gene product of *Pto* is probably a serine-threonine protein kinase which may have a role in signaling the presence of the pathogen's elicitor. As a result, the HR-regulating genes are activated (Martin et al., 1994). Recently, it was shown that this may happen indirectly. Zhou et al. (1995) identified the second element of the signaling cascade, the *Pti1* gene. The product of this gene is specifically phosphorylated by the gene product of *Pto*. Then, the phosphorylated Pti1 protein, which is a serine-threonine kinase, transfers a signal for HR. The quantity of Pti1 protein seems to be important because transgenic tobacco transformed with the *Pti1* gene responded to infection with a quick and intensive hypersensitive reaction.

There is another gene in tobacco, the *hsr203J* gene, that encodes a protein that has a role in signal transduction. The promoter of the gene does not respond to stresses, how-

ever, it is specifically activated by infection of avirulent strains of *Pseudomonas solanacearum*, and, as a result, transcription of a specific mRNA is stimulated and the end result is the elicitation of HR (Pointer et al., 1994).

Recognition is encoded in an incompatible plant/fungus interaction by the *Cf-9* resistance gene in tomato (Jones et al., 1994). Tomato cultivars carrying this nucleic acid sequence exhibit resistance to those strains of the fungus *Cladosporium fulvum* that carry the *avr9* avirulence gene. The protein predicted from the nucleotide sequence of the *Cf-9* gene is probably a transmembrane glucoprotein with extracellular leucin-rich regions. One can suppose that a receptor-like domain is responsible for binding the elicitor of the fungus carrying the *avr9* gene. Binding hypothetically corresponds to recognition and that is the first step in the elicitaion of HR. May et al., (1996) have shown that subsequent to the interaction of the *Cf/avr* genes, the host response resulted in severe oxidative stress and, as a consequence, in HR.

Interestingly, the mechanism of recognition was analyzed by a peculiar experiment of Hammond-Kosack et al. (1994). The *avr9* gene of *C. fulvum* was incorporated into a susceptible tomato line lacking the *Cf-9* resistance gene. After crossing this line with the resistant line, the uninfected progenies died because the interaction between the product of the resistance gene and the pathogen's avirulence gene resulted in the elicitation of the hypersensitive response (HR). However, if the *Cf-9* gene was inactivated in the resistant tomato, the progenies of the crossing with this mutant survived (there was no binding between the two gene products). In other words, recognition, i.e. interaction between the host gene products and pathogen's gene products, did not occur. Another possibility to circumvent the resistance mediated by the gene products of the host *Cf* gene is to produce by the pathogen unstable *avr* gene products. Joosten et al. (1997) have shown that during pathogenesis, similar to the expression of the *avr* genes, virulent *avr* alleles are also induced. Because of the instability of the isoform products of the *avr* allels, the fungus remains virulent, i.e. there will be no binding between the products of the *R* gene and the *avr* allele. Consequently, there will be no recognition event and the pathogenic fungus circumvents recognition.

Additional resistance genes have been cloned from flax (*L6* gene) and *Oryza* spp. (*Xa21* gene). *L6* confers resistance to the fungal pathogen *Melampsora lini* (flax rust) and this sequence is responsible for two gene products (Lawrence et al., 1995). The larger gene product resembles the products of the *N* gene and that of the *RPS2* gene. It contains leucine-rich repeats and the gene has an ATP/GTP binding site. The *Xa21* gene was the first gene isolated from cereals. It confers resistance to *Xanthomonas oryzae* and it was cloned from *Oryza longistaminata*. The *Xa21* gene is effective in different rice species (Song et al., 1995). The gene product possesses leucine-rich repeats and a serine-threonine kinase domain. The gene products of both *L6* and *Xa21* supposedly recognize the elicitors of pathogens. On the basis of these examples now we can better understand how resistance genes control the recognition of plant pathogens and initiate the resistance response.

It is interesting that *RPS2*, *N*, *Cf-9*, *L6* and *Xa21* resistance genes encode proteins that contain leucine-rich repeats. Whether this characteristics has significance in the process of recognition remains to be seen.

A toxin-inactivating resistance gene

The *Hm1* maize gene is not involved in encoding receptors of pathogen elicitors and has no role in signal transduction. *Hm1* encodes a reductase that inactivates the HC-toxin of *Cochliobolus carbonum*. The enzyme, NADP-dependent HC toxin reductase, has been identified in extracts from resistant genotypes. Johal and Briggs (1992) cloned the resistance gene from maize. This gene confers race specific resistance to race 1 of the fungus *C. carbonum*. The *Hm1* gene product, HC toxin reductase, represents an inactivation mechanism used by the host plant to inhibit or block the infecting pathogen. This resembles the mechanism used by microorganisms for antibiotic inactivation.

Transgenic plants carrying alien nucleic acid sequences for disease resistance

Scientists have worked during the past 30 years on the mechanism of pathogenicity of microorganisms and host plant defence thereby permitting more effective disease control and breeding for disease resistance (Michelmore, 1995).

In transgenic plants, foreign genes control the mechanism of disease resistance. These "transgenes" may be derived from different plant species, even from plants that are non-hosts of the disease causing pathogens. Transgenes have also been isolated in plant pathogens (mostly in viruses) and transferred to plants resulting in pathogen-derived resistance (Lomonossoff, 1995). In special cases, resistance genes can be isolated from nonpathogenic microorganisms or even from animals.

Incorporation of pathogen-derived sequences

The wildfire disease of tobacco is caused by tabtoxin, a phytotoxic dipeptide of the bacterial pathogen *Pseudomonas syringae* pv. *tabaci*. The bacterium defends itself by enzymatic detoxification of tabtoxin. The enzyme acetyl transferase is responsible for detoxification. The enzyme is encoded by the tabtoxin resistance gene (*ttr*). Anzai et al. (1989) cloned this gene and incorporated it into tobacco which became resistant to tabtoxin.

Pathogen-derived resistance has been induced successfully when viral sequences that can confer resistance were incorporated into plant genomes (cf. Baulcombe, 1996). Pathogen-derived resistance means transformation of plants with portions of viral genomes. Transgenic plants become resistant to the virus from which the genome was derived. Resistance can be RNA-mediated or protein-mediated. The first type is highly specific and usually protects against high levels of inoculum. On the other hand, protein-mediated resistance has a broader spectrum with relatively lower levels of defence. As regards the mechanism of the protein-mediated resistance, experiments have shown a correlation between the level of coat protein expression in transgenic plants and the level

of resistance. In plants expressing the coat protein, the removal of viral subunits from the viral capsid (uncoating) may be inhibited by the transgene product. As a consequence, replication of the viral genome will not be initiated. Another hypothesis suggests that the coat protein encoded by the transgene blocks the host receptors to which the virus binds before removal of the viral coat. In transgenic plants in which viral genes encoding the movement protein have been introduced the transgene product binds to sites (perhaps on plasmodesmata) in host cells and compete with the functional movement protein encoded by the infecting virus. As a consequence the virus cannot move from cell to cell. Another possibility is that the movement protein encoded by the transgene is a mutant protein which binds nonproductively to the viral nucleic acid and, therefore, it competes with the functional protein thus inhibiting the movement of the virus.

The RNA-mediated resistance is very specific. The levels of transcripts are lower in the resistant transgenic plants. The mechanism seems complicated. The virus inhibition may be due to a specific RNA-degradation process. In the presence of the virus, the transgenic plant cells signal very high levels of a particular transcript. As a consequence, a specific RNA-degradation is initiated and both the induced transcript and the genomic RNA from the virus from which the transgene was derived are inhibited (Lomonossoff, 1995).

The first paper that described transgenic plant that expressed the coat protein gene was published by Powell et al. (1986). The transformed tobacco plant was resistant to tobacco mosaic virus (TMV). Since then, several transgenic plants have been created that express the viral coat protein gene and exhibit resistance to the following viruses: alfalfa mosaic virus (Tumer et al., 1987), cucumber mosaic virus (Couzzo et al., 1988; Quemada et al., 1991), potato virus X (Hemenway et al., 1988; Fehér et al., 1992), potato virus Y (Kollár et al., 1993b), tobacco streak virus (Van Dun, 1988), potato leaf roll virus (Kawchuk et al., 1990), different potyviruses (Lawson et al., 1990; Lindbo and Dougherty, 1992; Ling et al., 1991; Stark and Beachy, 1989; Palkovics et al., 1995). One of the best summaries on the engineered resistance against plant viruses which includes the coat protein gene technique has been published relatively early by Nejidat et al. (1990).

Several other parts of the viral genome have been incorporated into crop plants which increased resistance to viral infections to different degrees, e.g. subgenic TMV RNA (the 54 kD gene) by Golemboski et al. (1990), the protease genes of several potyviruses (Maiti et al., 1993; Vardi et al., 1993), movement protein genes of tobamo-, bromo- and potex viruses (Lapidot et al., 1993; Malishenko et al., 1993; Beck et al., 1944), the full or reduced replicase genes of a series of viruses (Anderson et al., 1992; Rubino et al., 1993; Audy et al., 1994; Zaitlin et al., 1994) and the 3′ untranslated region of turnip mosaic virus (Zaccomer et al., 1993). According to Lomonossoff (1995), probably many or all viral sequences are suitable to induce some degree of resistance to viruses in transgenic plants.

Some other techniques were also applied to induce resistance to viruses. Several years ago, Harrison et al. (1987) incorporated the *satellite RNA* of cucumber mosaic virus (CMV) into tobacco to increase protection against CMV and the related aspermy mosaic virus infection. Another technique was applied by Kollár et al. (1993a) when the *defec-*

tive interfering RNA of the cymbidium ringspot tombusvirus (CyRSV) was expressed in *Nicotiana benthamiana*. The defective RNA-mediated resistance fully suppressed top necrosis and decline of *N. benthamiana* plants after infection with CyRSV.

Transgenes from distantly related plants

Broglie et al. (1991) successfully transformed tobacco and rape with the ethylene-inducible *chitinase gene* derived from French bean. Transgenic plants expressed resistance to *Rhizoctonia solani* which has chitin in its cell walls. On the other hand, *Pythium aphanidermatum*, having cellulose-containing cell walls, was insensitive to chitinase of transgenic plants after infection. Contrary to that, transformation of tobacco with chitinase derived from sugar beet infected with *Cercospora beticola* did not result in resistance to *Cercospora nicotianae*.

Phytoalexin genes encoding stilbene synthase (Vst1, Vst2) have been cloned from grapes and groundnut and transferred to tobacco that does not contain this enzyme. Stilbene synthase is a key enzyme in the synthesis of the phytoalexin *resveratrol*. Transgenic plants expressing this gene accumulate resveratrol because its precursors, p-coumaroyl coenzyme-A and malonyl coenzyme-A, are available in tobacco cells. As a result, resistance in transgenic tobacco plants against *Botrytis cinerea* slightly increased (Hain et al., 1990, 1993).

Virus resistance was expressed by several *ribosome-inactivating proteins* (RIPs) when genes encoding RIPs were incorporated into transgenic plants. Specificity of RIPs is variable and the toxicity to eucaryotic cells is also variable. The gene encoding a RIP of *Phytolacca americana*, when expressed in tobacco, resulted in heavy damage to some tobaccos. However, plants expressing this gene on a low level remained undamaged and exhibited resistance to potato viruses X and Y as well as to cucumber mosaic virus (Lodge et al., 1993). In another interesting experiment Logemann et al. (1992) have shown that expression of a barley RIP in transgenic tobacco leads to resistance to the fungus *Rhizoctonia solani*. Tobacco has been transformed with a construction of a barley RIP gene driven by a wound-inducible promoter from potato (*wun1*). The transgene was effective only against the fungus. However, the transgenic plants remained undamaged.

Genes from higher animals are also suitable for increasing plant disease resistance

Tavladoraki et al. (1993) created resistance in *Nicotiana benthamiana* against artichoke mottled crinkle virus (AMCV) by a unique method. They produced monoclonal antibodies against this virus. The gene encoding this mammalian immunoglobulin was then transferred into *Nicotiana bethamiana*. Those plants that expressed this gene constitutively, exhibited virus resistance. Truve et al. (1993) transformed potato with a rat gene encoding 2-5A synthase. The latter enzyme participates in the antiviral protection on mammalian cells induced by interferon. Actually, it activates a ribonuclease that decomposes viral nucleic acid molecules.

Table 2

Cloned genes from plants and micoorganisms that have role in plant disease resistance

Gene	Source	Encoded properties	Reference
CHI1, *CHI2*, *CHI3*	cucumber	resistance (chitinase)	Lawton et al., 1994
chi3-17	tomato	resistance (chitinase)	Danhash et al., 1993
Cf-9, Cf-2	tomato	resistance (HR, receptor, signal transduction)	Jones et al., 1994 Dixon et al., 1996
CP	tobacco mosaic virus (TMV)	coat protein elicitor (avirulence)	Powell-Abel et al., 1986
Di-RNA	cymbidium ringspot tombusvirus (CyRSV)	symptom suppression	Kollár et al., 1993a
hmg1, *hmg2*, *hmg3*	potato	resistance (biosynthesis of phyoalexin)	Choi et al., 1992
Hm1	maize	resistance (HC toxin reductase)	Johal and Briggs, 1992
hsr203J	tobacco	resistance (HR, signal transduction)	Pontier et al., 1994
L6	flax	resistance (recognition, HR)	Lawrence et al., 1995
N	tobacco	resistance (HR, against TMV infection)	Whitham et al., 1994
PAP	*Phytolacca americana*	inactivation of ribosomes (virus resistance)	Lodge et al., 1993
Pto	tomato	resistance (signal transduction), HR	Martin et al., 1994
Pti1	tomato	resistance (signal transduction), HR	Zhou et al., 1995
RPS2	*Arabidopsis thaliana*	receptor (signal transduction), HR	Bent et al., 1994
satRNA	satellite RNA from CMV	symptom attenuation	Harrison et al., 1987
subRNA	subgenic RNA of TMV	symptom attenuation	Golemboski et al., 1990
ttr	*Pseudomonas syringae* pv. *tabaci*	resistance (detoxification of tabtoxin)	Anzai et al., 1989
Vst1, *Vst2*	grapes, groundnut	resistance (biosynthesis of the phytoalexin resveratrol)	Hain et al., 1990, 1993
wun1-RIP	potato, barley	inactivation of ribosomes (resistance to *Rhizoctonia solani*)	Logemann et al., 1992
Xa21	*Oryza longistaminata*	resistance (receptor kinase, recognition)	Song et al., 1995
–	*Aspergillus niger*	glucose oxidase (H_2O_2 generation, HR)	Wu et al., 1995
mlo	barley	race non-specific resistance, no HR	Büschges et. al., 1997

Genes from non-phytopathogenic microorganisms may also be used improving plant disease resistance

Several fungal species possess *virus-like particles* (VLP) with double stranded RNA (dsRNA). Most fungi live in a peaceful coexistence with VLP, however, a few specis protect themselves against the virus-like particles. *Schizosaccharomyces pombe* has the *pac1* gene the (Iino et al., 1991) that encodes an RNase which decomposes dsRNA. This gene has been cloned and expressed in tobacco. Transgenic plants became

resistant to tomato mosaic virus (ToMV) and delayed symptom expression in the case of cucumber mosaic virus and potato virus Y infections (Watanabe et al., 1995). Explanation of resistance could be due to the RNase activity of a fungal gene. This decomposes double-stranded replication intermediates of single-stranded plant viruses.

Recently, Beffa et al. (1995) transformed tobacco plants with a chimeric gene encoding the Al subunit of the *cholera toxin*, driven by the light-inducible wheat *Cab1* promoter. Transgenic plants expressing the toxin became relatively resistant to the bacterial pathogen *Pseudomonas syringae* pv. *tabaci* and accumulated high levels of salicylic acid, which is regarded as a resistance factor.

The *glucose oxidase* gene of the fungus *Aspergillus niger* was transferred and expressed in potato (Wu et al., 1995). Higher plants do not possess this gene. The enzyme glucose oxidase catalyzes generation of the reactive oxygen species hydrogen peroxide (H_2O_2). As is known, reactive oxygen species, including H_2O_2, are involved in the hypersensitive response of host plants. This response is associated with resistance and expression of the transgene glucose oxidase in potato induced resistance to the phytopathogens *Erwinia carotovora* and *Phytophthora infestans*.

In summary, new prospects are opening up for plant breeding by cloning pathogenicity genes of plant pathogens as well as resistance genes of host plants (Table 2) to gain a deeper insight into the mechanism of host/pathogen interaction. Transfer of new genes or even modified sequences into crop plants makes it possible to enhance resistance to economically important pathogen infections.

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Notes added-in-proof. Anderson et al. (Plant Cell 9, 641–651, 1997) recently have shown that another cloned *R* gene of flax (*M* gene) confers resistance to a flax rust fungus pathotype and encodes a protein with leucine-rich repeats (LRR) and a nucleotide binding site (NBS). It turned out that several loss-of-function mutations fall within the LRR-encoding domain of the gene, giving evidence that the LRR domain in this *R* gene is involved in recognition of the pathogen.

In another paper Ori et al. (Plant Cell 9, 521–532, 1997) demonstrated that the *I2C–1* tomato *R* gene indeed interacts with the race 2 of the pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* during the recognition process. Transgenic *I2* tomato plants expressing a portion of the *I2C-1* gene in an antisense orientation became susceptible to race 2 of the *Fusarium* fungus. Untransformed plants remained resistant to the pathogen. Furthermore, recognition of race 1 of the pathogen, that is recognized by a different *R* locus, was not affected. Thus, the antisense strategy can be used in understanding the *R* gene structure and function relationships.

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A Repetitive DNA Sequence Associated with Karyotype Variability in *Fusarium poae*

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A moderately repetitive DNA element has been cloned from *Fusarium poae* a strictly asexual fungus, which is frequently occurring secondary invader of small-grain cereals. The element, named ZIT1 selectively hybridised to the polymorphic, 1.0–3.7 Mb chromosomal region of the fungus, but no hybridisation signal was observed on any of the large chromosomes. Nucleotide sequence analysis indicated that ZIT1 shares only a moderate level of similarity to *gag* genes of fungal retrotransposons which was mainly due to a small cysteine-reach motif, known as zinc-finger DNA binding domain. No significant homology was found with any other published nucleotide sequence. The coincidence of the polymorphic chromosome region and the chromosomal distribution of ZIT1 in several strains of *F. poae* suggests that this repeat, that could be a remnant of a formerly active element still may generate chromosome rearrangements through recombination among its scattered homologous sequences.

Section 'Sporotrichiella' of the genus *Fusarium* includes certain species that are weak pathogens of cereals, invade stroged grains and cause serious toxicological problems by excreting secondary metabolites, mainly 12,13-epoxytrichothecenes (Logrieco et al., 1990). Two of the most prominent members of this group of fungi are *Fusarium poae* (Peck) Wollenw. and *Fusarium sporotrichioides* Sherb. These morphologically similar, closely related species are strictly asexual, moreover attempts to demonstrate the complete parasexual process within their life cycle proved also to be unsuccessful (Cullen et al., 1983).

Many examples show high levels of intraspecific karyotype variability in imperfect fungi that rarely or never undergo meiosis (Kistler and Miao, 1992). In our experiments noteworthy interspecific (Fekete et al., 1993) and inter-subspecific (Nagy and Hornok, 1994) karyotype differences were observed for fusaria. As far as the intraspecific variability is concerned, it was obvious only within the mini-chromosome region in the case of *F. sporotrichioides* (Nagy et al., 1995). On the other hand, *F. poae* showed polymorphisms in the medium-sized chromosome region, as well (this paper).

Chromosome rearrangements are regarded to play an important role in the evolution of fungi (Smith et al., 1991) that lack sexual recombination. Karyotype polymorphisms may be accompained by phenotype differences including morphology (Suzuki et al., 1991), antibiotic production (Walz and Kück, 1991) or host range (Cooley and Caten,

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1991). Early studies indicated that repetitive DNA sequences may be associated with chromosomal variations in fungi (Scherer and Stevens, 1988).

In the present article we describe the cloning and sequencing of a repetitive DNA fragment which selectively hybridised to small- and medium-sized chromosomes in *F. poae* strains.

Materials and Methods

Strains

F. Poae isolates A-11, A-13, A-14, A-15, A-18, TAPO-2, TAPO-4 and TAPO-5 were isolated and identified by us (Tóth et al., 1993), strain 72 187 was kindly supplied by Prof. A. Ylimäki (Finland). *Escherichia coli* DH5α and pEMBL18 were used for transformation and cloning experiments.

Pulsed-field gel electrophoresis

Separation of chromosome-sized DNA bands have already been described in detail (Fekete et al., 1993). In order to facilitate the evaluation of the figures electrophoretic conditions are briefly recalled. CHEF-DR II system was used under the following parameters: (1) in 0.6% agarose at 40 V for the whole run with 3 sec pulse time for 48 h, followed by 53 min/96 h, 45 min/72 h, and 37 min/72 h to separate the large chromosomes; (2) in 0.7% agarose 40 V/1600 sec/24 h, 40 V/1200 sec/24 h, 180 V/120 sec/12 h, and 180 V/180 sec/12 h to separate the small chromosomes. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* chromosome size standards (Bio-Rad Laboratories, Richmond, USA) served as markers.

Isolation and manipulation of DNA

DNA minipreparation was performed as described earlier (Fekete et al., 1995). Standard procedures were used for Southern hybridisation (Southern, 1975). Restriction endonucleases were obtained from Amersham (Amersham International, Amersham, UK) and used according to the manufacturer's recommendations.

Nucleotide sequence analysis

DNA sequencing was performed by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase, Version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, USA). The nucleotide sequence identified in this study appears in EMBL, GenBank and DDBJ nucleotide sequence databases under the accession num-

ber Z71706. The nucleotide sequence, as well as the deduced polypeptide sequence were compared with the EMBL sequence library (release 38.0) using the GCG software package (Devereux et al., 1984).

Results

Genomic DNA from *F. poae* strain 72 187 was digested with *Bam*HI. Fragments were directly cloned into pEMBL18 and minilibraries were prepared after transformation of *E. coli* DH5α cells. Plasmids recovered from approximately 500 recombinant colonies were *Bam*HI digested and after electrophoretic separation 20 fragments ranging between 0.6 and 8.0 kb were isolated then tested for specificity and abundance. Nineteen of the clones gave single hybridisation signals when *Bam*HI digested total DNA samples of nine *F. poae* strains were probed, therefore they were not further analysed. One 1.2 kb clone, however hybridised to 4–8 *Bam*HI fragments of these strains and revealed strain specific patterns (Fig. 1). The hybridisation patterns obtained by this repetitive clone, named hereinafter ZIT1 were different from the patterns revealed by hybridisaiton with a 2.3 kb *Eco*RI–*Bam*HI fragment of the *rDNA* gene from *A. nidulans* (data not shown), indicating that ZIT1 is not a ribosomal repeated sequence. (The same probe proved to be suitable for mapping rRNA loci on *Fusarium* chromosomes in an earlier study – Fekete et al., 1993.)

Chromosome-sized DNAs of the nine *F. poae* strains were separated by pulsed-field gel electrophoresis. Figs 2 and 3 show electrophoretic karyotypes obtained under conditions in which large and small chromosomes, respectively, were resolved. Figure 4 is a schematic diagram which summarizes the results of the two different electrophoretic runs. The number of chromosome-sized DNAs resolved varied between five and six among the strains with sizes ranging 1.0 to ~ 6.5 Mb. One large, intersively-stained band was obtained within the 4.6–5.7 Mb range of the size standard, *S. pombe* chromosomes representing at least two comigrating chromosomes, but this band may even comprise two doublets. Another, more diffuse band appeared at around 6.5 Mb range; its intensity and distinctness varied greatly among isolates, the best resolution was achieved in strain TAPO-5. This band represents one, possibly two chromosomes. Additional chromosome-sized DNAs could be detected within the 2.2–4.6 Mb range (Fig. 2 Left), but in that case only, if the final concentrations of protoplasts embedded into agarose blocks were extremely high, approaching 5×10^9 ml^{-1}. A disadvantageous side-effect of this elevated concentration of protoplasts resulted in a greater degree of degeneration as indicated by a smear over the lanes. Separation of chromosomes smaller than 2.2 Mb in size was more easily performed, since higher concentration of agarose could be used and the electrophoretic parameters were favourable to the formation of sharp, distinct bands (Fig. 3 Left). The two large bands were present in the same position in all strains, no variation, except for staning intensity occurred among the isolates in this range of chromosomes. On the other hand, significant interstrain karyotype polymorphisms were observed within the 1.0–3.7 Mb chromosome region. All but one strain harboured one or two mini-

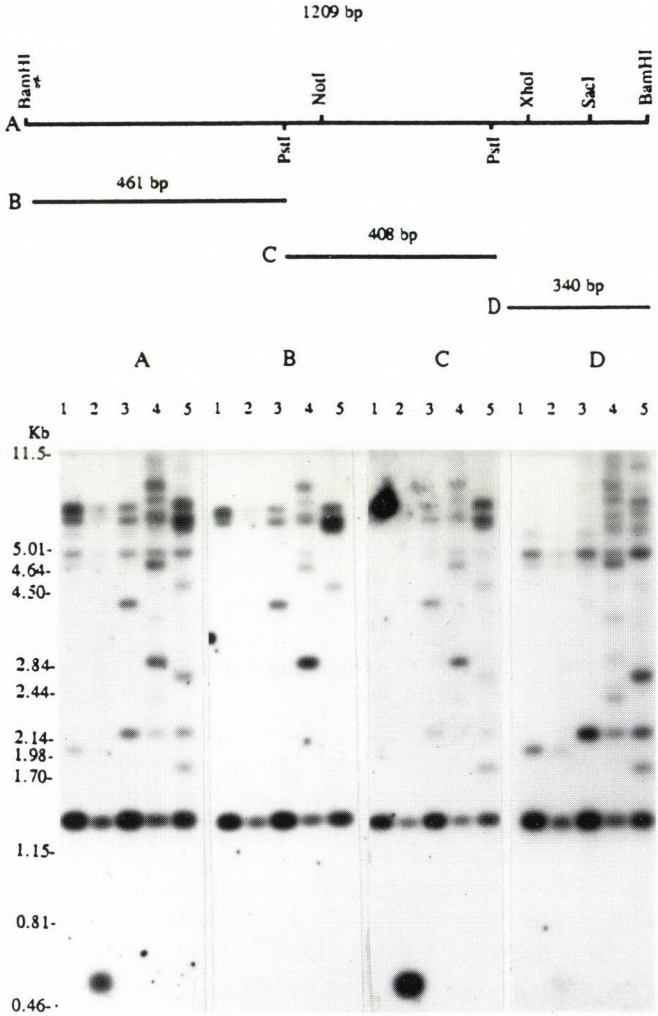

Fig. 1. Southern blot of genomic DNA of 5 strains of *F. poae* probed with the 1.2 kb element cloned *from F. poae* strain 72 187. A, hybridisation with the entire clone. B-D, hybridisation patterns obtained with the three subclones. *Lanes 1-5* are strains A-13, A-14, 72 187, TAPO-2 and TAPO-4, respectively

chromosomes, smaller than 1.6 Mb. Small (> 1.6–2.2 Mb) and medium-sized (> 2.2–3.7 Mb) chromosomes were present in 7 out of 9 strains.

When pulse field gels were probed with radiolabelled ZIT1, no hybridisation signal was observed on any of the large chromosomes (Fig. 2 Right) indicating that this element was absent in the non-polymorphic chromosomal region of *F. poae*. On the contrary, the probe did hybridise to all small- and medium-sized chromosomes in each strains (Figs 2 Right, 3 Right). The distribution of this repetitive element was thus limited to the polymorphic, 1.0–3.7 Mb chromosomal region of *F. poae*.

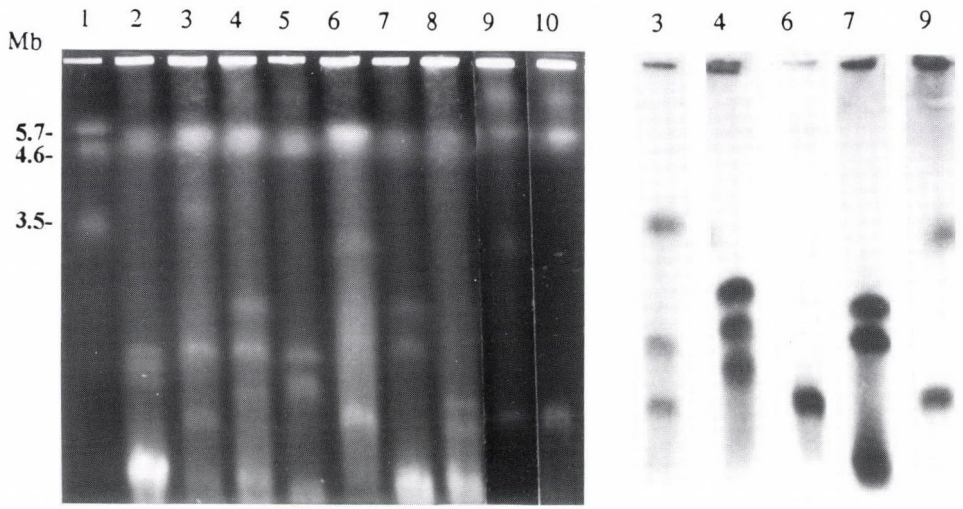

Fig. 2. Separation of the large- and medium-sized chromosomal DNAs of various *F. poae* strains by pulsed-field gel electrophoresis. Left, ethidium-bromide stained gel. Right, hybridisation with radiolabelled ZIT1. *Lanes* are: *1, S. pombe* size standard; *2–10, F. poae* strains A-11, A-13, A-14, A-15, A-18, 72 187, TAPO-2, TAPO-4, TAPO-5, respectively

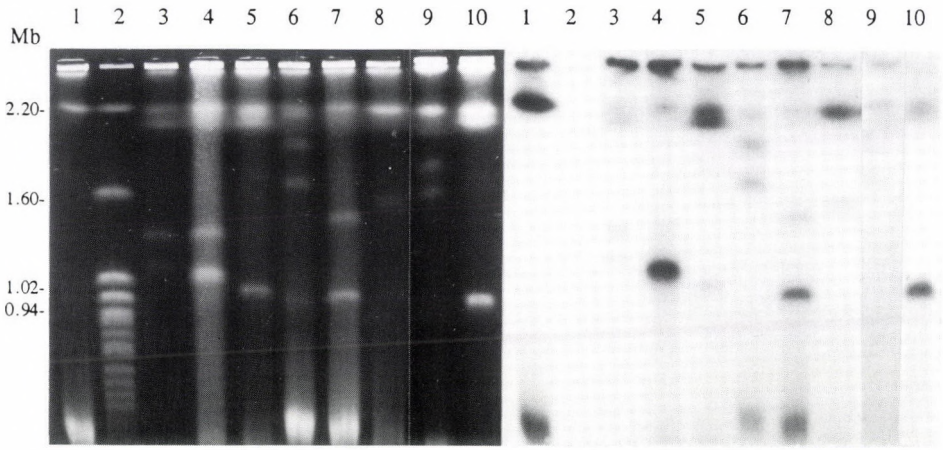

Fig. 3. Separation of the small-sized chromosomal DNAs of various *F. poae* strains. Left, ethidium-bromide stained gel. Right, hybridisation with radiolabelled ZIT1. *Lanes* are: *1, F. poae* strain 72 187, *2, S. cerevisiae* size standard; *3-10, F. poae* strains TAPO-4, TAPO-5, A-13, A-11, TAPO-2, A-14, A-15, A-18, respectively

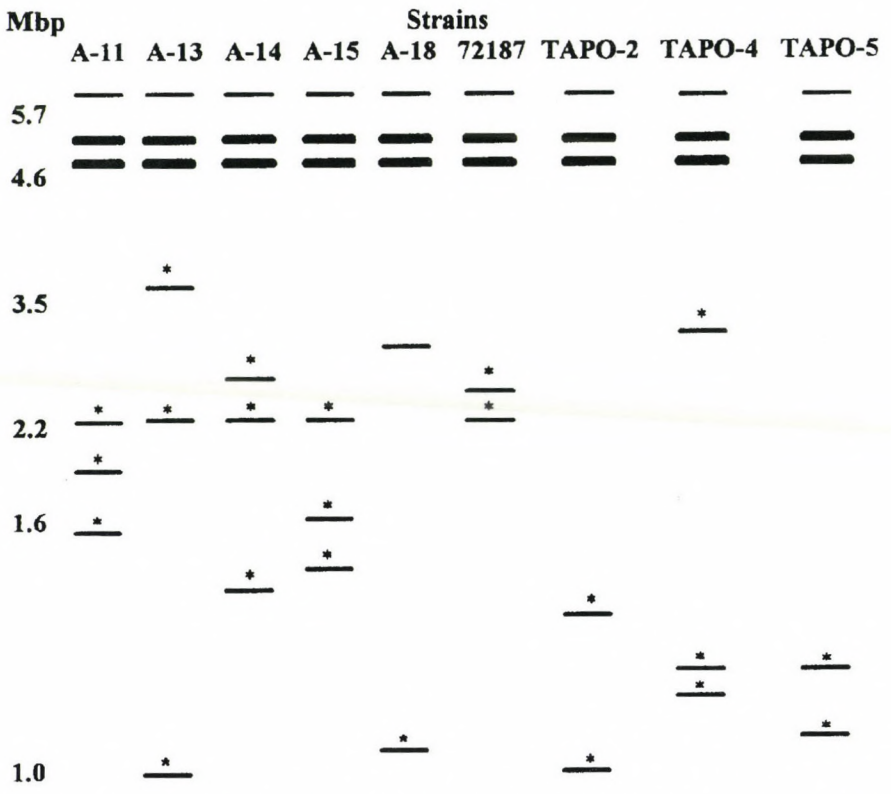

Fig. 4. Scheme of chromosome-sized DNA profiles of the *F. poae* strains; * indicates the bands that gave positive signals when probed with ZIT1

ZIT1 was restriction mapped and three non-overlapping internal subclones were further examined in order to determine what parts of the element are responsible for repetitiveness. Figure 1 shows the results of this experiment in which *Bam*HI digested genomic DNAs of five strains were probed with the entire 1.2 kb clone (A), as well as with the three subclones, designated B, C and D, respectively. As all the three subclones showed repetitive properties the whole ZIT1 was characterised by DNA sequence analysis; the nucleotide sequence and the deduced amino acid sequence are given in Fig. 5.

Examination of ZIT1 for conserved motifs known to be present in repetitive elements resulted in identification of a 501 nt region starting from an ATG codon (nt 708) not followed by a nearby stop codon and stretching to the end of the clone. Computer search revealed that this predicted sequence of 167 aa has similarity to *gag* genes of fungal retrotransposons. The most related sequences were the *gag* genes known from *Cf*T-1 (43.4%), *skippy* (37.7%) and *grh* (37.3%) described by McHale et al. (1992), Anaya

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1     GGATCCGCGTTTCTTAGACGCTTCATTGACATGGGAAGTTGGCGCTGCTTCAGGCTCGGG
61    TTCTGGTATGGATGCTGGTGCTAGTTGAGGAGCTTCTTCCCCCTGTGCTTCCCTAGATCG
121   TCTCGTGCTCCTACTAGTGATGGTCTCTGAGTCACGTTGATTTCTCGCCCAACCGACGCG
181   TTACCACTCGCTTTTTGTCTGCTGCTCTTCCTCGTCGTGGCTGTGTCATCGCGTTCATTT
241   CTTTCTCGGTGCCTCTTTCACATCGTTCCATTAGTCGTCGGTGCCTTAACAGCGTGTTGC
301   TACGCCGCAGGTCGTCCATTTGTAGGCTTCTTCACTGCTCTGCCTTTGGCTCTAGCACTA
361   CTTCCGGCTTCGGTTCATCTGTCTTGTCCGTCTTAATTCTCTTCGATTTTCGCACAAATG
421   GCAAGTCGTCGTCGTACTCATAATCTGTCGCGCGCCGCTGCAGCGAGACGCTTGCTCAAC
481   CGTCGCCCGTTCTCGTTGCCCATGCTAATTACTTGAAGCGGCCGCCGAGTCATCACTAGA
541   GTAGTCATGGGTGGCGCATTCGGGGCCGTTGACGACGTGATTTTAGATTCCCGAGGTTGT
601   GATGCCAAGAAGTTATCGGTGCGGCGCGGTACGGCCAGTAGTGGACGCACGTCGCGTTGT
661   TATTTGCGTCTTCTCAATGATTAAAACACGCTGCTCAACACGCTCAAATGCTGTTGCAAT
                                                      M  L  L  Q  C
721   GCAATTCGTGATGTCGGCTAGTTTGCGGCGTCGTCAATGGAGGTTTGCGCTTCCCCACGT
        N  S  G  C  R  L  V  C  G  V  V  N  G  G  L  R  F  P  T  C
781   GTTTTCTTGAGAATTTTTCTGCTTCATATCCTCGTGTTGTGGCTCTGTCGGTGCCCTCGG
        F  L  E  N  F  S  A  S  Y  P  R  V  V  A  L  S  V  P  S  G
841   GCACCATATGTTGCAACCGTGATACCTGCAGTCTCCCCCCAGCCATCGGCTTTGTCTGTT
        T  I  C  C  N  R  D  T  C  S  L  P  P  A  I  G  F  V  C  L
901   TGCTGATCTCCCTCGAGCCCCCCGGGTTTTTGTGCGTACTCTTATGTTCCGTGTGTTTCT
        L  I  S  L  E  P  P  G  F  L  C  V  L  L  C  S  V  C  F  C
961   GTTGTTGTTGCCTCCGACCGGGTCACTATGTGGCATGCTGTACTGTGAAGTCAACCGCTG
        C  C  C  L  R  P  G  H  Y  V  A  C  C  T  V  K  S  T  A  G
1021  GCGACATTAACCATAGCCGTCATCAACCACCTCATGTAGTGCAAGCTGGAATCGTTTCGT
        D  I  N  H  S  R  H  Q  P  P  H  V  V  Q  A  G  I  V  S  Y
1081  ACATGGTGAATACTGAGTATGAGAGCTCCTCACGGCCTCCTGAGACCGATCAGCCGGGTG
        M  V  N  T  E  Y  E  S  S  S  R  P  P  E  T  D  Q  P  G  G
1141  GCGGAGGGTGCCAAGCCTCCGTAACCGTAGTTTGGGGGGAGCGACAGTGGGCAAGTCTGT
        G  G  C  Q  A  S  V  T  V  V  W  G  E  R  Q  W  A  S  L  S
1201  CTGGGATCC
        G  I
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Fig. 5. Nucleotide and deduced amino acid sequences of the ZIT1 element. The putative zinc-finger motif is underlined

and Roncero (1995), and Dobinson et al. (1993), respectively. These, otherwise moderate similarities were mainly due to a small cysteine-reach motif, known as zinc-finger DNA binding domain (C-X_2-C-X_4-H-X_4-C) located at positions between nt 960 and 1001. No significant homology was found with any other published nucleotide sequence.

Discussion

The presence of repetitive elements that may be amplified under certain conditions are regarded to be important determinants of genetic variability in clonal eukaryotic organisms, like most hyphomycete fungi in which recombination through sexual or parasexual cycles is rare or, in fact absent. Mobile repetitive elements characterised in

fungi include transposons, retroposons and retrotransposons. Features of these element have recently been summarized by Kachroo et al. (1995). ZIT1 is most probably not related to these elements because it has no significant homology with transposons (Daboussi et al., 1992; Langin et al., 1995) or retrotransposons (Anaya and Roncero, 1995; Julien et al., 1992) cloned from *Fusarium oxysporum* and showed no valuable similarity to mobile genetic elements known from other organisms. A further counter-argument is, that the ZIT1 probe hybridized only to the small- and medium-sized chromosomes, contrary to the *grh* element, which was found on all resolvable chromosomes of *Magnaporthe grisea* (Dobinson et al., 1993). Transposons and retroelements either of animal or plant origins are also more evenly distributed in the genome of their hosts (Smyth, 1991).

Other repeated sequences in fungi were characterised as telomere-associated elements, present on all or almost all chromosomes. Such telomeric sequences, like Ca7 (Sadhu et al., 1991) or the Rel-2 element (Thrash-Bingham and Gorman, 1993) of *Candida albicans* are considered essential for proper chromosome maintenance. The largest three chromosomes of *F. poae* that comprise nearly 80% of the genome do not carry a single copy of ZIT1, therefore this repetitive sequence appears to be non-essential for chromosome function and hence not related to Ca7 or Rel-2.

The third class of non-rDNA repeats is represented by the *CARE-1* element (Lasker et al., 1991) and the RPS sequences cloned from *C. albicans* (Chindamporn et al., 1995; Iwaguchi et al., 1992). These and the ZIT1 clone share one common trait, *i.e.* they were not found on all resolvable chromosomes of one or more strains that had been tested for their occurrence. *CARE-1* was identified as a nontelomeric, probably non-essential sequence with a copy number ranging between two and twelve per haploid genome, depending on strain, while the RPS1 element or its homologous sequences, represented by some 80 copies were found to be present on all chromosomes of *C. albicans* except for chromosome 4.

The novelty of the moderately dispersed ZIT1 element is that its distribution is restricted to the polymorphic chromosome region of *F. poae.* The limited sequence data do not allow any detailed speculation about the origin of this unusual repeat, but one may suggest that it is a remnant of a formerly active foreign DNA that had been eliminated from the large chromosomes by a defensive mechanism suitable to prevent the disruption of necessary genes. However, even this remnant may generate chromosome rearrangements through recombination among its scattered homologous sequences. The coincidence of the polymorphic chromosome region and the chromosomal distribution of ZIT1 in several strains of *F. poae* supports this hypothesis. Alternatively, ZIT1 may be a footprint of genetic duplication, a mechanism found to contribute to chromosome polymorphism within *Fusarium oxysporum* f. sp. *cubense* (Kistler et al., 1995).

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Selective Effect of Wheat Germplasm upon Isolates of *Mycosphaerella graminicola*

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The selection of *Septoria tritici* biotypes by Bobwhite'S' resistant wheat germplasm was studied. Seedlings of cultivars with Bobwhite'S' germplasm and with dwarf-mexican germplasm were inoculated in the greenhouse. Two *Septoria tritici* isolates (atypical yeast-like variant coming from Bobwhite'S' germplasm and stromatic from traditional argentine germplasm) were used. Isolates of *S. triciti* from each isolate × cultivar combination were analyzed, and the percentage of regular or variant colonies was registered. Significant pathogen population effect and cultivar effect were demonstrated. The regular isolate produced chlorotic and necrotic lesions with pycnidia in all the cultivar; the atypical variant had quite distinct reaction according to the cultivars, being predominant the non-pycnidial necrotic reaction. Pathogen population isolated from Bobwhite'S' germplasm produced higher levels of variant colonies, than did population isolated from dwarf mexican cultivar. Inoculation with the variant produced a high number of variant colonies in cultivars with both germplasm, indicating that Bobwhite'S' germplasm could induce the origin of less pathogenic variants from a heterogeneous pathogen population through a remarkable mechanism of selective effect and any genetic change.

The selection pressure exerted by diverse hosts upon their pathogens has been largely studied (Lincoln, 1940; Watson and Luig, 1968; Leonard, 1969; Brasier, 1987; Cordo et al., 1989; Rapilly et al., 1989).

The pathogens progressively adapt to their resistant hosts through a selective effect on such hosts. They produce a selective pressure (Krupinsky, 1982; Cunfer and Youmans, 1983) on the pathogen, showed throughout the increase or decrease of pathogenicity (Skajennikoff and Rapilly, 1983; Osburn et al., 1986). Moreover the lack of virulence in a biotype of the population is due to the mutants that have lost the genetic factors conditioning such feature (Stackman, 1947).

Spontaneous mutation, sexual recombination and somatic hybridisation are mechanisms of change is pathogenicity by which the new virulence combinations could be generated in a pathogen population. Additional variations occur from other pathogen populations or due to molecular and cytoplasmic changes (Burdón, 1992). Such examples as delay in chromosome separation in mitosis (Mc Clusky and Mills, 1990); suppressions producing polymorphism in nuclear DNA restriction fragment sequences (Mc Donald and Martinez, 1990); reduction in the pathogenicity of aggressive isolates because of the host cytoplasm or nucleus (Brasier, 1987; Rapilly et al., 1989), support such statement.

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Brasier (1977, 1982, 1983, 1987) studied aggressiveness changes in *Ophiostoma (Ceratocystis) ulmi* in elms populations. The reduction in pathogenicity would appear because of a combination of mutations that occurred in nuclear genes and for the influence of a cytoplasmic factor.

In Argentina, the population of *Septoria tritici* isolates, obtained from cultivars having a Bobwhite'S' resistant germplasm, showed new cultural types, with diminished pathogenicity, and a different physiological behaviour (Perelló et al., 1990). Besides, one of these isolates (P_3 isolate which produces microspores in cultures and pycnidia and microspores on leaves) could be selected from the pathogen local population (Sanderson, pers. comm. 1990).

This paper studies the evidence to support that Bobwhite'S' resistant germplasm selects *S. tritici* biotypes morphoculturally different and less pathogenic than the regular ones.

The spreading of wheat cultivars with resistant germplasm could cause alterations on the composition of *S. tritici* population with an increase of biotypes with diminished pathogenicity.

Materials and Methods

Cultural and morphometrical studies were carried out upon 10 pathogen isolates, obtained from cultivars with Bobwhite'S' (CM 33203) and Kavkaz, germplasm collected from naturally infected plants (Table 1).

Each fungal isolates was isolate from infected tissue samples or by spore transfer from a pycnidium. The cultures (6 replication for isolate) were grown on 2% PDA (Potato Dextrose Agar) under laboratory conditions (means temperature 20 °C, diffused

Table 1

Origin, cultural types and colony colour of ten *Septoria tritici* isolates

Isolate	Cultivar	Germplasm	Colour	Collected in
P_3	LPI/BW'S'	BW'S'	45 Buff	Pergamino
P_1	Bw/4/... Laj 3139	BW'S'	45 Buff	Pergamino
E_0	CST 169	BW'S'	45 Buff	Uruguay
35_B	CM 76751 × 0 (F_1)	BW'S'	61 Rossy Buff	Barrow
P_{3-4}, P_{3-6}	LPI/BW'S'	BW'S'	45 Buff	Pergamino
19_N	CM 61830	Kavkaz	45 Buff	Necochea
5_E	Millalew	Kavkaz	61 Rossy Buff	Uruguay
E_1	LI7	BW'S'	45 Buff	Uruguay
LH_{SM}	Los Hornos improved line	physiologic mutant	45 Buff	Los Hornos

Table 2

Size of budding cells and conidia of ten *Septoria tritici* variants developed "in vitro" on PDA media (Fitzgerald and Cook method) after seven days

Isolate	Cultivar type	Thallus type	In vitro length (µm)	In vitro width (µm)
P_3	mucous (yeast-like)	short and long cylindrical budding cells	(1.93) 2.15 3.66	(1.02) 1.25 (1.59) 1.50
P_1	albinic, mycelial plastery, "cordeé type"	elliptical budding cells	3.92	2.02
E_9	stromatic, albinic, dusty	elliptical conidia	6.02	3.75
35_B	albinic, mycelial, "cordeé type"	elliptical conidia	(4.3) 5.73 (6.0)	2.86
P_{3-4} P_{3-6}	mycelial, filamentous	unicellular and bicellular elliptical conidia	(4.5) 6.0 (7.5)	3.0
19_N	albinic, powdery, plastery, mycelial	secondary budding cells and conidia	(6.0) 7.5 (10.5) *	3.0 *
5_E	albinic, powdery plastery, stromatic	filiform conidia, secondary conidia chlamidospora	(7.15) 11.4 (12.) **	1.43 **
E_1	albinic, powdery plastery, stromatic	elliptical conidia	6.02	3.75
LH_{5M}	albinic, mycelial, "cordeé type"	prismatic budding cells	(5.78) 9.5 (13.5)	(1.8) 2.38 (2.6)

* These sizes correspond to budding cells only
** These sizes correspond to filiform conidia only

light) for 21 days. The aspect, colour (following Rayner, 1970), form, margin, internal structure, size and spore type were determined. Colonies were described after Garassini 1958 and Negroni 1938. Direct microscopic observations microculture techniques, micrometrical measurements and SEM spore observations were made. Fitzgerald and Cooke's method (1989, pers. comm.) was applied to determine the germination of each vegetative structure (Table 2).

Monospore colonies arising from resistant germplasm were studied and comparing with the phenotypes usually isolated from Argentine germplasm (pycnidial-stromatic).

Two isolates were assayed in order to test the selective effect of the Bobwhite'S' germplasm through artificial inoculation technique: P_3 atypical yeast-like and mucous isolate, as a variant and M89, regular and stromatic, as a control. Every isolate come from hosts with different genetic origin: P_3 from La Paz INTA/Bobwhite'S' line and M_{89} from Buck Poncho with argentine traditional germplasm.

Three wheat cultivars of different germplasm were chosen as hosts: Don Ernesto INTA (D. E. I.) and La Paz INTA/Bobwhite'S' line with CM 33203, AU/KAL/BB/

/3/WOP'S' ancestry, and Marcos Juarez INTA (M. J. I.) with Sonora 64/KLRE genealogy.

Artifical inoculations of the two isolates on the cultivars mentioned were perfomed on the 3rd leaf stage. The inoculum concentration for each isolate ranged between 1.8×10^7 for M_{89} and 2.2×10^7 for P_3. The inoculum was applied by pulverization until run off from the wheat leaves. The incubation period under wet chamber was of 96 hours. At the end all pots inoculated were maintained under greenhouse with controlled condition of temperature and humidity. The pycnidial coverage percentage (PCP) was registered on the 21st days and after the assessment of the disease all lesions of the middle part of the third leaf inoculated were cut out in 0,5 cm long pieces. Three pieces were randomly selected and, once desinfected (70% ethanol for 1/2 minute and 1‰ Cl_2Hg for 1 1/2 minute) were incubated on PDA. Within 7 to 10 days, the percentage of regular colonies (RCP) and variant colonies (VCP) for each selected piece were determined. The experimental design used was a random block with 10 replications. A variance analysis of PCP and VCP was carried out. The average differences were analyzed by Tukey test.

Results

The different isolates studied comprised two types according to thallus structure: yeast-like and albinic filamentous. The largest part of the material studied belonged to the latter (Table 2, Fig. 1).

Most isolates comprised cell types that germinated through terminal cell by budding or by fission. True conidia germinated by a tube.

A highly significant effect on cultivars and isolates ($P \leq 0.05$) was shown by the ANOVA for PCP (Table 3). The highest PCP was observed in all cultivars with the M_{89} isolate (Fig. 2).

The PCP has always been higher on LPI/Bbw'S', for the two isolates studied. No differences were found between other two cultivars (Fig. 3 and Table 4). Analyzing each isolate in particular, M_{89} caused chlorotic and necrotic lesions with pycnidia, when it was inoculated on 3 cultivars.

The P_3 isolate produced different reactions on inoculated cultivars. Very restricted or absent lesions on M. J. I. (Fig. 4), only small marginal lesions without pycnidia on D.E. I. (Fig. 5) and small lesions with or without pycnidia on LPI/Bbw'S' (Fig. 6).

A significant effect on cultivars and nonsignificant effect on isolates were showed by the ANOVA for VCP ($P \leq 0.05$) (Table 5). Among cultivars, M. J. I. did not produce variant colonies for M_{89} with respect the other two cultivars (Fig. 7). The cultivars behaviour with respect to each isolate was different (Fig. 8). The VCP produced by the inoculation of M_{89} isolate was significantly high in D. E. I. and LPI/Bbw'S' compared to M. J. I. With P_3 variant, VCP did not differ significantly among the three cultivars.

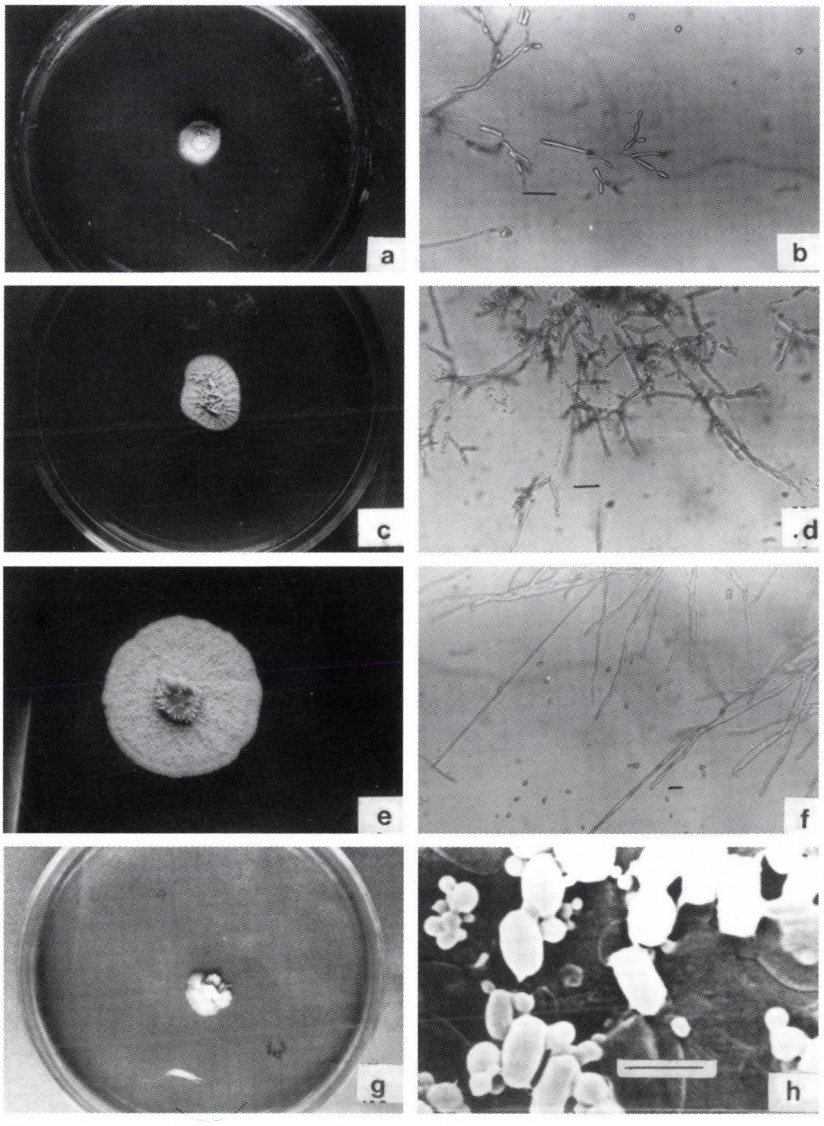

Fig. 1. Types of variants of *Septoria tritici* developed "in vitro" on PDA media after 21 days.
a) isolate LH_{5m}; monosporic colony upon a potato – dextrose agar. (PDA) (× 0.8)
b) isolate LH_{5m}; pseudomycelium hyphas with apex budding cells; bar = 20 μm
c) isolate P_1; monosporic colony upon PDA (× 0.8)
d) isolate P_1; pseudomycelium with end budding cells; bar = 1 μm
e) isolate 5_E; monosporic colony upon PDA (× 0.8)
f) isolate 5_E; elliptical hyaline conidia and secondary conidia; bar = 18 μm
g) isolate P_3; yeast-like monosporic colony upon PDA (× 0.8)
h) isolate P_3; cylindrical budding cells; bar = 2 μm

Table 3

ANOVA for percentage pycnidial coverage (PCP)

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	3869.0979	12	322.4248	16.374	0.0000
cultivar	286.5103	2	143.2551	7.275	0.0018
isolate	3297.3246	1	3297.3246	167.453	0.0000
block	285.2630	9	31.6959	1.610	0.1415
FACTOR INTERACTIONS	45.704698	2	22.852349	1.161	0.3225
cultivar isolate	45.704698	2	22.852349	1.161	0.3225
RESIDUAL	886.09738	45	19.691053		
TOTAL (CORR.)	4800.9000	59			

0 missing values have been excluded

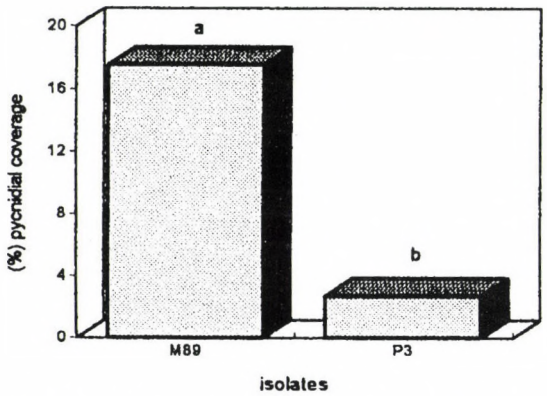

Fig. 2. Pycnidial coverage percentage of wheat cultivars inoculated with M_{89} and P_3 isolates. Different letters show significant differences by Tukey test

Table 4

Mean values (10 replications) for percetage pycnidial coverage (PC) and percentage variant colonies (VC)

Cultivar	Isolate	Pycnidial coverage %		Variant colonies %	
		M_{89}	P_3 (variant)	M_{89}	P_3 (variant)
MJI (Mexican Dwarf germplasm)		17.56	0.34	0	34
LPI/Bw'S' (Bbw'S' germplasm)		19.7	6.51	70	59
Don E. INTA (Bbw'S' germplasm)		15.1	1.12	55	58

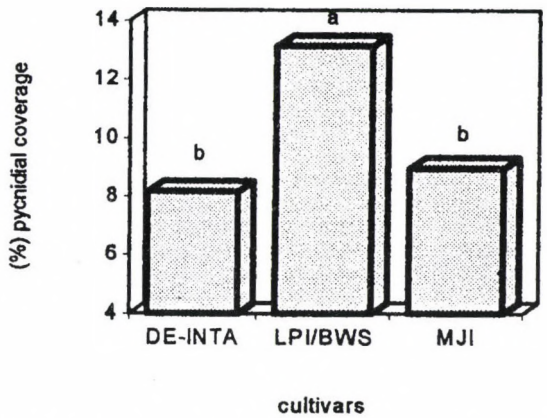

Fig. 3. Pycnidial coverage percentage on DE-INTA (Don Ernesto INTA); LPI/BWS (LPI/Bobwhite'S') and MJI (Marcos Juárez INTA) cultivars, inoculated with M_{89} and P_3 isolates. Different letters show significant diffrences by Tukey test

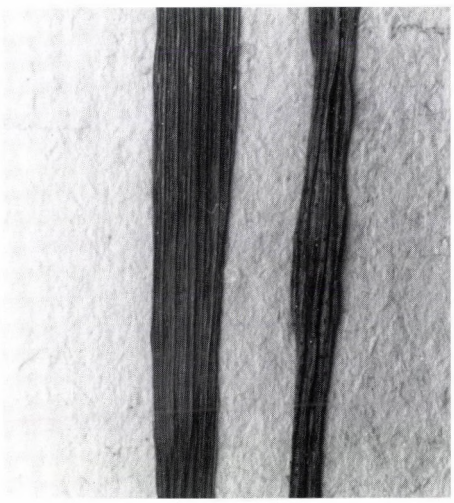

Fig. 4. Foliaceous lesions without pycnidia for P_3 × MJI (isolate × cultivar) interaction

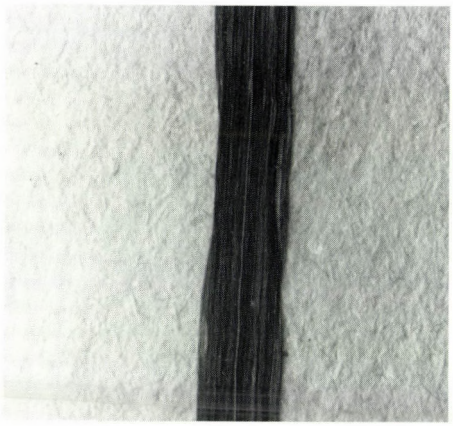

Fig. 5. Foliaceous lesions without pycnidia for P_3 × Don Ernesto INTA (isolate × cultivar) interaction

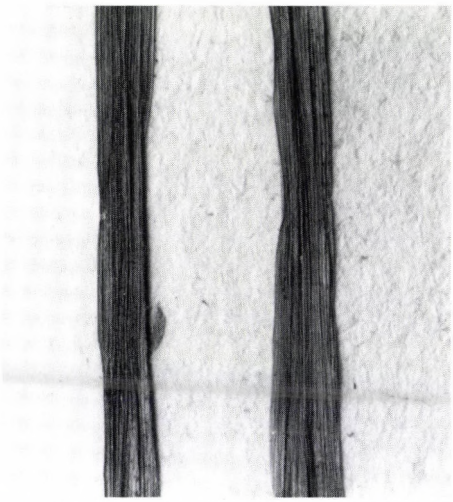

Fig. 6. Foliaceous lesions with or without pycnidia for P_3 × LPI/Bobwhite'S' (isolate × cultivar) interaction

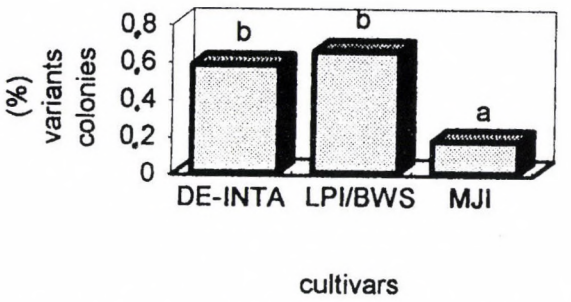

Fig. 7. Mean values of the variant colony percentage of isolates on DE-INTA (Don Ernesto INTA); LPI/BWS (LPI/Bobwhite'S') and MJI (Marcos Juárez INTA) cultivars. Different letters show significant differences by Tukey test

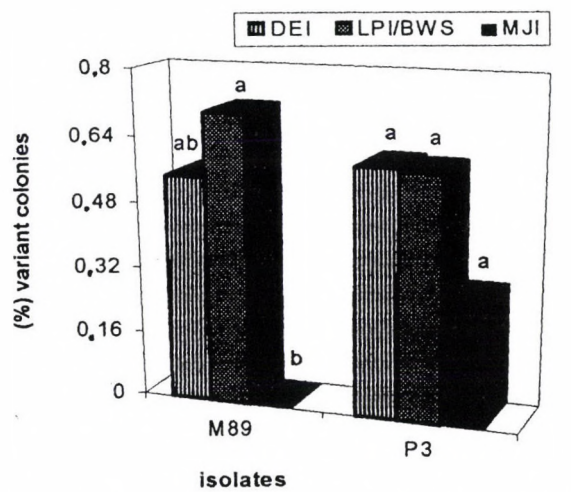

Fig. 8. Variant colonies percentage in DE-INTA (Don Ernesto INTA); LPI/Bobwhite'S' and MJI (Marcos Juárez INTA). Different letters show significant differences by Tukey test

Table 5

ANOVA for percentage variant colony (VCP)

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	4.8113400	12	0.4009450	3.141	0.0026
cultivar	2.3070400	2	1.1535200	9.036	0.0005
isolate	0.0299267	1	0.0299267	0.234	0.6357
block	2.4743733	9	0.2749304	2.154	0.0441
FACTOR INTERACTIONS	0.2465733	2	0.1232867	0.966	0.3884
cultivar isolate	0.2465733	2	0.1232867	0.966	0.3884
RESIDUAL	5.7443267	45	0.1276517		
TOTAL (CORR.)	10.802240	59			

0 missing values have been excluded

Discussion

The results obtained in this work showed the effect of wheat germplasm (specially Bobwhite'S'-CM 33203) in variants selection of *S. tritici* with lower pathogenicity. Rapilly et al. (1989) verified the selection pressure exerted by triticale isogenic lines upon *Septoria nodorum* cytoplasm.

Cordo et al. (1989) detected qualitative changes in the virulence of two *S. tritici* isolates with only one generation of subculture from a resistant wheat line with Bbw'S' germplasm. The virulence either increased or remained stable with regard to their original generation.

Current biochemical methods have allowed to study the genetic variability of *S. tritici* (Mc Donald and Martinez, 1990) suggested a great genetic variability among small isolates samples collected from small geographic areas. They found that different genotypes were present in the same lesion of the leaf.

The Bbw'S' germplasm had selected atypical yeast-like and albinic mycelial variants, from the total population of *S. tritici*. Besides, it has been demostrated that the P_3 isolate had lost pathogenicity. In order to show the selective effect of Bbw'S' wheat germplasm it must be marked that M89 isolate, did not produce variants colonies by passed throughout M. J. I. cultivar (control) but was affected when passed the first time throughout varieties with Bobwhite'S' germplasm. The evidence coincides with Stackman (1947) and Lincoln (1940) statements for different selection pressure examples. In both cases, the less pathogenic variants would have been caused by mutant selection during fungus multiplication in its host with loss of the pathogenicity factor.

In this work, as in Brasier's experiences (1977, 1982, 1983, 1987) polymorphic colonies (mycelial-albinic, stromatic-dusty-albinic and mycelial-filamentous) and less

pathogenic isolates have been described. They are morpho-cultural different from those traditionally stromatic (wild type in Argentina). This would suggest that these cultural types with low associated pathogenicity may be brought about the combination of a mutagenic effect and some cytoplasmic factor as Brasier mentioned for *Ceratocystis ulmi*.

The selective effect of Bbw'S' germplasm has been seen through various paths: 1) when inoculating a regular or a variant isolate on cultivars having such germplasm, variant isolates appeared in a high percentage, compared with the isolates obtained from the cultivar with dwarf-mexican germplasm. 2) the P_3 variant isolate was characterized by its weak pathogenicity on the 3 cultivars assayed.

The variants frequently isolated from cultivars with Bbw'S' germplasm could be originated by a serie of adaptative changes in the pathogen due to diverse mechanism. It could be thought that Bbw'S' germplasm act as a variant selector substratum. Also, certain character (pathogenicity, pigment production, spores feasibility) could been ruled by specific genes affected by the selection. These genes would be present and active in the regular isolate, but when infect the host could suffer an inactivation as a result of the adaptative change (Stackman and Harrar, 1957). If the combination of a mutagenic effect and cytoplasmatic factors lasted long, it could be expected that a more durable resistance would occur in cultivars containing this germplasm.

Acknowledgements

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Decomposed Coconut Coirpith – A Conducive Medium for Colonization of *Trichoderma viride*

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The effect of Decomposed Coconut Coirpith (DCCP), when added to normal nursey media, on the survival of *Trichoderma viride* was tested under sterilized and unsterilized conditions. The unsterilized media, except pure DCCP, caused reduction of *T. viride* population. Among the sterilized media, the Raw Coconut Coirpith (RCCP) and Normal Nursery Mix (NNM) did not support the multiplication of *T. viride*. In dual inoculation experiments in media mixes, with *Fusarium equiseti* (Sacc.) Corda., the population of *T. viride* did not reduce significantly but drastic decline in *F. equiseti* was observed. The seed and soil application of *T. viride* was carried out and rhizosphere and non-rhizosphere colonization of *T. viride* was tested. Seed treatment favoured greater colonization of rhizosphere than soil application. The media mix DCCP50 and DCCP25 significantly reduced the damping off of *Eucalyptus camaldulensis* caused by *F. equiseti*.

The success of biological control with introduced microorganisms mainly depends on the ability of biological control agents to colonize the rhizosphere region of host plants (Deacon, 1991). The rhizosphere colonization of biological control agents has been greatly influenced by quality and quantity of root exudates, nutrient availability from the substrates, competition with other microorganisms, genetic capability of the biocontrol agents etc. Addition of substrates or food base either into soil or on seed coat which enable the biocontrol agents to proliferate in the spermosphere or rhizosphere is another likely approach (Papavizas and Lewis, 1981). We conducted an investigation to study the effect of Decomposed Coconut Coirpith (DCCP) on the colonization of *T. viride*, applied either through seed treatment or soil application and its effect on *Fusarium equiseti*, the causal agent of damping off of *Eucalyptus* seedling.

Materials and Methods

Preparation of Decomposed Coconut Coirpith (DCCP)

The DCCP was prepared by inoculating the Raw Coconut Coirpith (RCCP) with *Pleurotus platypus* (Theradimani and Marimuthu, 1992). One month old compost was shade dried before use.

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Preparation of Normal Nursery Mix (NNM) and other media mixes

The Normal Nursery Mix (NNM) was prepared by mixing equal volume of red earth, finely powdered farmyard manure and fine sand. The DCCP was mixed with NNM in different combinations to get the following media mixes.

1. DCCP25% + NNM75% V/V – DCCP25
2. DCCP50% + NNM50% V/V – DCCP50
3. DCCP75% + NNM25% V/V – DCCP75

The pure NNM, DCCP and RCCP were also included for comparison.

Population of T. viride *in media mixes*

The media mixes were filled in polypropylene bags of size 15 × 10 cm and sterilized in an autoclave for 1 h at 1.5 kg/cm^2 for three successive days. Media mixes were thoroughly mixed with 12.5 g (5% w/w) of talc based *T. viride* formulation. The initial level of propagules immediately after blending with media mixes and subsequently at 7 days interval up to 30 days were assessed by dilution plate technique using a selective medium (Elad and Chet, 1983). The moisture content of the media mixes was maintained at 65% throughout the study.

Similar study was also made in unsterilized media mixes. The reduction or increase in the population of *T. viride* was calculated and presented as percentage.

Population of T. viride *and* F. equiseti *in dualy inoculated media*

The procedure was similar to the abovedescribed method. Media mixes were inoculated with 12.5 g each of talc based *T. viride* and *F. equiseti* (multipled in sand maize medium) simultaneously. Periodical sampling was done to find out the survival of pathogen and antagonist. Similar experiment was also done in unsterilized media mixes.

Seed treatment with T. viride

Talc based *T. viride* formulation obtained from BIOCONTROL LABORATORY, TNAU, COIMBATORE was used to coat the seeds of *Eucalyptus camaldulensis*. Carboxy methyl cellulose (3% w/v) was added to increase the adherence of propagules. The initial level of propagules was assessed using the dilution plate technique. Treated seeds were sown in unsterilized media mixes and standard nursery practices were followed up to 45 days.

Soil application of T. viride

Trichoderma viride formulation was mixed with unsterilized media mixes @ 5 g/kg of media mix and incubated for five days at 28 ± 2 °C temperature.

The seeds were sown in treated media mixes and the pots were arranged randomly in greenhouse. The nursery practices were continued up to 35 days. *Fusarium equiseti* multiplied in sand maize media was used to make the media mixes sick (5 g/kg of soil).

Enumeration of rhizosphere and non-rhizosphere population of T. viride

The rhizosphere colonization was observed by taking samples at different intervals (15 and 30 Days After Sowing) while non-rhizosphere colonization was assessed on 0, 15 and 30 DAS. Selective media (Elad and Chet, 1983) was used to count the propagules as Colony Forming Units (CFU)/gram of dry media mixes. Damping off due to *F. equiseti* was observed at different intervals and cumulative disease incidence was calculated and presented.

Results and Discussion

Trichoderma viride when introduced into different sterilized media mixes showed steady increase in population except in RCCP and NNM (Fig. 1, Table 1). Availability of large quantity of macro and micro nutrients from DCCP (Nagarajan et al., 1985) and absence of competition might have contributed for the successful colonization of *T. viride* in sterilized media. The nutrient status of the compost has a great role in counteracting fungistasis and to enhance the antagonistic activity of biological control agents (Burbee, 1990) and it was true with *Trichoderma* and *Gliocladium* (Papavizas and Lewis, 1989).

In unsterilized condition the DCCP alone supported the multiplication of *T. viride*. Drastic reduction in population of *T. viride* was observed in RCCP followed by NNM. However, no significant reduction was observed in other combination of DCCP and NNM (Fig. 2). The rhizosphere and non-rhizosphere colonization of *T. viride* was more favoured in DCCP50 and DCCP25 when compared to NNM (Tables 2, 3). Colonization in the rhizosphere was better when *T. viride* was applied to seed and subsequently sown in DCCP amended media than to media mixes as soil application. In the non-rhizosphere region the population of *T. viride* was almost stable throughout the period of study both in soil and seed application (Figs 3, 4). In DCCP amended media mixes the rhizosphere population of *T. viride* when applied either through seed or soil was well above the minimum level of propagules (1×10^5 CFU/g of dry soil) required to contain the soil-borne diseases (Adams, 1990).

Trichoderma viride when applied either to seed or DCCP amended media contained damping off of *E. camaldulensis* (Table 2). The possible reason could be the

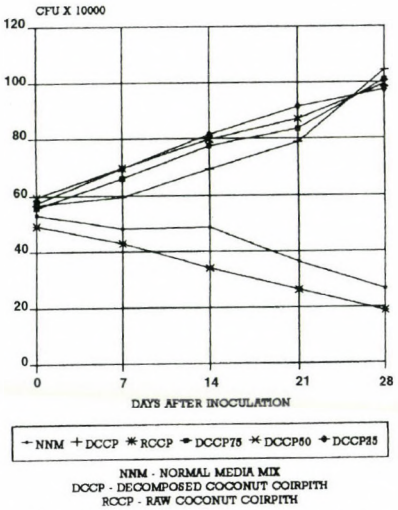

Fig. 1. Population of *Trichoderma viride* in sterilized media mixes

Table 1

Survival of *T. viride** in media mixes

Media mixes	Sampling interval (Days)			
	0		28	
	sterilized	unsterilized	sterilized	unsterilized
NNM	53.25 (7.29)	46.5 (6.82)	26.5 (5.15)	25.75 (5.07)
DCCP	56.75 (7.53)	43.75 (6.61)	104.5 (10.22)	49.5 (7.04)
RCCP	49.25 (7.02)	39.00 (6.24)	18.75 (4.33)	9.50 (3.05)
DCCP75	55.75 (7.74)	44.5 (6.67)	101.0 (10.05)	39.75 (6.3)
DCCP50	59.50 (7.71)	45.75 (6.76)	99.5 (9.97)	41.5 (6.44)
DCCP25	57.5 (7.58)	46.5 (6.82)	97.5 (9.87)	38.75 (6.22)

* CFU × 10000
CD (P=0.05) Sterilized media = 0.44
Unsterilized media = 0.33
Figures in the parentheses are square root transformed values

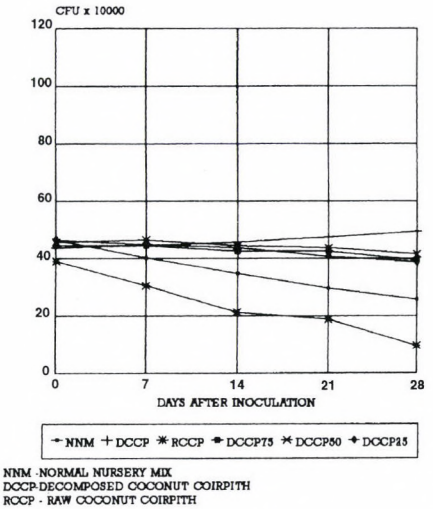

Fig. 2. Population of *Trichoderma viride* in unsterilized media mixes

Table 2

Colonization* of *T. viride* due to soil application and its effects on damping off of *E. camaldulensis*

Media mixes	Rhizosphere (cfu**)	Non-rhizosphere (cfu***)	Damping off (%)	
			T. viride	Untreated
NNM	16.93 (4.07)	28.00 (5.21)	5.57 (13.55)	32.91 (33.00)
DCCP25	27.57 (5.24)	40.76 (6.38)	1.74 (7.53)	14.48 (22.37)
DCCP50	30.29 (5.49)	40.78 (6.36)	0.78 (5.01)	6.80 (15.05)
CD (P=0.05)	0.21	0.16	2.02	

* = CFU × 10000
** = mean of two samplings replicated thrice
*** = mean of three samplings replicated thrice
Figures in the parentheses are transformed values

Table 3

Colonization* of *T. viride* (seed application) and its effect on damping off

Media mixes	Rhizosphere (cfu**)	Non-rhizosphere (cfu***)	Damping off (%)	
			T. viride	Untreated
NNM	27.93 (5.19)	17.52 (4.16)	7.48 (15.84)	54.74 (42.95)
DCCP25	56.57 (7.53)	34.81 (5.88)	1.51 (6.98)	26.91 (31.19)
DCCP50	55.21 (7.43)	35.29 (5.92)	0.45 (3.73)	13.33 (21.37)
CD (P=0.05)	0.19	0.18	4.23	

* = CFU × 10000
** = mean of two samplings replicated thrice
*** = mean of three samplings replicated thrice
Figures in the parentheses are transformed values

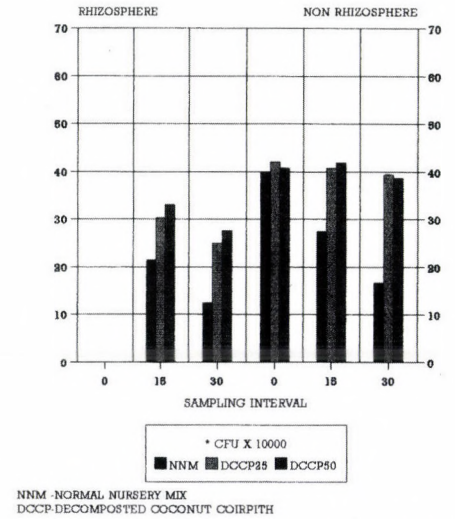

Fig. 3. Colonization of *Trichoderma viride* (soil application)

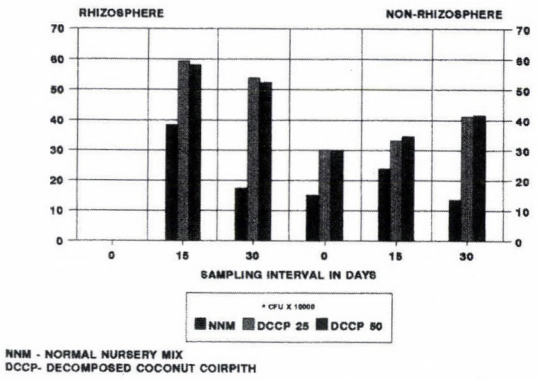

Fig. 4. Colonization of *Trichoderma viride* (seed application)

availability of adequate nutrients from the media, which favoured the colonization of *T. viride*. It may be noted that the DCCP amended media are not only supportive to *Trichoderma* but also suppressive to *F. equiseti*.

Trichoderma species can be excellent biocontrol agents when applied to soil or soilless mix in the greehouse (Marois and Locke, 1985). Incorporation of composted hard wood bark which contain high level of *Trichoderma* or composted house hold waste into potting mixes offered better control of damping off caused by *Pythium* in a range of plant species (Hoitink, 1980; Chen et al., 1988). Similar incorporation of organic and inorganic material termed 'S-H' mixture has also been shown to give control of a range of soilborne plant pathogens (Lin and Lo, 1988; Lin et al., 1990). From the present study it is clear that DCCP enhanced the multiplication of *T. viride* which could be exploited for the management of damping off in *Eucalyptus* seedlings caused by *F. equiseti*.

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The Effect of 1,3;1,6-β-D-Glucans on the Phytoalexin Set and the Activity of Carbohydrases in the Soybean Callus Cultures and Shoots

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1,3-β-D-Glucanase, 1,4-α-D-glucanase, 1,6-β-D-glucanase, lytic β-D-glucanase, β-D-glucosidase, β- and α-D-galactosidase, N-acetyl-β-D-glucosaminidase were found in the soybean callus cultures; 1,4-β-D-glucanase and 1,3;1,4-β-D-glucanase (lichenanase) were absent. The specific activities (units/mg protein) of these enzymes in the callus cultures exhibited more than tenfold of those in the shoots. The addition of 1,3;1,6-β-D-glucans differing in the structure to the culture media does not affect the 1,3-β-D-glucanase and β-D-glucosidase activities in these sources.

In contrast, the quantitative and qualitative contents of phytoalexins (PA) detected by HPLC were shown to be dependent on the presence of 1,3;1,6-β-D-glucans in the media. Isoflavonoid fraction isolated from the hypocotile-derived callus cultures was separated on four subfraction, designed PAI, PAII, PAIII, and PAIV. Isoflavonoid fraction isolated from the cotyledon-derived callus cultures contained only PAI and PAII. The soybean callus cultures and shoots produced diverse sets of PA when 1,3;1,6-β-D-glucan at low concentration was added to the culture medium. Moreover, the additional fifth substance, PAV, appeared when the culture medium contained 1,3;1,6-β-D-glucans. But PAI and PAII were the main components when the culture medium contained 1,3;1,6-β-D-glucan at high concentration. UV-spectra and the behavior on HPLC of PAII and PAIII were similar to the same of formononetin and genistein, respectively.

In the presence of β-D-glucan 4, which suppresses Rhizoctonia infection is soybean shoots, the level of PA was shown to drop 2-fold in comparison with the healthy plants. Infection with *Rhizoctonia solani* of shoots pretreated with β-D-glucan 4 restores the PA level to healthy one.

During the last years appreciable progress was achieved in understanding the biochemistry of the plant-pathogen system. Substances acting as inductors of the defense response were isolated from some phytopathogens (Albersheim et al., 1992). Among them, the carbohydrates, β-D-glucooligo- and polysaccharides in particular, are known to reveal often the immunomodulatory properties. Thus, elicitor isolated from the cell walls of the pathogenic fungus *Phytophthora megasperma sojae* was identified as 1,6;1,3-β-D-glucoheptasaccharide (Sharp et al., 1984). The enzymes of the host plant, carbohydrases among them, seem to be able to split the pathogen cell walls up to oligomers, some of which being the elicitors (Ham et al., 1991; Keen and Yoshikawa, 1983).

The hypersensitivity response as well as the formation of antibiotics phytoalexins, are the most significant defense mechanisms of plants. On response to the infection the leguminous plants start to synthesize isoflavonoids, the compounds which are rare occurred in the other plants (Ingham, 1982; Hahn et al., 1985).

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The aim of this study was to examine the effect of different 1,3;1,6-β-D-glucooligo- and polysaccharides on the composition of isoflavonoid fractions and the levels of activity of carbohydrases in the soybean callus cultures and both in intact and in *Rhizoctonia solani* infected soybean shoots.

Materials and Methods

The soybean shoots were grown according to (Hahn et al., 1985). The callus cultures were obtained from hypocotile and cotyledon of the 3-day-old soybean shoots and were cultivated in the dark at 24 ± 2 °C in the Murashige-Skoog medium (Murashige and Skoog, 1962) containing naphthalene acetic acid (2 mg/L) and benzylaminopurine (0.5 mg/L).

The shoots were infected by *Rhizoctonia solani* according to (Rakitin, 1966) and in 4 days after inoculation the whole plants were freezed and stored at –20 °C.

Laminaran and the products of its enzymatic conversion (β-glucans 1-4, Table 1) were added to the culture medium before autoclaving to test their ability for defense response induction. In each experiment fifteen plants were grown in the same conditions, and from 6 to 13 plants have remained to the end of different experiments.

The callus cultures and shoots were powdered and extracted twice with 0.05 M Na-succinate buffer, pH 5.2 (1 ml/0.1 g of tissue). The extracts were joined, centrifuged, and the supernatants were tested on the carbohydrases activity.

Amylose, CM-cellulose, lichenan, p-nitrophenyl derivatives of β-D-gluco-, N-acetyl-β-D-gluco-, β- and α-D-galactopyranoses, were the commercial preparations. Laminaran (water soluble 1,3;1,6-β-D-glucan) was obtained from the brown seaweed *Laminaria cichorioides* (Elyakova and Zvyagintseva, 1974), pustulan was purified from the lichen *Umbellicaria russica* (Zvyagintseva et al., 1988), and yeast glucan (water insoluble 1,3;1,6-β-D-glucan) vas isolated from the baker' yeast cell wall according to (Duffus et al., 1982).

Glucans 1-4 were isolated from the products of laminaran enzymatic transformation by endo-1,3-β-D-glucanase from shellfish *Chlamys albidus* according to (Zvyagintseva et al., 1995).

Glycosidases were detected using the corresponding p-nitrophenyl glycopyranosides as substrates. The reaction mixture containing 50 µl of the extract under study and 100 µl of the corresponding substrate (2 mg/ml) was incubated in 2 hours at 37 °C. The reaction was stopped by addition of 100 µl of 1M Na_2CO_3, and the absorbance of p-nitrophenol liberated was measured at 405 nm. The enzyme unit corresponds to the enzyme quantity which catalyzes the formation of 1 µmol of p-nitrophenol in minute at the reaction conditions.

Glucanases were detected using corresponding glucans as substrates. The reaction mixture containing 10 µl of the extract under study and 100 µl of the corresponding substrate (5 mg/ml) was incubated at 37 °C in 3 hours. Then the bicinchoniate reagent

Table 1

The structure characteristics of 1,3;1,6-β-D-glucans

No.	Compound	Degree of polymerization	Relation of β-1,3 to β-1,6-linkage
1	Glucan 1	10	85 : 15
2	Glucan 2	15	80 : 20
3	Glucan 3	20	80 : 20
4	Glucan 4	25	75 : 25
5	Native laminaran	30	90 : 10

(100 µl) was added, and the mixture was kept at 100 °C in 10 min. After cooling, the solution extinction was measured at 570 nm. The enzyme unit is equal to those enzyme quantity which catalyzes the formation of 1 µmol of glucose in minute at the reaction conditions.

The extraction of phytoalexins from the soybean callus tissues and shoots was carried out according to (Ingham et al., 1981). The analysis of isoflavonoid fractions was performed on HPL chromatograph "Milichrom" (Russia) using the KAX-1 column (64 × 2 mm) and Silosorb 600 (4 µm) as sorbent. The fraction tested was dissolved in ethyl acetate (100 µl/g of the initial tissue), applied onto the column and eluted with the mixture of chloroform-aceton-ammonia (50:50:1). The phytoalexin elution was monitored at 260 nm. The retention volumes for PAI, PAII, PAIII, PAIV and PAV were 230, 345, 450, 515, and 770 µl, respectively. The individual phytoalexin quantity was evaluated as the square of the corresponding peak on chromatograms. The weight content of the individual peak was calculated using genistein as standard (4 µg of genistein was equal to the square of 1 cm^2 at 260 nm). UV-Spectra of phytoalexins were recorded on Cary-219 (England).

Results and Discussion

The results of the carbohydrase testing in the soybean callus cultures and shoots are summarized in Table 2. It is obvious that callus cultures do not contain cellulase and lichenanase, but activities of 1,3-β-D-, 1,6-β-D-, 1,4-α-D-glucanase and also β-D-glucosidase and N-acetyl-β-D-glucosaminidase are significant in these sources. The carbohydrase activities in shoots were more than 10-fold less of the callus cultures one. The levels of 1,3-β-D-glucanase and β-D-glucosidase activities were stable during the callus growth (Table 3). The induction of these enzymes in shoots and in callus cultures was not noted neither in the presence of different 1,3;1,6-β-D-glucans (Tables 3, 4) nor after the shoots infection with *R. solani* (Table 4).

Table 2

Carbohydrases of the soybean callus tissues and shoots

Carbohydrase	Substrate	The total activity (U/g of tissue)	
		Callus tissue	Shoot
Glucanases			
1,3-β-D-glucanase	laminaran	0.05	0.0012
1,6-β-D-glucanase	pustulan	0.008	
lytic β-D-glucanase	yeast glucan	0.009	
1,4-β-D-glucanase	CM-cellulose	0	
1,3;1,4-β-D-glucanase	lichenan	0	
1,4-α-D-glucanase	amylose	0.09	
Glucosidases	p-nitrophenyl		
N-acetyl-β-D-glucosaminidase	Np-N-acetyl β-D-glucopyranosaminide	0.07	0.0020
β-D-glucosidase	Np-β-D-glucopyranoside	0.05	0.0015
β-D-galactosidase	Np-β-D-galactopyranoside	0.055	0.0010
α-D-galactosidase	Np-α-D-galactopyranoside	0.03	0.0013

Table 3

The effect of 1,3;1,6-β-D-glucans on the carbohydrase levels in the soybean callus tissues

1,3;1,6-β-D-glucan	Concentration of glucan, μg/ml	Time of growth, days	Cotyledon-derived callus tissue	Hypocotyle-derived callus tissue	
			1,3-β-D-glucanase*	1,3-β-D-glucanase*	β-D-glucosidase**
Control	0	7	0.39 + 0.13***	0.43 + 0.10	0.80 + 0.40
Glucan 1	500	7	0.40 + 0.07	0.40 + 0.10	1.01 + 0.26
Control	0	14	0.42 + 0.10	0.42 + 0.11	0.95 + 0.37
Glucan 1	500	14	0.42 + 0.15	0.34 + 0.08	1.27 + 0.30
Glucan 3	500	14	0.54 + 0.12	0.52 + 0.12	1.05 + 0.30
Control	0	30	0.57 + 0.27	0.36 + 0.21	
Glucan 1	100	30	0.41 + 0.19	0.40 + 0.21	
	500	30	0.55 + 0.22	0.24 + 0.18	
Glucan 2	100	30	0.37 + 0.13	0.15 + 0.12	
	500	30	0.39 + 0.16	0.36 + 0.18	
Glucan 3	100	30	0.44 + 0.29	0.34 + 0.14	
	500	30	0.48 + 0.23	0.43 + 0.15	
Glucan 4	100	30	0.35 + 0.24	0.54 + 0.08	
	500	30	0.43 + 0.24	0.47 + 0.13	
Laminaran	100	30	0.25 + 0.13	0.37 + 0.28	
	500	30	0.49 + 0.19	1.04 + 0.27	

* OD_{570}; ** OD_{410};α *** the deviation was estimated by the square method

Table 4

The level of 1,3-β-D-glucanase in the soybean shoots intact and infected with *R. solani*

The growth condition	Enzyme activity*
Control	0.39 ± 0.11*
Culture medium with glucan 4 (100 μg/ml)	0.48 ± 0.08
Infection with *R. solani*	0.42 ± 0.10
Infection with *R. solani*, culture medium with glucan 4 (100 μg/ml)	0.43 ± 0.13

* OD_{750} of the reaction mixture

Table 5

The qualitative and quantitative contents of phytoalexins from the soybean callus tissues grown on the media with 1,3;1,6-β-D-glucans

1,3;1,6-β-D-glucan	Phytoalexin*					
	PAI	PAII	PAIII	PAIV	PAV	Sum PA
Cotyledon-derived callus tissue						
Control	3.5 (8)	3.6 (8)	0	0	0	7.1 (8)
Glucan 1 (100 μg/ml)	7.2 (10)	4.4 (10)	3.5 (4)	10.0 (2)	0	25.1 (10)
Glucan 3 (500 μg/ml)	0.6 (6)	1.4 (7)	2.3 (6)	3.7 (5)	0	8.0 (7)
Hypocotyle-derived callus tissue						
Control	0.5 (6)	1.3 (6)	9.7 (6)	1.1 (6)	0	12.6 (6)
Glucan 1 (100 μg/ml)	0.8 (7)	1.8 (4)	5.9 (7)	7.0 (5)	2.0 (4)	17.5 (7)
Glucan 1 (500 μg/ml)	7.6 (8)	9.2 (10)	2.4 (7)	2.0 (1)	0	21.2 (10)
Glucan 2 (100 μg/ml)	4.0 (5)	6.4 (5)	2.4 (2)	0	1.0 (4)	13.8 (5)
Glucan 3 (500 μg/ml)	1.2 (13)	3.1 (13)	4.0 (13)	30.4 (7)	1.2 (1)	38.9 (13)
Glucan 4 (100 μg/ml)	4.7 (10)	6.4 (13)	10.0 (9)	0	0	21.1 (13)
Glucan 4 (500 μg/ml)	3.6 (6)	6.0 (13)	5.2 (13)	13.2 (7)	0	28.0 (13)
Laminaran (100 μg/ml)	0.8 (9)	1.1 (7)	4.1 (8)	11.9 (9)	6.0 (8)	23.9 (9)
Laminaran (500 μg/ml)	1.9 (9)	3.8 (7)	3.6 (2)	14.4 (2)	1.6 (1)	25.3 (9)

* The concentration of phytoalexin is expressed in μg/0.1 g of callus tissue; the numbers in parantheses correspond to the numbers of plants survived in parallel experiment.

Table 6

The qualitative and quantitative contents of phytoalexins from the soybean shoots

The growth conditions	Phytoalexin, μg/0.1 g of issue			
	PAI	PAII	PAIII	Sum of PA
Control	13.6	3.2	0	16.8
Glucan 4	5.2	0.3	2.8	8.3
R. solani	8.0	3.2	0	11.2
R. solani + glucan 4	4.4	8.4	4.4	17.2

The composition of PA isolated from shoots and callus cultures appeared to be more susceptible to change of environment (Tables 5, 6). Only two peaks, PAI and PAII, were observed at HPLC for isoflavonoid fraction of the cotyledon-derived callus tissue (Fig. 1A). The isoflavonoid fraction obtained from the hypocotyle-derived callus tissue was separated in the same conditions onto 4 peaks: PAI, PAII, PAIII and PAIV (Fig. 1B). PAI, PAII and PAIV were purified up to homogeneity by the repeated separation on HPLC; PAIII was contaminated with PAII. UV-spectra of the individual peaks are represented in Fig. 2. UV-spectrum of PAIII was like to UV-spectrum of genistein (Ingham, 1982), and the behavior of PAIII on HPLC was identical to the same of genistein. The difference observed at 250 nm might be caused by PAII admixture. UV-spectrum of PAII was similar to UV-spectrum of formononetin (Fig. 2) (Ingham, 1982). The behavior of PAII on HPLC was also identical to the same formononetin. These findings suggest that PAII and PAIII are formononetin and genistein, respectively. PAIV seemed to be a formononetin derivative, as its UV-spectrum is like to UV-spectrum of formononetin.

A comparative study of PA sets from cotyledon- and hypocotyle-derived callus tissues was conducted to elucidate how the presence of various 1,3;1,6-β-D-glucans in the culture media affects the content and composition of isoflavonoid fraction. 1,3;1,6-β-D-glucans were added to the culture media in two concentrations – 100 and 500 μg/ml. Each variant accounted from 6 to 13 parallel probes obtained from the plants grown in the same conditions. The results of the qualitative and quantitative HPLC analyses of isoflavonoid fractions are listed in Table 5. It should be noted that to the end of experiment the highest number of plants was saved in the presence of β-glucan 4 so-called "Antivir" (Elyakova et al., 1994).

The most stable composition of PA was in the control when the callus tissues were grown in the standard culture medium without any glucan. For example, six hypocotyle-derived callus tissues saved in these conditions contained all of four phytoalexin (Table 5). When 1,3;1,6-β-D-glucans were added to the culture medium the synthesis of new phytoalexins was noted. So, the synthesis of new PAV was observed in the presence of some β-glucans in the culture medium (Table 5).

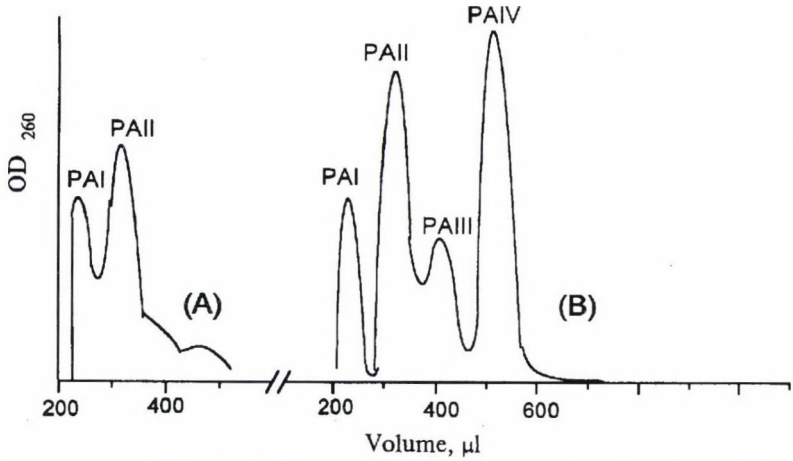

Fig. 1. Chromatography of the PA fractions obtained from cotyledon- (A) and from hypocotyle-derived (B) callus tissues. HPLC was performed on Silosorb-600 (4 µm silica; 2 mm × 64 mm) in cloroform:aceton:water (50:50:1, v/v) at flow rate 50 µl/min. Column effluent was monitored at 260 nm

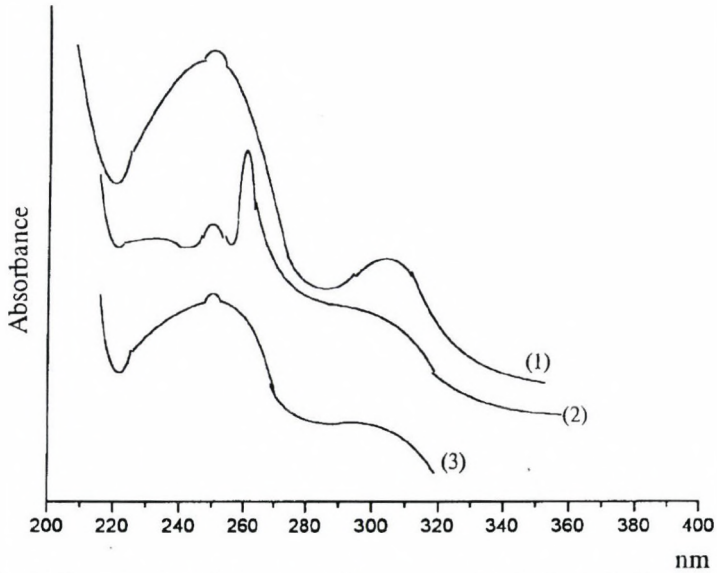

Fig. 2. Ultraviolet absorptions spectra of the compounds PAII (1), PAIII (2) and PAIV (3) in methanol. UV-spectrum of formononetin is analogous to (1). UV-spectrum of genistein in methanol is similar to (2)

The rich phytoalexin pattern characteristic for hypocotyle-derived callus tissue was observed for cotyledon-derived callus tissue grown in the culture media with the β-glucans addition (Table 5). The behavior on HPLC and UV-spectra of these new synthesized phytoalexins were analogous to the same for PAIII and PAIV isolated from hypocotyle-derived callus tissue. It is of interest that the amount of these new synthesized phytoalexins was often considerable, e.g. the PAIII and PAIV formation in cotyledon-derived callus tissue in the presence of β-glucan 1 (Table 5). Addition of β-glucan 1 and laminaran at higher concentration (500 μg/ml) to the culture media made the PA spectrum in hypocotyle-derived callus tissue scanty. So, in the presence of β-glucan 1 the PAV synthesis was suppressed, and PAIV was found only in the one probe of 10 ones. On the whole, the PA content in hypocotyle-derived callus tissue is slightly higher in comparison with the same of cotyledon-derived callus tissue. The addition of β-glucans to the culture medium induce mainly the increase of the total amount of PA (Table 5).

As is obvious from the above, the sum content of PA reflects not always the point of the processes taking place in plants. The isoflavonoid sets induced in almost equal amounts by different β-glucans are significantly differed in the qualitative composition. So, PAIII, probably genistein, was the main component fo the isoflavonoid fraction in the untreated (control) hypocotyle-derived callus tissue (Table 5). But in the presence of β-glucan the level of PAII was increased. As noted above, PAII is like to formononetin which has the higher fungicydic activity than genistein (Ingham, 1982), and the synthesis of which might be more preferable for the struggle against the infection. The change of PA spectrum at the elicitor action was early noted by Dixon with colleagues (Dixon et al., 1989).

The effect of β-glucan on the biochemistry of the soybean shoots was studied at *R. solani* infection of the shoot roots. The damage square of roots was visually diminished when β-glucan 4 was added to the culture medium. It is noteworthy that in presence of β-glucan 4 the infection of callus tissues was also deminished (Table 5). The results of the study of isoflavonoid fractions isolated from the soybean shoot's roots are listed in Table 6. Phytoalexins of these fractions are designed as PAI*, PAII* and PAIII* (Table 6). The *R. solani* infection inhibited the PAI* synthesis. The more pronounced inhibition of PAI* and, especially, PAII* syntheses was observed at the presence of β-glucan 4. But in this case PAIII* was synthesized, which was absent in the untreated and infected shoot roots. When the shoots were infected with *R. solani* at the presence of β-glucan 4, the considerable amounts of all three phytoalexins were produced.

Taken together, the present data indicate that the addition of 1,3;1,6-β-D-glucans to the culture medium favored the synthesis of more diverse spectrum of phytoalexins in both the soybean callus tissues and the soybean shoots. It has probably to give a positive effect when the plants meet the unpredictable infection in the field. The most pronounced effect was observed when the medium contained β-glucan 4 having the highest molecular weight and the most concent of 1 → 6-linked glucose residues.

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Some Foliage Necrosis Causing *Coelomycetes* on Broad Leaved Forest Trees and Shrubs in the Surrounding of Sopron, Hungary

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Thirthy species of leaf necrosis causing *Coelomycetes* are related from broad leaved forest trees and shrubs, based on observations and collections executed in 1990–1995 in the forests near Sopron. The occurrence of 5 species is reported for the first time in Hungary.

The present paper intend to contribute to the enlargement of the knowledge about a special group of parasitic fungi on a special group of host species and their occurrence in the explored region. The knowledge of necrosis causing fungi and their symptoms on leaves, occasionally on shoots and twigs of broad leaved forest trees above the mycological and phytopathological interest plays a part in the correct establishment of the causes of the leaf damages by the investigations of changes in the healthy state of the trees. Leaf necrosis causing fungi treated in this paper develop their anamorph phase in the growing season in the diseased leaf and twig tissue. The conidiomata (pycnidia or acervuli) appear regularly on the host plants some time after the onset of the symptoms. The teleomorph phase (where it is known) develops on the fallen, dead leaves. The ascomata (perithecia or apothecia) appear after wintering, excepted *Glomerella miyabeana* (Fukushi) Arx whose perithecia form soon in the season in the leaf and twig necrosis.

Materials and Methods

The investigations were executed in the forests near Sopron between 1990–1995. The material with symptoms were collected in repeated field investigations executed every two weeks in average in the growing season for know the starting time of the symptoms, the symptom modifications during the season, the period of formation and maturation of anamorph fruit-bodies. The investigations and muster collections were effectuated in different types of forsts by making tours of the forest sections and watching the trees and shrubs on the way. For following the development and maturation of teleomorph fruit-bodies were executed series of muster collections from the leaf litter under the marked infected trees from early March to the end of June.

The collected pathological material, after a desinfection in 2 g/l active chlorine containing NaOCl solution and rinsing in sterile water was hold in humid chamber for several days on room temperature. This stimulated the production of the conidial spores inside the conidiomata formed mostly soon on the trees. Then the fungi were identified. Occasionally for the correct identification of the fungi and examination of their cultural characters cultures were made on 2% malt containing artifical media. The identification of fungi was executed on the base of morphological characters (van der Aa, 1973;

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von Arx, 1970; Bánhegyi et al., 1985; Brandenburger, 1985; Butin, 1960, 1989; Ellis and Ellis, 1985; Grove, 1935, 1937; Monod, 1983; Spiers, 1988; Sutton, 1980; Wulf, 1994). We executed microscopical measurements (minimum 100 conidia) and calculated the mean and standard deviation values. The material collected from leaf litter was likewise examined, the presence and the period of maturation of teleomorphs was established through the repeated microscopic investigations. It was prepared a pathological herbarium founded on the collected material.

Results and Discussion

The species of fungi found during the investigations, their hosts and conidium size are comprised in Table 1. The occurrence in Hungary of *Asteroma tiliae* Rud., *Colletotrichum gloeosporioides* Penz. as anamorph of *Glomerella miyabeana* (Fukushi) Arx on *Salix* species, *Marssonina salicicola* (Bres.) Magn., *Phyllosticta minima* (Berk. et Curt.). Underwood et Earle and *Septogloeum carthusianum* Sacc. is reported for the first time.

The followings include short observations and specifications about the morphology of the fungi, caused symptoms, period of appearance of the symptoms and fruitbodies, occurrence of ascomycetous forms. This work do not intend a completely detailed treatment of the morphology (structure of conidiomata, mode of conidiogenesis, description of teleomorph) either a fully description of the symptoms when the observed aspects correspond with the descriptions in the cited literature.

Asteroma alneum (Pers. ex Fr.) Sutton causes rounded, brown, 5–10 mm diameter necrotic spots on leaves of *Alnus glutinosa* (L.) Gaertn., which appear mostly in early June. The acervuli developing hypophyllous and subcuticular measure 100–130 μm, the one celled, fusiform, hyaline conidia are 9–13 × 2.5–3.5 μm sized. Teleomorph of the fungus (*Gnomoniella tubiformis* (Fr.) Sacc.) were not found.

Asteroma carpini (Lib.) Sutton, the most common leaf fungus of *Carpinus betulus* L. causes 5–20 mm sized, roundish, reticular delimited leaf spots appearing at the end of season (August, September) on the senescent leaves. The numerous, 70–110 μm sized, subcuticular acervuli develop hypophyllous in the spots. The fusoid-cylindrical, one-celled, hyaline conidia measure 7–13 × 1–1.5 μm. The belonging teleomorph is not certainly known (Monod, 1983). On the over-wintered infected leaves were found the Ascomycet *Apioplagiostoma carpinicolum* (Höhn.) Barr. but the connection is not certified.

The symptoms of *Asteroma tiliae* Rud. on *Tilia cordata* Mill. and *T. platyphyllos* Scop. appear only by the end of the vegetation time (end of August – early September). There are especially characteristic the remarkably large sized, on the most part of leaf blade extended roundish, retuculated spots, on which the presence of subcuticular radiating hyphae is typically observable on the over leaf surface. The formation of acervuli in the most of cases cannot be observed, so the identification of the pathogen was possible only by the characteristic symptoms. Teleomorph is not known.

Table 1

Leaf necrosis causing *Coelomycetes* on broad leaved forest trees and shrubs

Fungi	Hosts	Conidial spore size (µm)
Asteroma alneum	*Alnus glutinosa*	9–13 × 2.5–3.5
Asteroma carpini	*Carpinus betulus*	7–13 × 1–1.5
Asteroma tiliae	*Tilia platyphyllos*	
	Tilia cordata	
Asteroma sp.	*Acer pseudoplatanus*	6–10 × 1.5–2
	A. campestre	
Colletotrichum gloeosporioides	*Salix caprea*	15–22 × 4–5
	S. alba	
Coniothyrium rhamni	*Rhamnus catharticus*	4–7 × 3–4.5
Didymosporina aceris	*Acer campestre*	10–13 × 3–4
Diplodina acerina	*Acer pseudoplatanus*	11–18 × 3–4
	A. tataricum	
Discula betulina	*Betula pendula*	6–16 × 2.4–4
Discula campestris	*Acer campestre*	6–10 × 3–4
Discula umbrinella	*Fagus sylvatica*	9–16 × 3.5–5
	Platanus acerifolia	8–16 × 4–5
	Quercus robur	9–16 × 3.5–5
	Quercus petraea	9–16 × 3.5–5
	Quercus pubescens	
	Tilia cordata	8–17 × 3–6
Marssonina castagnei	*Populus alba*	16–20 × 4.5–8
Marssonina brunnea	*Populus xeuramericana*	13–17 × 4–6
Marssonina rosae	*Rosa* spp.	18–24 × 7–8
Marssonina salicicola	*Salix alba v. vitellina f. pendula*	11–20 × 4–6
Microdiplodia melaena	*Ulmus campestris*	7–11 × 3–4
Monostichella robergei	*Carpinus betulus*	11–17 × 5–7
Monostichella salicis	*Salix fragilis*	11–17 × 5–7
	Salix alba	
Phloeospora aceris	*Acer campestre*	26–42 × 3–6
	Acer pseudoplatanus	
Phloeospora robiniae	*Robinia pseudoacacia*	22–60 × 2–3
Phloeosporella padi	*Prunus avium*	38–64 × 3–3.5
Phloeospora sp.	*Castanea sativa*	27–44 × 2–2.5
Phoma hedericola	*Hedera helix*	4–6 × 2.5–3
Phyllosticta minima	*Acer pseudoplatanus*	10–18 × 9–10
Phyllosticta sphaeropsidea	*Aesculus hippocastanum*	10–18 × 9–12
Septogloeum carthusianum	*Euonymus europaeus*	26–34.5 × 10–12
Septoria cornicola	*Cornus sanguinea*	29–42 × 2
Septoria populi	*Populus nigra "Italica"*	32–51 × 2.5–4
	P. trichocarpa	
Septoria pyricola	*Pyrus pyraster*	51–73 × 3–4
Septoria rubi	*Rubus* spp.	40–89 × 2–3

The symptoms of *Asteroma* sp. on *Acer pseudoplatanus* L. appear also in autumn, in form of 5–20 mm sized, grey-brown spots limited by the secondary leaf nervure. By reason of different symptoms and conidial size, this species seems to be not identical with *A. pseudoplatani* Butin et Wulf described by Butin and Wulf (1987). While *A. pseudoplatani*, the agent of "giant leaf blots disease" causes large sized (20–50 mm) leaf spots beginning along the nerves, frequently on the leaf basis, appearing already in June (Wulf, 1994), *Asteroma* sp. causes necrosis of the leaf tissue delimited by the nerves and only at early September. The conidia of *A. pseudoplatani* are oblong and measure 6–7 × 2–3 µm (Butin, 1989; Wulf, 1994), while the conidia of *Asteroma* sp. are fusoid to aciculate and 6–10 × 1.5–2 µm sized. A teleomorph to *Asteroma* sp. were not found.

Colletotrichum gloeosporioides Penz. (teleomorph *Glomerella cingulata* (Stonem.) Spauld. et Schrenk) is a complex fungal species which occurs on several hundred host species (von Arx, 1957). The form occurring on willows initially described in Japan as *Physalospora miyabeana* Fukushi with *Gloesporium* anamorph was integrated at this species by von Arx (1957) but it was proposed to be considered as deviate form with the distinct name *Glomerella miyabeana* (Fukushi) Arx (von Arx, 1957). This fungus was frequent on *Salix caprea* L. in 1991–1992 in the forests near Sopron (Szabó, 1992). The symptoms beginning at the end of May were extended both on the leaves and on the twigs in accordance with the special literature (Butin, 1960, 1989): It causes necrosis of anthracnose type on leaves and twig tops, lenticular shaped, sinking decays on the bark of the twigs. In the necrotized tissue first appeared the acervuli in May–June then the perithecia at the end of June. The anamorph of this species was found on the died top of twigs of *Salix alba* L. too. The one celled, hyaline, cylindrical conidia measure 15–22 × 4–5 µm. In culture the colonies have dark rot reverse and do not produce acervuli, only solitary conidia. The specific hosts, cultural characters and lack of the acervular setae distinguish this form from the ground type of the species.

Coniothyrium rhamni (Westend.) Keissl. causes roundish-oval, 2–8 mm diameter, light brown coloured, then whitish, dark limited spots on the leaves of *Rhamnus catharticus* L. The pycnidia form epiphyllous, the brown, one-celled conidia are 4–7 × 3–4.5 µm sized.

Didymosporina aceris (Lib.) Höhn. is a characteristic foliar pathogen of *Acer campestre* L. Its considerable occurrence was observed in 1995. This fungus causes 2–5 mm sized, dark brown angulat spots, starting from early June. Acervuli appear mostly on the over surface of the leaves, the two-celled, brown conidia measure 10–13 × 3–4 µm. Teleomorph is not known.

Diplodina acerina (Pass.) Sutton causes on the *Acer* species in autumn appearing, irregular shaped, frequently along the nerves or on the margins of leaf limb formed necrosis, or it can be found in strong relation with leaf inhabiting gall insects (*Dasineura vitrina* Kieffer). The two-celled, hyaline, fusiform conidia measure 11–18 × 3–4 µm. Teleomorph, *Cryptodiaporthe hystrix* (Tode) Petrak, was not found. Wulf (1994) proved the association with *Gnomonia ceratis* (Riess) Ces et de Not. This *Ascomycete* was neither found.

Table 2

Comparation of the length of conidia by host specific isolates of *Discula umbrinella* (t-test)

	Nr. measurements	Length mean µm	Standard deviation	Calculated *t*-values			
				Quercus petraea	*Quercus robur*	*Fagus silvatica*	*Tilia cordata*
Quercus petraea	200	11.88	1.54				
Quercus robur	400	11.69	1.35	1.58			
Fagus sylvatica	300	11.63	1.24	2.06	0.63		
Tilia cordata	600	11.45	1.31	3.88*	2.79	1.88	
Platanus xacerifolia	500	10.92	1.38	8.47*	8.53*	7.24*	6.42*

* significantly different on the 0.1% level

Discula betulina (Westend.) Arx is a leaf pathogen on *Betula pendula* Roth. It causes 1–10 mm sized, brown, angular sports, beginning at early June. The infected leaves wither and fall prematurely. Acervuli (100–180 µm diameter) form on the abaxial leaf surface, the one-celled, hyaline conidia measure 6–16 × 2.5–4 µm. Teleomorph is not known.

Discula campestris (Pass.) Arx is a sporadic occurring leaf pathogen on *Acer campestre.* It causes small, roundish, light brown, darker limited spots starting from early summer on. The spots are distributed mostly on the distal part of the leaf limb. The hypophyllous acervuli are 100–200 sized, the cylindrical-ellipsoid, hyaline, one-celled conidia measure 6–10 × 3–4 µm. Teleomorph is not known.

Discula umbrinella (Berk. et Br.) Sutton (teleomorph *Apiognomonia errabunda* [Rob.] Höhn.) according to the conception of von Arx (1970) accepted by Sutton (1980) is a cosmopolitan leaf and twigs pathogen fungus. By us it was found on *Quercus petraea* L., *Q. robur* L., *Q. pubescens* Willd., *Fagus sylvatica* L., *Tilia cordata*, *T. argentea* Desf. and *Platanus xacerifolia* (Ait.) Willd.. On *Q. pubescens* and *T. argentea* the symptoms appeared rarely, mostly only on the border of leaf injuries caused by other factors (abiotic or insects). Monod (1980) treats on the *Platanus* occurring fungus as separated species with *Apiognomonia veneta* (Sacc. et. Speg.) Höh. teleomorph. Other authors take the forms from different hosts traditionally as separated *Gloeosporium,* respectively, *Apiognomonia* species (Butin, 1989). The pathogen causes on its hosts leaf necrosis and shoot dieback of anthracnose type. In oaks these symptoms appear only from the middle of June, while on the other hosts the first symptoms can be observed from early May. The fungus often is associated with leaf inhabiting galls. The most frequently observed such cases: *Neuroterus numismalis* Ol. on *Q. robur*, *Eriophyes* spp. on *Tilia cordata*, *Mikiola fagi* Htg. on *Fagus sylvatica*. *D. umbrinella* as Endophyte can live without symptom in hosts organs. Acervuli develop subcuticular mostly hypophyllous, frequent along the nerves, and in periderma of infected shoots. They measure

135–165 µm. The one-celled, hyaline, oblong conidia are 8–16 × 3.5–5 µm. Although the length ranges of conidia are very similar by the isolates from different hosts (Table 1), a statistical analysis (t-probe) of the data show the conidia originated from *Platanus* significantly smaller than the conidia from the other hosts (Table 2). Morphometric difference among the host-specific strains of this fungus was studied also by Toti et al. (1992), who found difference between the isolates from beech, respectively, oak and European chestnut. Teleomorph was found on all the examined hosts. By *Fagus* and *Tilia* perithecia were found in the leaf litter also when in the precedent season were not observed symptoms. The ascospore release in the case of oak species occurs at the end of April–May, in the other hosts earlier, at the end of March–April.

Marssonnina castagnei (Desm. et Mont.) Magn. It was found on *Populus alba* L., causing 2–5 mm diameter, confluing brown spots dotted with white, punctiform acervuli, observable on the over surface of the leaves. The hayaline, ovoid, unequal two-celled, slightly curved conidia of the specimen examined were 16–20 × 6.5–8 µm sized.

Marssonina brunnea (Ellis et Everh.) Magn. was found on the leaves of *Populus xeuramericana* (Dode) Giunier nm. '*Marilandica*'. It causes 1–2 mm sized round brown spots associated with prematur leaf fall. On the over surface, subcuticular formed acervuli produce ovoid, hyaline, unequal two, celled, 13–17 × 4–5 µm sized conidia. Teleomorph, *Drepanopeziza punctiformis* Gremmen was, found regularly, the ascospora maturation started at the end of April.

To investigate the complete range of poplars *Marssonina* species and their hosts in Hungary it would be necessary to execute detailed examination in the mass occurrence and cultivation areas of poplars, which is not characteristic for the surroundings of Sopron. According to earlier investigations related to the occurrence of *Marssonina* species on poplars in Hungary (Gergácz, 1967) *M. brunnea* occurs on all the cultivated euramerican hybrid poplars and *M. populi-nigrae* (Lib.) Kleb. on black poplars.

Marssonina rosae (Lib.) Desm. was found frequent on leaves of *Rosa* spp. In the ornamental plant cultivation well-known pathogen occurs also on wild *Rosa* species, causing similar symptoms: 2–6 mm sized, confluent, reticular bordered black spots, observable on the over surface of the leaves. Under the stereo-microscope one can observe the subcuticular radiating hyphae. The hyaline, two-celled conidia measure: 18–24 × 7–8 µm. The septa are near of the middle of conidia. Teleomorph (*Diplocarpon rosae* Wolf) was not found.

Marssonina salicicola (Bres.) Magn. caused during 1991–1992 epiedemic leaf and twig disease on Weeping yellow willow, that was the first evidence of this fungus in Hungary (Szabó, 1992). The symptoms were as described by Butin (1960, 1989), brown spots, yellow and wither of the leaves, twig dieback and oval patches on the bark of the twigs. The first symptoms appeared at the end of April on the young leaves and twigs in form of 1–2 mm sized dark brown spots, with punctiform acervuli. The spots increased, the leaves withered and the twig tops died. The subcuticularly formed acervuli were 150–200 µm sized, the hyaline, two-celled conidial spores measured 11–20 × 4–6 µm. Teleomorph (*Drepanopeziza sphaeroides* (Fr.) Nannf.) was rarely found. The spring

infection was realised mostly by the conidial spores produced in the over-wintered acervuli on the twigs.

Microdiplodia melaena Allesch. is a foliar pathogen of *Ulmus minor* Mill. It was observed in 1995. Its symptom: 5–15 mm sized, roundish well-delimited light brown spots appearing from June. The brownish, two-celled conidia measure 7–11 × 3–4 µm. Teleomorph is not known.

In the necrotized tissue of small, withered leaves of decaying branches of *Carpinus betulus* appear large numbers the acervuli of *Monostichella robergei* (Desm.) Höhn, from the end of May. Schneider and Sauthoff (1972) hold this fungus responsible for the decline of *Carpinus betulus*. The ellipsoid conidial spores are one-celled, hyaline and measure 11–17 × 5–7 µm. Teleomorph (*Gnomoniella carpinea* (Fr.) Monod) was not found.

The small, dark, on the surface of the leaves observable round spots caused by *Monostichella salicis* (Westend.) Arx appear from middle summer on the *Salix fragilis* L. and *Salix alba* L. The symptoms appear regularly every year, with different degree. The infected leaves are yellow and fall prematurely. The subcuticular epiphyllous formed acervuli measure 120–170 µm. Conidial spores are 11–17 × 5–7 µm sized. The apothecia of the teleomorph (*Drepanopeziza salicis* (Tul.) Höhn.) appear on the fallen leaves but not too frequently. The ascospore release started at middle April.

Phloeospora aceris (Lib.) Sacc. causes small, point-like necrosis on the leaves of the maples and in the case of *Acer campestre* L. premature wither and falling of the leaves, from the second part of the summer. In *Acer pseudoplatanus* L. blotch (acervuli) without necrosis can be observed on the reverse of the leaves. In the acervuli produced conidia are hyaline, cylindrical, with 2–4 (5) transversal septa, constricted at the septa. They measure 26–42 × 3–6 µm. From August in the leaf spots dark grey, small pycnidia, were observed which produced 2–4 × 0.5–1 µm sized microconidia. This formation known as *Phyllosticta platanoidis* Sacc. belong as spermatial state to the leaf cycle of *Phloespora. aceris. Mycosphaerella latebrosa* (Cook) Schröt., the teleomorph of the fungus was not found.

Phloeospora robiniae (Lib.) Höhn. is a widespread leaf necrotising fungus on *Robinia pseudoacacia* L., which in the dry years causes an important disease and premature falling of the folioli. The leaf spots are 5–20 mm sized, brown, well delimited, irregular shaped. They appear first in early June (Szabó, 1993). The acervuli appear mostly hypophyllous, but they can be found on the upper surface of the leaves too. They are small, 60–90 µm. The hyaline, cylindrical, curved, mostly three-celled conidia measure 22–60 × 2–3 µm. They are not constricted at the septa. Teleomorph is not known.

On *Castanea sativa* Mill. *Phloeospora* sp., is widespread. The traditionally *Cylindrosporium castaneae* (Lev.) Krenner foliage disease causing fungus. It causes small, angular, by the small nerves delimited, joining spots appearing from June. The acervuli develop in the epidermal hypophyllous. They measure 200–250 µm. The conidia are cylindrical, hyaline, mostly 3-septate, curved, 27–44 × 2–2.5 µm sized. At the end of the growing season small, dark pycnidia appear which produce 4–7 × 0.7 sized

bacilliform spermatia. This fungus is associated with the ascomycete *Mycosphaerella punctiformis* (Pers.) Strab., which occurs on dead leaves of a wide ranges of plant species. On over-wintered *Castanea* leaves it can be found regularly.

On the leaves of forestry *Prunus* species especially on *P. avium* L. *Phloeosporella padi* (Lib.) Arx. is rather frequent. It causes 1–2 mm sized, red brown spots, early senescence and leaf falling from middle summer. Acervuli form hypophyllous, conidia are hyaline worm-like elongated, two-celled, 38–64 × 3–4 µm sized. *Blumeriella jaapi* (Rehm.) Arx, the teleomorph of the fungus was not found.

Phoma hedericola (Dur. et. Mont.) Boerema occurs frequent on the leaves of *Hedera helix* L., causing 4–10 mm sized brown spots. The mostly epiphyllously formed pycnidia produce one-celled, oval, hyaline, 4–6 × 2–3 µm sized conidia. Teleomorph is not known.

Phyllosticta minima (Berk. Et Curt.) Underwood et Earle was found on *Acer pseudoplatanus*. It causes from June starting, initial small (3–4 mm), later several cm increasing, light brown leaf spots with reddish border, sometimes with yellow halo. The globular pycnidia measure 180–200 µm. The hyaline, one-celled, ovoidal, by slime layer surrounded, apical slime appendage possessing conidia are 10–18 × 9–10 µm sized. The measured sizes of the pycnidia and conidia were larger than the values given by Van der Aa (1973) for *Ph. minima* and similar to the *Ph. spaeropsidea* Ellis et Everh. The symptoms on the leaves differs from the description in the literature, especially the size of the spots, which in the literature rarely exceed a diameter of 5 mm (van der Aa 1973; Wulf, 1994). So the identification of *Ph. minima* was based principally on the host plant. The *Leptodothiorella* spermatial state of the fungus was also found from the second part of the season. The teleomorph to *Ph. minima* is not specified by Van der Aa (1973).

Phyllosticta sphaeropsidea Ellis et Everh. is everywhere common on the leaves of *Aesculus hippocastanum* L. The observed symptoms and the pathogenesis correspond to the discription of Schneider (1961). The pycnidia were 150–170 µm and the conidia 10–18 × 9–12 µm sized. The *Leptodothiorella* spermatial state was observed and it was found the teleomorph too (*Guignardia aesculi* (Peck.) Steward). The maturation of the ascospore starts at the end of April, and their mass release happens during May.

Septogloeum carthusianum Sacc. causes leaf disease on *Euonymus europaeus* L. This fungus was found in the years 1993–1994. It causes 1–2 cm sized, roundish, light brown, by a thin dark brown line delimited spots, beginning early summer. The acervuli develop under the epidermis, hypophyllously. The conidia are hyaline, broad-cylindrical, curved, they have 0–3 transversal septa and measure 26–34.5 × 10–12 µm. The morphological characters correspond to the description given by Sutton (1980). Teleomorph is not known.

Septoria cornicola Desm. occurs frequently on the leaves of *Cornus sanguinea* L. It causes small, 3–7 mm sized, roudish, red brown delimited spots, with whitish becoming centre. The hyaline, septate conidia (3–6, most frequently 5 septa) measure 29–42 × 2 µm.

Septoria populi Desm. causes small, 2–4 mm sized, by a narrow, brown line well delimited, in the centre whitish becoming, angular spots on the leaves of *Populus* species.

It was found on *P. nigra* L. *'Italica'* and *P. trichocarpa*. The conidia are hyaline, cylindrical, curved, 1- rarely 2-septate, 32–51 × 2.5 × 4 µm sized. Teleomorph (*Mycosphaerella populi* (Auersw.) Schröt.) can be found regularly, the maturation of the ascospore begins early, soon at the second part of March.

Septoria pyricola Desm. occurs on the leaves of *Pyrus pyraster* L. It causes round-oval, whitish becoming, 2–6 mm sized spots. The 1–3 septa possessing, paleolivaceous, curved, cylindrical conidia measure 51–73 × 3–4 µm. The appearance of the teleomorph (*Mycosphaerella sentina* (Fr.) Shröt. was not observed.

Septoria rubi (Westend.) Sacc. causes on the leaves of *Rubus* species 1–5 mm sized, well delimited, in the centre lightening spots. The 40–89 × 2–3 µm measuring conidia are hyaline, long cylindrical, with 1–9 transversal septa. The occurrence of *Mycosphaerella rubi* Roark, the teleomorph of the fungus, was not studied.

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Interaction of Hydrolytic Enzymes Produced by *Rhizoctonia bataticola* during Rot Development

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Rhizoctonia bataticola (Taub) Briton produced enzymes with pectin-methyl-esterase (PME), polygalacturonase (PG), C_x cellulase and C_1 cellulase activities in culture. Generally, mycelial biomass, enzyme production, pH and protein contents of the culture medium increased with incubation period and attained maximum values between the fourth and sixth day. Optimum production of PME and PG occurred under slightly alkaline conditions whereas acid condition favoured the production of C_x and C_1 cellulases. PG was primarily responsible for tissue maceration and cell death of potato tissues whereas cellulase (C_x) seem to play a secondary role in the process. PG and C_x cellulase were constitutively secreted althrough increased synthesis occurred in the presence of their specific substrates.

Many phytopathogenic fungi produce extracellular enzymes which hydrolyse pectic and cellulase substrates (Talboys and Busch, 1970; Wood, 1960). Pectin-methyl-esterase (PME) and polygalacturonase (PG) have been associated with a number of plant diseases. Pectin-methyl-esterase has been implicated as the main factor causing wilting and vascular discolouration in vascular wilt disease (Winstead and Walker, 1954) whereas PG has been reported as major agent of tissue maceration (Bateman, 1963; Wood, 1967; Bateman and Miller, 1966). These enzymes also appear to play an essential role in initial infection process, as loss in virulence in mutants of the soft-rot pathogen *Erwinia carotovora*, is associated with loss of PG synthesis (Friendman, 1962). There is evidence for a correlation between production of PG and PME and virulence (Paquin and Coulombe, 1962).

Cellulolytic enzymes may serve as invasive agents which enable the pathogen to penetrate the tissues of its host or as digestive agents which enable plant tissues to be penetrated by other enzymes and enable cellulose itself to be used as a carbon source (Olutiola and Cole, 1976). A cellulase complex has been isolated in many fungi (Wood, 1968; Umezurike, 1970; Selby and Maitland, 1967). These organisms produce, C_1 cellulase, which hydrolyses the crystalline cellulose to the simpler and soluble anhydroglucose units which can be further degraded by C_x cellulase into cellobiose. A cellobiase (β-glucosidase) reduces the cellobiose to glucose units.

In the present investigation, a study of the production in culture, of cellulolytic and pectic enzymes of *Rhizoctonia bataticola* as well as their interaction in rot development and the effect of carbon sources on the production of the enzymes was undertaken.

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Materials and Methods

Source of potato and causal organism

The experiments were conducted with two species of potato: Irish Cobbler (Yellow/creamy tuber), and Red Pontiac (Red tuber) extensively cultivated in Nigeria. They were obtained from local market stalls when required. The organism used for inoculations was *Rhizoctonia bataticola* (IMI 298277) associated with black rot of potato tubers in storage in Nigeria.

Extraction of enzymes from healthy and infected potato tissues

The method described by Bateman (1963) was used. Extracts were prepared from tubers of *S. tuberosum* inoculated with a fungal disc (5 mm diameter) of *R. bataticola*. Lesion areas and surrounding tissue were removed with a scalpel, weighed (10 g) and extracted by grinding in a Waring blender for 2 min in 20 ml of distilled water. The ground tissue was strained through three layers of cheese cloth to remove the pulp. The liquid fraction was clarified by centrifuging at 3000 g for 20 min at 4 °C. Filtrates extracted from healthy (uninoculated) tubers of *S. tuberosum* served as controls. The activity of enzyme filtrates from both sources was measured as described below.

Media for hydrolytic enzyme production

The growth medium for enzyme production consisted of KNO_3, 10.0 g; K_2HPO_4, 1.0 g; $MgSO_4$. 7 H_2O, 0.5 g; thiamine, 0.1 mg; biotin, 0.005 mg (Olutiola and Ayres, 1973) per litre of distilled water plus 0.5% (w/v) citrus pectin or carboxymethyl cellulose as carbon source for PG and PME production and for 1,4-glucan glucanohydrolase (C_x) production, respectively. Microcrystalline cellulose was employed as carbon source for 1,4-glucan cellobiohydrolase or exo-β-1,4-gluconase (C_1) production. The pathogen was grown in four replicates in 250 ml Erlenmeyer flasks containing 30 ml of liquid medium adjusted initially to pH 5.6 with 0.1 M NaOH. The flasks were inoculated with one disc (5 mm diameter) of 5-day-old PDA culture of the pathogen and incubated at 27 °C for 10 d. The content of each flask was filtered through a sterile glass-fibre filter paper and dried to a constant weight at 80 °C. The filtrate was clarified by centrifugation for 20 min at 4 °C and 3000 g. The clear supernatant (crude enzyme preparation) was dialyzed after measuring the protein content by the method described by Lowry et al. (1951) and stored under toluene at 4 °C until used.

Enzyme assay

Pectin-methyl-esterase was determined by the procedure described by Winstead and Walker (1954). The assay method is based on the hydrolytic removal of methyl groups from the pectin molecule by the action of the enzyme. Ten ml of 0.5 M acetate

buffer (pH 4.5) were added to 75 ml of 1.5% pectin solution in a 250 ml beaker. The pH of the mixture was initially adjusted to pH 7.0 with 0.1 M NaOH. Assay samples of 5, 10 and 15 ml (brought up to 15 ml in each case with distilled water) were stirred into the pectin buffer mixture and incubated for 3 h. Controls contained 5, 10 and 15 ml autoclaved culture filtrate. The reaction mixtures were titrated to pH 7.0 with 0.1 M NaOH. The difference between the number of ml of 0.1 M NaOH required for the sample and that of the control equals the ml of 0.1 M acid released by the enzyme in the culture filtrate. This figure multiplied by 3.1 gave the mg methoxyl group removed by the enzyme in 3 h.

Polygalacturonase activity was determined spectrophotometrically by measuring the reducing sugars released from pectin hydrolysis by the DNS method of Miller (1959). The reaction mixture is made up of 0.4 ml of enzyme plus 1 ml of 2 mg/ml citrus pectin in citrate-phosphate buffer (pH 5.0) incubated at 35 °C for 2 h. Boiled enzyme was used as control. One unit of PG activity was defined as the amount of enzyme per ml of the reaction mixture that under the assay conditions, catalysed the release of reducing sugars equivalent to 10 μg/h of glucose.

β-1,4-glucan glucanohydrolase or endo-β-1,4-glucanase (C_x) activity was assayed by the DNS method of Miller (1959) by measuring the reducing sugars released from carboxymethyl cellulose degradation. The reaction mixture is made up of 1 ml of 6 mg/ml carboxymethyl cellulose in citrate-phosphate buffer (pH 5.0) and 0.4 ml of enzyme incubated at 35 °C for 2 h. One unit of enzyme activity was defined as the amount of the enzyme in 1 ml of the reaction mixture required to release reducing sugars equivalent to 10 μg glucose/h under the assay conditions.

β-1,4-glucan cellobiohydrolase or exo-β-1,4-glucanase (C_1) activity was assayed by measuring the reducing sugars released from the hydrolysis of microcrystalline cellulose powder spectrophotometrically by the DNS method as previously described except that the reaction mixture contained 0.5 ml of enzyme and 5.0 g microcrystalline cellulose powder, suspended in 2.5 ml of citrate-phosphate buffer (pH 5.0). The control mixture contained boiled enzyme. The enzyme-substrate mixture was stirred thoroughly and incubated at 35 °C for 48 h. The unhydrolysed cellulose left after incubation was removed by filtration through glass-fibre paper and the filtrate assayed spectrophotometrically for reducing sugars. One unit of cellulase was defined as the amount of enzyme in 1 ml of the reaction mixture required to liberate, per h, reducing sugars equivalent to 10 μg of glucose under assay conditions.

Synergistic action of the hydrolysing enzymes

The culture filtrates of *R. bataticola* obtained as described above were concentrated by ammonium sulphate fractionation and dialyzed against distilled water at 4 °C for at least 15 h. The dialysate was then passed through a diethyl-aminoethyl cellulose ion exchange column (1.5 × 15 cm column) at 4 °C. The column was caliberated with a 50 mM tris-HCl buffer (pH 8.0).The filtrate applied to the column was washed with 30 ml of equilibrating buffer and then eluted with the same buffer-500 mM NaCl gradient.

Fractions were collected in 15 ml portions and assayed for enzyme activity. Peaks of activity were then purified in an AcA 54 ultragel column at 4 °C using a 50 mM phosphate buffer (pH 7.0) as eluant. Fractions were also collected in 15 ml portions and assayed for peak of activity which were used separately or in a 1:1 mixture.

The macerating activity of the culture filtrates was determined by the use of potato tissue as described by Bateman (1963) and by the determination of reducing sugar released into the reaction medium during the maceration process. Cores of potato tissue were cut from surface-sterilized potato tuber with the aid of sterile size 5 cork-borer. The cores were cut into discs of tissue (1 mm thick) and washed by constant agitation in four changes of distilled water to remove loose cell fragments and starch grains.

Each maceration mixture consisted of 3 washed potato discs, 3 cm^3 of 0.1 M citrate buffer (pH 5.0) and 2 cm^3 of the culture filtrate contained in a test tube and incubated at 27 °C. The maceration index was based on the linear scale of 0–5, on which 0 indicated cellular cohesion (no maceration) and 5 indicated no cohesion (tissue was macerated). In control experiments boiled enzymes were used in place of active enzyme. The macerating enzyme activity was assessed as previously described.

Cellular death was estimated by the Neutral Red technique of Tribe (1955). Each test tube contained 3 potato discs, 3 cm^3 of 0.1 M citrate buffer (pH 5.0) and 2 cm^3 of the culture filtrate and incubated at 27 °C. At hourly intervals, the discs were recovered and stained for 20 min in a plasmolysing solution containing 8.5 cm^3 of 0.1 M KNO_3, 1 ml of 0.1% Neutral Red chloride and 0.5 cm^3 of 0.1 M citrate buffer. After washing in 0.1 M KNO3, the cells were checked microscopically for dye retention and scored on 0–5 cell death index (Neutral Red Index), on which 0 indicated that all cells accumulated the dye (all living) and 5 indicated that no cell accumulation of dye (complete cell death).

Three replicate determinations were carried out in each case and the mean of the three values was taken.

Effect of carbon sources on PG and C_x cellulase enzyme synthesis

Culture medium prepared as previously described was suplemented with 0.5% (w/v) of carboxymethyl cellulose (CMC), pectin, lactose, glucose, sucrose and cellulose as carbon source. The vegetative growth of the growth medium and enzyme production were monitored and assayed as previously described.

Results

Rhizoctonia bataticola induced black (charcoal) rot in the inoculated potato tuber during the 10 d incubation period. The organism grew and secreted both pectolytic and cellulolytic enzymes as well as protein into the culture medium. The results (Table 1) showed that both PG and PME synthesis, mycelial biomass, protein production and pH of the culture medium increased with incubation period and attained maximum levels between 4–6 d. Optimum PG and PME production by the fungus occurred at pH (7.4–8.0).

Table 1

Pectolytic enzyme and protein production by *Rhizoctonia bataticola* in culture

Incubation period (day)	Polygalacturonase (PG) enzyme				Pectin-methylesterase (PME) enzyme			
	Dry wt. (mg/ml)	PG (mg/ml)	pH	Protein (µg/ml)	Dry wt. (mg/ml)	PME (mg/ml)	pH	Protein (µg/ml)
1	26.3±1.2	34.1	6.1	125	28.2±0.8	9.5	6.3	129
2	40.1±0.9	43.8	7.2	130	42.0±1.4	19.6	7.2	130
4	59.0±1.6	49.4	7.4	138	62.1±1.5	22.4	7.7	136
6	86.2±0.8	50.8	8.0	145	86.4±0.9	26.6	7.8	140
8	82.7±2.4	45.2	7.3	140	80.6±1.9	22.4	7.4	134
10	74.3±1.6	42.4	6.8	136	78.8±1.2	20.5	7.0	132

Each value is the means of three replicates

Table 2

Cellulolytic enzyme and protein production by *Rhizoctonia bataticola* in culture

Incubation period (day)	C_x cellulase enzyme				C_1 cellulase enzyme			
	Dry wt. (mg/ml)	C_x	pH	Protein (µg/ml)	Dry wt. (mg/ml)	C_1	pH	Protein (µg/ml)
1	30.4±1.8	28.5	5.4	134	30.3±0.9	0.8	5.1	32.1
2	37.6±1.4	79.8	5.7	136	35.8±1.7	1.2	5.4	35.0
4	54.2±2.3	91.2	5.8	142	67.2±1.5	2.0	5.6	36.4
6	76.3±1.2	96.9	6.0	146	87.1±1.8	3.8	5.8	39.3
8	72.9±0.9	79.8	6.0	133	84.2±1.4	3.6	5.7	32.2
10	69.5±1.6	74.1	5.8	131	81.6±1.2	3.2	5.7	30.7

Each value is the means of three replicates

It was also found that maximum C_1 and C_x cellulase enzyme production as well as optimum mycelial biomass production and protein secretion occurred simultaneously between the fourth and sixth d of incubation (Table 2). Maximum C_1 and C_x cellulase production generally occurred in acid medium (pH 5.8–6.0).

Table 3 shows that *R. bataticola* secreted both PG and C_x cellulase enzymes *in vitro* in the presence of each of the six carbon sources tested. However, the quantity of PG and C_x cellulase produced in the presence of the different carbon sources varied. Pectin was the best source for extracellular PG production whereas cellulose was the least. Carboxymethyl cellulose, sucrose and lactose were also very poor carbon sources for PG production. Maximum C_x cellulase production occurred in carboxymethyl cellu-

Table 3

Effect of carbon source on production of polygalacturonase and C_x cellulase enzymes by *Rhizoctonia bataticola*

Carbon source (0.5% w/v)	Polygalacturonase (PG) activity		C_x cellulase activity	
	Dry wt. (mg/ml)	PG (mg/ml)	Dry wt. (mg/ml)	C_x (mg/ml)
Pectin	80.2±1.8	59.6±1.5	81.8±1.4	43.2±2.5
Lactose	91.6±2.1	21.8±1.1	82.2±2.5	33.8±2.3
Carboxymethyl cellulose	85.6±1.9	27.4±1.7	69.8±2.8	68.6±1.2
Glucose	98.8±0.5	24.5±1.4	93.3±1.6	41.7±1.5
Sucrose	100.2±1.4	25.7±1.8	96.5±1.2	36.4±1.6
Cellulose	78.6±1.6	18.8±0.6	63.8±2.3	38.2±0.8

Each value is the mean of three replicates with standard deviation

Table 4

Maceration and cell death of potato tissue by pectolytic and cellulolytic enzymes produced by *Rhizoctonia bataticola*

Time (h)	Control		PG		PME		C_x		PG + PME		PG + C_x	
	MI	CD	MI	CD	MI	CD	MI	CD	MI	CD	MI	CD
1	0	0	1	0	0	0	0	0	1	1	2	1
2	0	0	2	1	0	0	0	0	2	3	3	3
4	0	0	3	3	0	0	0	3	4	4	4	5
6	0	0	4	5	0	0	0	0	4	5	5	5
8	0	0	5	5	0	1	0	0	5	5	5	5
10	0	0	5	5	0	1	0	1	5	5	5	5
12	0	0	5	5	0	2	0	2	5	5	5	5

PG = Polygalacturonase
PME = Pectin-methylesterase
CD = Cell death
C_x = C_x Cellulase
MI = Maceration Index

A value of 0 indicates no maceration or cell death whereas a value of 5 indicates complete maceration or cell death

lose followed by cellulose, whereas the minimum enzymic activities occurred in the presence of lactose, sucrose, and glucose. The production of PG and C_x cellulase enzymes which is not related to the mycelial biomass, is at least in part constitutive.

Result in Table 4 show that PG alone started to macerate potato tissue within 1 h of the experiment and the tissue was fully macerated within 8 h and cell death was no-

ticed from 6 to 12 h at which time all cells failed to accumulate the Neutral Red stain. With C_x cellulase and PME enzyme alone, potato tissue was not macerated even after 12 h. However, cell death occurred within 10 h of the experiment but maximum kill was not achieved. A mixture of PG and C_x cellulase enzymes fully macerated the potato tissue within 6 h and completely killed the tissue in 4 h. PG and PME mixture had the same maceration index as PG alone but appreciable cell death was achieved within 4 h. The controls did not cause cell death or maceration of potato tissue.

Discussion

Rot-causing fungi are known to produce extracellular hydrolytic enzymes. The involvement of pectolytic enzymes in the degradation of the pectic constituents of cell walls and middle lamellae has been demonstrated in sweet potato (Arinze et al., 1976), yam (Ogundana et al., 1971), and fruits (Adisa and Fajola, 1982). PG and PME are pectin hydrolysing enzymes. PME occurs naturally in higher plants and is also produced by numerous fungi and bacteria (Srivastava et al., 1959). The present study reveals that *R. bataticola* produced both PG and PME in culture. These enzymes could in part be involved in the hydrolysis of the pectic constituents of cell walls and middle lamellae of *S. tuberosum* during pathogenesis by the fungus.

The ability of any pathogenic fungus to degrade cellulose which forms an integral part of the plant cell wall depends largely on the ability of the fungus to produce cellulase. The activity of C_x cellulase secreted by many plant pathogenic fungi has been implicated in the degradation and tissue disorganisation (Whitney et al., 1969; Shiketa and Nisizawa, 1975). C_1 cellulase enzyme is believed to serve as an inversive agent which enables the pathogen to penetrate the host's tissues or as digestive agent which enables the structural cellulose to be hydrolysed (Olutiola and Cole, 1976). The present investigation demonstrated the ability of *R. bataticola* to produce cellulases, C_x and C_1 enzymes suggesting that the cellulolytic enzymes produced by the rot-causing organism are involved in the breakdown of cellulolytic components of cell walls of *S. tuberosum* tissues invaded by it. The C_1 enzyme act on the crystalline parts of the cellulose chain thus loosening the microfibrils in such a way that subsequent action by C_x enzyme in breaking the β-1,4-glucosidic bonds become possible (Reese and Mandels, 1963).

There was a decline in the production of both pectolytic and cellulolytic enzymes after the sixth d. This tends to suggest that the age of the culture influences the production of both enzymes and this may also be attributed to the effects of toxic metabolic wastes such as acids secreted by the aging fungus into the growth medium (Pelczar and Reid, 1972).

The products of the enzymic activity may provide the pathogen with useful carbohydrate sources of energy (Husain and Dimond, 1960). The present study has shown that the growth and production of PG and C_x cellulase were associated with the utilization of the carbon sources in the media. Consequently, for this fungus, PG and C_x production are in part constitutive. The fungus utilized both oligosaccharides and polysaccharides

suggesting that it may produce enzymes capable of breaking down a wide variety of complex carbohydrates, thus allowing breakdown of cell wall and subsequent rots of potato since the tuber contains appreciable amount of these complex carbon sources (Talburt et al., 1975). The carbohydrate content of the host tissue probably regulates enzyme production or enzymic activity is repressed in the presence of non-substrate sources.

Cellulolytic enzymes did not macerate potato tissue in the absence of PG, suggesting a probable synergistic relation between PG and cellulase in the maceration process. Nevertheless, PG appeared essential for the maceration process and cell death since filtrates that contained primarily PG macerated tissue best than did filtrates which contained primarily cellulase (Table 4).

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Seed Health Testing of Peas for *Ascochyta* spp. and their Impact on Foliage Seed Health and Seed Yield

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Seed health testing of 83 local and 9 foreign pea samples belonging to 14 cultivars for *Ascochyta* spp. showed that the average infection percentage was higher in the local than in the foreign samples. In general, average infection percentage by *A. pinodes* exceeded that occurred by *A. pinodella* and *A. pisi* in most samples examined. The tree *Ascochyta* spp. were mostly located in the seed coat than in the cotyledons, whereas embryo infection was the least. *A. pinodella* and *A. pinodes* were isolated in more higher frequences than *A. pisi* from plant debris mixed with pea seeds. *A. pinodes* caused significant blight infection on leaves, stems, pods, significant seed infection and significant reducation in seed yield as compared with check. *A. pinodella* caused significant blight infection on, stems, pods, seeds and significant reducation in seed yield of cv. little marvel, however, in cv. lincolin, it affected only seed health. *A pisi* did not cause pronounced blight, seed infection or reducation in seed yield of c. little marvel, but caused significant blight on stems and pods of cv. lincolin without affecting seed health and seed yield. Seed wight (g/100 seeds) had not been affected by any of the tree species of *Ascochyta* in both cultivars tested.

Pea (*Pisum sativum* L.) is grown in Egypt for edible green and dry seeds. It comes next to cereals as a source of food (Hagedorn, 1989). Hewett (1987) recommended the agar method for seven days incubation as a substrate medium for detection of *Ascochyta pisi* in pea seed samples. In Egypt, Abo-El-Gasim (1976) and Ramadan (1989) reported *A. pisi* in agar test on pea seed samples at 0.5% and 5%, respectively. Fahim et al. (1978) isolated *Ascochyta* pinodella from naturally-infected seeds, leaves and pods of pea. Ali et al. (1982) stated that out of 214 commercial field pea seed samples in agar test, 90 and 31 samples were infected with *A. pinodella* and *A. pinodes,* respectively, Moravcik (1979) noted that *A. pisi*, *A. pinodella* and *A. pinodes* caused necrosis on pea seeds, and seed with more than 25% necrosis on the seed coat did not germinate. Guerrero (1987) reported that *Phoma medicaginis* var. *pinodella* on pea caused death of seeds and seedlings. Wallen (1974) reported that *A. pinodes* and *A. pinodella* caused severe leaf infection, early defoliation and reduced the yield but *A. pisi* caused slight reduction in the yield. Furgal-Wegrzycka (1984) noted that *A. pisi*, *A. pinodella* and *A. pinodes* caused leaf spot in field and green pea. Pokacka (1992) reported *A. pisi* and *Mycosphaerella pinodes* as the most frequent causal agents of pea blight.

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Materials and Methods

Seed health testing

Ninety two local and foreign pea seed samples of 14 cultivars were obtained from the Agricultural Research Centre (ARC), Nobaria seed Production Company (Noba Seed Co.) and seed dealers during 1988–1991. The foreign pea seeds were introduced from England, Hungary, Netherlands and U. S. A. Two hundred seeds of each sample were tested by the standard Agar method according to Hewett (1987). The seeds were first washed, surface-sterilized in 1% sodium hypochlorite solution for 5 minutes, plated on PDA at the rate of 10 seeds/dish. Dishes were incubated at 20 °C under 12 hours alternating cycles of NUV light and darkness for 7 days. The developing *Ascochyta* spp. were examined and the percentage of infection of each sample was recorded.

Location of Ascochyta spp. *in the different seed parts*

One hundred pea seeds each of three samples were used. Seeds of each sample were soaked in tap water for 60 minutes which was the shorest period to facilitate separation of the different seed parts of pea. The different parts of each seed were immersed in 1% sodium hypochlorite solution for 3 minutes, then equidistantly plated on PDA in 9 cm Petri dishes. Dishes were incubated at the conditions mentioned before.

Isolation from debris mixed with seed samples

One hundred pieces of plant debris mixed with each of three pea seed samples were collected. Pieces of each seed sample were washed under running tap water for 10 minutes, surface-sterilized in 1% sodium hypochlorite solution for 3 minutes, then plated on PDA at the rate of 5 pieces/dish. Dishes were incubated at the same conditions as indicated earlier.

Pathogenicity tests

Pathogenicity tests were carried out in the Experimental station of Noba Seed Co. using cv. Lincolin and Little marvel seeds were sown in rows 45 m long and 0.6 m wide, then the developing seedlings were thinned to 50 seedlings/row after 20 days. Five days later, three rows (replicates of one treatment) were sprayed with a spore suspension of a single fungus of the isolated fungi. Seedlings of each row received 250 ml of the spore suspension. Check treatment received only water. Treatments were arranged in Randomized Comple Block Desing (RCBD). The spore suspension was prepared by collecting the spores of a fungal isolate which was grown on PDA and adjusting the suspension to 10^5/ml using a haemocytometer. At plant maturity, infection percentage on leaves, stems, pods, as well as seed yield, seed health and seed weight (g/100 seeds) were achieved. Number and diameter of spots on leaves, stems and pods of 50 randomized plants of each

replicate were recorded. Disease severity was assessed according to this index. Leaves and pods: 0 = healthy, 1 = 0.5–2 mm, 2 = 2.5–4 mm, 3 = 4.5–6 mm, 4 = 6.5–8 mm, 5 = 8.5–10 mm. stems: < 1 mm = healthy, 1 = 1–10 mm, 2 = 1.5–3 cm, 3 = 3.5–5 cm, 4 = 5.5–7 cm, 5 = 7.5–10 cm, 6 = > 10 cm.

Data were statistically analyzed according to analysis of variance (Steel and Torrie, 1980).

Results

Data on seed health testing of 92 local and foreign pea samples of 14 cultivars (5 local and 9 foreign) are presented in Table 1. From Table 1 it is evident that percentage of seed infection by the three *Ascochyta* spp. was higher in the local than the foreign samples. Average seed infection by *A. pinodella* and *A. pinodes* was higher than that occurred by *A. pisi* in both local and foreign pea samples examined. In general, average seed infection percentage by *A. pinodes* exceeded that by *A. pinodella* in both local and foreign samples tested with some exceptions. In the local cv. Licolin, the average infec-

Table 1

Seed health testing of 92 local and foreign pea samples tested on PDA medium, incubated at 20 °C under 12 hours alternating cycles of NUV light and darkness for 7 days (200 seeds/sample)

Cultivar	Local/Foreign	No. of samples	Average infection percentage by *Ascochyta* spp.					
			A. pinodella		*A. piodes*		*A. pisi*	
			Rang	av.	Rang	av.	Rang	av.
	Local							
Lincolin		43	0–23	6.6	0–27	6.2	0–10	1.7
Little marvel		21	0–6	1.5	0–24	8.7	0–2	0.2
Perfection		11	0–4	1.6	0–5	1.6	0–6	1.1
Progress no. 9		1	0	0	0	0	0	0
Victory Freezer		7	0–6	2.1	0–9	4.7	0	0
	Foreign							
Alaska		1	0	0	3	3	0	0
Debricini		1	1	1	0	0	1	1
Forestery		1	0	0	2	2	0	0
Julium lium		1	7	7	0	0	1	1
Moro		1	3	3	0	0	0	0
Robi		1	0	0	6	6	0	0
Rondo		1	0	0	7	7	0	0
Target		1	0	0	3	3	0	0
Telephono		1	0	0	1	1	0	0

Table 2

Location of *Ascochyta* spp. in pea seed parts on PDA at 20 °C under 12 hours alternating cycles of NUV light and darkness for 7 days (100 seeds/sample)

No. of samples	*Ascochyta* spp.	Average infection percentage		
		Coat	Cotyledons	Embryo
51	*A. Pinodella*	8	6	1
	A. Pinodes	30	26	4
	A. Pisi	0	0	0
53	*A. Pinodella*	25	19	0
	A. Pinodes	0	0	0
	A. Pisi	0	0	0
56	*A. Pinodella*	8	6	1
	A. Pinodes	12	10	0
	A. Pisi	4	3	1

tion percentage by each of the two species was more or less equal. However, in the foreign cvs. Debrocin, Julium Liun and Moro, the average percentage infection by *A. pinodella* was higher than that occurred by *A. pinodes*.

Location of Ascochyta spp. *in the different seed parts*

Location of *Ascochyta* spp. in the different pea seed parts of three samples which showed highest infection percentages in health test is shown in Table 2. It is evident that the average infection percentage by *Ascochyta* spp. was higher on seed coat than the cotyledons. Embryo infection was the least. The average infection percentage caused by *A. pinodes* was higher than that caused by *A. pinodella* or *A. pisi* on the different seed parts of samples No. 51 and 56 with the exception. It was lacking in embryo of sample No. 56. In sample No. 53, *A. pinodella* was the only species isolated from seed coat and cotyledons.

Detection of Ascochyta spp. *in plant debris mixed with seeds*

Isolations from plant debris mixed with seeds of three seed samples are presented in Table 3. Data show that *A. pinodes* appeared in more higher frequency than *A. pinodella* and *A. pisi* in seed sample Nos 83 and 85. However, *A. pinodella* was more frequent in plant debris mixed with seed sample No. 84 than the other two samples.

Table 3

Percentage of *Ascochyta* spp. in plant debris mixed with pea seed samples (100 pieces/sample) on PDA at 20 °C under 12 hours alternating cycles of NUV light and darkness for 7 days

No. of samples	Average infection percentage of *Ascochyta* spp.		
	A. Pinodella	*A. Pinodes*	*A. Pisi*
83	28	32	2
84	34	30	0
85	0	27	5

Table 4

Pathogenicity tests by *Ascochyta* spp. on foliage, seed health, seed yield and seed weight of cv. lincolin and cv. Little marvel

Cultivar	*Ascochyta* spp.	Infection % of vegetative parts*			Yield (g)	Seed weight (g/100 seeds)	Seed health testing %
		Leaves	Stems	Pods			
Lincolin	Control	0 (0)^{A}	0 (0)^{A}	0 (0)^{A}	520A	16.3A	0 (0)^{A}
	A. Pinodella	12 (20.2)^{A}	24 (29.3)^{A}	20 (26.6)^{A}	240A	15.3A	17 (24.4)^{B}
	A. Pinodes	37 (37.5)^{B}	32 (34.4)^{C}	31 (33.8)^{C}	180B	14.0A	22 (28)^{C}
	A. Pisi	12 (20.3)^{A}	9 (17.4)^{B}	12 (20.3)^{B}	292A	13.8A	11 (19.3)^{A}
Little marvel	Control	0 (0)^{A}	0 (0)^{A}	0 (0)^{A}	370A	17.8A	0 (0)^{A}
	A. Pinodella	24 (29.3)^{A}	34 (36.9)^{B}	30.3 (33.3)^{B}	158.3B	13.3A	24 (29.3)^{B}
	A. Pinodes	50 (45)^{B}	45 (43.4)^{C}	50.5 (45.3)^{C}	79.3C	12.8A	35 (36.3)^{C}
	A. Pisi	19 (25.8)^{A}	14 (22)^{A}	18.5 (25.5)^{A}	172.3A	13.2A	19 (25.8)^{A}
L. S. D. 0.05 for Lincolin		2.7	2.4	2.5	58.8		3.7
L. S. D. 0.05 for Little marvel		3.7	1.8	3.2	7.5		3.2

* Average of 50 readings of 3 replicates
Values between brackets are the means of transformed data
Means followed by the same letter are not significantly different

Pathogenicity tests

Pathogenicity tests using *Ascochyta* spp. on the foliar plant parts of cv. lincolin and little marvel are presented in Table 4. Data show that *A. pinodes* caused significant blight infection on leaves, stems, pods, seed infection and reducation in seed yield of both cultivars as compared with the check *A. pinodella* had pronounced yet not significant effect on leaves, stems, pods, seed yield of cv. Lincolin, however, in cv. Little marvel, there was significant blight on stems, pods and significant reducation in seed yield *A. pinodella* significant effected seed health of both cultivars tested. *A. pisi* significantly effected the stems and pods of cv. Lincolin, however it had no significant effect on leaves, stems pods of cv. Little marvel. *A. pisi* had no significant effect on seed yield and seed health of both cultivars tested. Seed weight (g/100 seeds) had not been affected by any of the three species of *Ascochyta* in both cultivars tested.

Discussion

Seed health testing of 92 local and foreign pea samples showed the presence of one or more *Ascochyta* spp., namely, *A. pinodella*, *A. pinodes* and *A. pisi*. *A. pinodes* was more frequently encountered, and *A. pisi* was the least isolated. This finding is in line with Jorgensen (1988) who tested 150 pea seed samples and found that *A. pinodes* was the most common species. Martens et al. (1984) noted that *A. pisi* since 1960 had been relatively rare on field pea. Bretag (1995) reported that out of 691 seed lots examined, 436 were infected with *Ascochyta*. 94.8% of isolates were *M. pinodes*, 42% *Phoma medicaginis* var., *A. pinodella* and 1% *A. pisi*.

Ascochyta spp. are externally and internally seed-borne in pea as they were found to be located in the seed coat, cotyledons and embryo at different frequencies. They were more frequent in the seed coat than other seed parts. Such finding substantiates the work of Moude (1966) who found that apparently normal seeds from pods bearing lesions of *A. pinodella* were often internally infected and when sown show disease lesions on seedlings at or below soil level 4–6 weeks after sowing. Hagedorn (1989) stated that when pea seeds were produced in an area of high rainfall, the *Asochyta* spp. were carried internally in the seeds. Plant debris mixed with pea seeds represent another source of infection as *A. pinodella* and *A. pinodes* were isolated in higher percentages, 34% and 32%, respectively, from such plant debris. This result suggests the exclusion of plant debris mixed with seeds before sowing.

Pathogenicity tests revealed that *A. pinodes* was more virulent than the other two *Ascochyta* spp. on pea plants of both cultivars tested. *A. pinodes* caused significant blight infection on leaves, stems, pods, seed infection and reducation in seed yield as compared to the check. *A. pinodella* did not cause significant blight infection or reducation in seed yield of cv. lincolin, however, it caused significant stem, pod blight and reducation in seed yield of cv. Little marvel. This species also significantly affected seed health of both cultivars tested. *A. pisi* did not cause significant blight or seed infection and did not re-

duce seed yield of cv. Little marvel, however, it caused significant stem and pod infection in cv. Lincolin without affecting seed yield and seed health of both cultivars. Such results support the work of Lepikhova (1974), Rudakov and Lepikhov (1974), Martens et al. (1984), Hagedorn (1989) and Wallen and Jeun (1968) who stated that *A. pinodes* became more common than the other two species. Wallen (1974) recorded yield lossos up to 50% in pea plots inoculated with *A. pinodes* and *A. pinodella*, however, slight reduction in yield occurred by *A. pisi*.

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Isolation and Identification of Thermophilic Cellulolytic Actinomycetes

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Twelve thermotolerant and thermophilic actinomycete strains have been isolated from the hot regions of horse manure and identified as *Thermomonospora fusca*, *T. alba* and *Micromonospora* spp. All strains were able to hydrolyse soluble-, microcrystalline- and crystalline-cellulose, as well as xylan, gelatine and Tween 80; all strains were able to grow on straw, as a sole carbon source, but their lignin solubilizing activity varied greatly. Temperature ranges required for growth were between 27 and 69 °C, six strains tolerated 66 °C. The large variety of hydrolytic enzymes secreted by these actinomycetes and the high temperature generated during their biodegradation activity are efficient means to eradicate phytopathogenic microorganisms in composted plant residues.

Plant biomass composed mainly of lignocellulose is the major carbon source in the terrestrial environment. In nature, fungi are the most important primary lignocellulose degraders, however under alkaline conditions, generated by the composting process, actinomycetes become the main agents of biodegradation (Stutzenberger, 1991). The alkaline conditions (pH 8–9) are caused by ammonification; the extent of pH increase depends on the chemical composition of the composted material. During the actinomycetes mediated degradation of aerated organic materials, temperature is elevated from mesophilic to thermophilic stage (Lacey, 1980).

Under the thermophilic stage temperature often exceeds 70–80 °C, and this is sufficient to destroy most pathogenic microbes, insects and weed seeds. Hadar and Gorodecki (1991) demonstrated that the viability of the sclerotia of *Sclerotium rolfsii* was strongly reduced after the thermophilic degradation of the grape marc compost. In an other experiment none of the test organisms (sclerotia of *Sclerotinia sclerotiorum*, egg sacs of *Meloidogyne incognita*, tobacco mosaic virus and tobacco seeds) were able to survive the high temperature in the central regions of the rotting windrows (Herrmann et al., 1994). Composted products obtained from various manures and wastes proved to be useful after a thermophilic degradation for biological control of phytopathogenic fungi, such as *Fusarium oxysporum* f. sp. *lini* and *Phomopsis sclerotioides* (Nelson and Craft, 1992; Reisinger et al., 1992). Soil amendments with composts degraded by thermophilic microorganisms were found to be efficient against soil-born diseases caused by *Pythium ultimum* and *Rhizoctonia solani* (Fuchs, 1995). Craft and Nelson (1996) have recently reported that the suppression of the *Pythium* disease of creeping bentgrass was directly

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related to the microbial activity of the various kinds of compost used for treating the plots. The antagonistic microflora was greatly stimulated in the rhizosphere of tomato after soil amendments with pathogen-free composted products and this effect was paralleled with an improved plant health (Alvarez et al., 1995).

Lignocellulose degradation accomplished by actinomycetes is achieved by the cooperative activity of a number of hydrolytic and oxidative enzymes, like hemicellulases, cellulases and peroxidases; substrates for these enzymes are hemicellulose, cellulose and lignin (Godden et al., 1989; Walker and Wilson, 1991). The primary products of lignocellulose degradation is a soluble, high-molecular weight, acid precipitable lignocarbohydrate complex (APPL). The ability for producing APPL by means of a non-haem peroxidase is a widespread trait amongst actinomycetes (Ball et al., 1990).

The biochemistry of cellulose hydrolysis is the most intensely studied aspect of actinomycete lignocellulose degradation. Three classes of enzymes are found in actinomycete cellulase systems: endoglucanases (1,4-β-D-glucan-4-glucanohydrolase, E.C.3.2.1.4.) which randomly cleave the internal cellulosic bonds, exoglucanases (1,4-β-D-glucan cellobiohydrolase, E.C.3.2.1.91.), that cleave from the non-reducing end of cellulose chains yielding cellobiose, and cellobiases (1,4-β-D-glucosidases, E.C.3.2.1.21.) which hydrolyse cellobiose (Woodward, 1991; Stutzenberger, 1991).

Although endoglucanase activity is common amongst actinomycetes, this is generally insufficient against native cellulose products, like filter paper or MN 300 cellulose powder. Only a few species show complete cellulase activity, i.e. they are capable of degrading native cellulose. These species are *Microbispora bispora* (Hu et al., 1993), *Streptomyces reticuli* (Sclochtermeier et al., 1992), *Thermomonospora curvata* (Lin et al., 1995) and *Thermomonospora fusca*. The molecular background of the cellulase systems of these organisms has recently become subject of interest; genes encoding endoglucanases, exoglucanases and endoxylanases have been cloned and sequenced from *T. fusca*, strain YX (Barr et al., 1996; Irwin et al., 1994; Lao et al., 1991).

The aims of this paper are to: (i) describe a new method for the isolation of thermophilic actinomycetes with complete cellulase activity from compost, (ii) identify and characterize these microorganisms and (iii) study the production of extracellular enzymes involved in lignocellulose degradation.

Materials and Methods

Isolation

Samples collected from the hot region (55–80 °C) of horse manure and litter compost were crushed in a sterile mortar and suspended in sterile distilled water. Cellulolytic isolates were obtained by plating the diluted suspension on MN 300-agar plate (MN 300 cellulose powder, Machery Nagel, Düren, Germany) 10 g, $NaNO_3$ 1 kg, KCl 0.3 g, $MgSO_4 \times H2O$ 0.5 g, K_2HPO_4 1 g, yeast extract (Sigma, St. Luis, USA) 0.5 g, peptone (Sigma) 0.5 g, agar 20 g, distilled water 1 l, pH 7.6) and incubating at 50 °C in

the dark. Colonies developed in the presence of native cellulose as a sole carbon source were suspended and transferred in appropriate dilutions to CMC-agar plates (the same medium as given above except MN 300 cellulose was replaced with carboxymethyl cellulose (Sigma, St. Luis, USA), 10 g l^{-1}). After 2–3 days incubation at 50 °C the crater forming, endoglucanase active colonies were repeatedly plated onto fresh medium until pure cultures were obtained.

Strains

T. curvata ATCC 19995 and *T. fusca* ATCC 27730 were used as reference strains throughout the experiments.

Maintenance, preservation

Isolates were maintained on MN 300 agar and transferred every two weeks to fresh medium. For long-term preservation spores and mycelia were suspended in 10% (v/v) skim milk and meso-inosit (40 g l^{-1}) and freeze-dried by conventional method. L-drying (liquid state lyophilization) was also used; in this case samples were kept in water bath (20 °C) throughout the primary drying phase to avoid freezing the samples (Lapage et al., 1970). Viability of the cells was continuously monitored for 2 years in order to compare the efficiency of the two preservation techniques.

Identification procedures

Morphological features of sporulating colonies were examined according to Cross and Goodfellow (1973) using light microscope. Cultures were grown on CMC agar at 44–55 °C for 7 days. Temperature ranges of growth were determined on MN 300 agar, at 27, 37, 44, 55, 61, 63, 66, and 69 °C. Standard protocols were used to determine oxidase and catalase activities, Gram-staining and acidification of glucose (Collins, 1976). Heat resistance of spores was determined by incubation the spore suspensions prepared on 0.1 M phosphate buffer (pH 7.6) at 90 °C for 30 minutes then plating on MN 300 agar and incubating for 7 days at 50 °C. Carbon source utilization tests were performed on a medium described by McCarty (1989). Various carbon sources (all from Difco, Michigan, USA) were filter-sterilized and added to the autoclaved medium at a final concentration of 1% (w/v). Bacterial growth observed in the presence of these carbon sources was compared to growth on negative (no added carbon source) and positive (D-glucose as carbon source) control plates.

Degradation of polymers

Test substrates (agar-agar, CMC, filter paper, chitin, xylan, amylose, pectin, Tween 80 and gelatine) were separately autoclaved and added to sterile MN 300 media (without cellulose) as homogenized suspensions at a final concentration of 1% (w/v). Clearing zones were measured after 4–14 days incubation. The agar medium for testing pectin hydrolysis was made up in 0.1 M phosphate buffer (pH 7.8).

Enzyme assays

Actinomycetes were cultivated on MN 300 liquid medium. Erlenmeyer flask (1000 ml) containing 200 ml medium were inoculated with 10^6 spores and incubated in an orbital shaker at 200 r.p.m. at 44–50 °C for 7 days. Bacteria and insoluble materials were removed by centrifugation at 4000 × g for 10 min, and the supernatant was used for enzyme assays.

Total cellulase activity was determined by measuring the amount of reducing sugars released from Whatman No. 1 filter paper. The reaction mixture contained 5 ml supernatant, 5 ml of 100 mM phosphate buffer (pH 7.6), and the filter paper strip. This mixture was incubated at 45 °C for 60 minutes. Reducing sugar level was determined by the dinitrosalicylic acid method (Miller, 1959); the absorbance was measured at 570 nm. β-glucosidase and cellobiohydrolase activities were determined by using chromogenic substrates, p-nitrophenil-β-D-glucopyranoside (Sigma) and p-nitrophenil-β-D-cellobiopyranoside (Sigma), respectively. The reaction mixture contained 1 ml supernatant, 1 ml phosphate buffer and 30 mM chromogenic substrate. The incubation was performed at 50 °C for 60 minutes. The reaction was terminated by adding equal volumes of borate buffer (pH 10) and the absorbance was measured at 400 nm. The reaction mixture for cellobiohydrolase assay contained 100 nm gluconoacid-δ-lactone for inhibition the cellobiase side reaction on p-NP-cellobioside substrate. Enzyme activity was expressed in units (U), which is the equivalent of nmoles nitrophenol released/minute in 1 ml reaction mixture. All enzyme assays, except for the endoglucanase test, were carried out in triplicates and the results were expressed as means. (Standard deviation were less than 0.05 in all the cases.) Endoglucanase activity was measured by viscosimetric method (Whitney, 1982). The reaction mixture contained 1 ml supernatant and 4 ml 0.5% (w/v) CMC solution. The reaction was stopped by adding 100 µl 1% (w/v) $HgCl_2$ solution after 2, 5, 10, 15 and 20 minutes. Decrease in relative viscosity was determined by an Oswald type viscosimeter. The results were graphically analyzed. One unit of enzyme activity was equivalent of a 50% decrease in relative viscosity after 10 min incubation at 37 °C.

Lignocellulose solubilization

Growth conditions were the same as for enzyme production, except MN 300 cellulose was replaced by straw powder as the sole carbon source. Acid precipitable lignin-carbohydrate complex (APPL) production was measured by recording the increase of absorbance at 600 nm for 5 minutes following acidification of 1 ml culture supernatant by adding 0.1 ml 1 M HCl (Ball et al., 1990).

Results and Discussion

The special isolation procedure used by us created selective conditions for those microbes that possess a complete cellulase system and able to grow at 50 °C. Under these conditions 12 thermotolerant bacterial colonies capable of utilizing cellulose powder as a

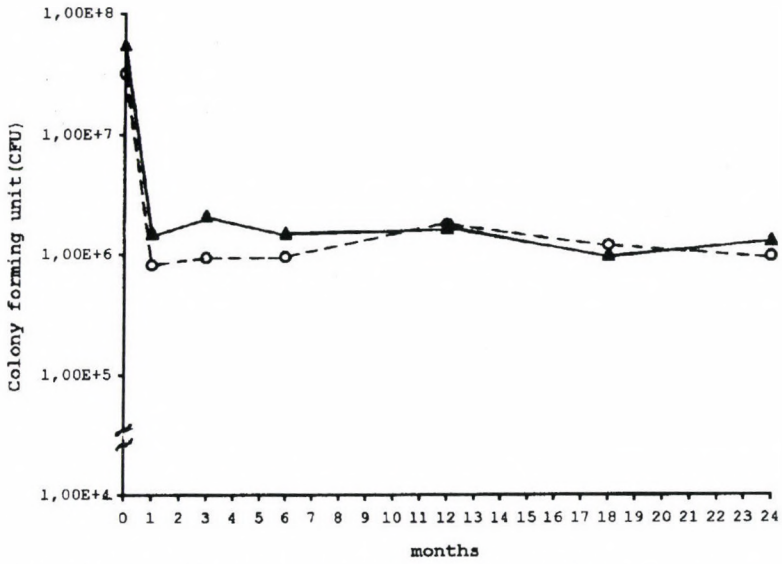

Fig. 1. Viability of *Thermomonospora fusca* strain ATCC 27730 preserved by freeze-drying (--o--) and L-drying (–▲–)

sole carbon source could be isolated. Pure cultures of these bacteria were named as TM514, TM513, TM51, TB110, TB108, TB107, TB105, TB102, TB100, K51, K52 and K21. In other experiments purporting the isolation of thermotolerant actinomycetes, the contaminating microorganisms, mainly *Bacillus* species were suppressed by means of selective antibiotics (McCarty, 1989). An alternative method is the use of a sedimental chamber, which allows the enrichment of the target actinomycetes spores (Lacey and Dutkiewicz 1976). The point of our isolation technique is a combination of selective culturing and enrichment. MN 300 cellulose powder, provided as a sole carbon source supports the growth of the true cellulolytic microorganisms and the transfer of these microbes to CMC-medium favors the rapid propagation of the endoglucanase producers. The latters can easily be identified without further staining procedures, as they intensively hydrolyse this substrate and form sharp craters on the agar surface within 2–3 days. Undesired microbial contamination is prevented by the short incubation period.

Preservation experiments were performed in order to avoid frequent subculturing of the strains. Due to their special environmental requirements and their slow growth, thermotolerant actinomycetes are easily contaminated by other microbes. Interestingly, the holotype strain of *T. curvata* proved to be a mixed culture in a subsequent examination (McCarty, 1989). The effects of freeze-drying and L-drying lyophilization are given in Fig. 1. The number of colony forming units (CFU) ranged between 3.2 and 5.5×10^7 per ampoule immediately after lyophilization, irrespective of the method used. After the first month of storage this number decreased by an order of magnitude but no further

Table 1

Characteristics differentiating the cellulolytic isolates

Strains	T. c	T. f.	TM 514	TM 513	TM 51	TB 110	TB 108	TB 107	TB 105	TB 102	TB 100	K 52	K 51	K 21
Gram-staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Morphology														
aerial mycelium	+	+	+	+	+	+	+	+	+	–	+	+	+	–
substrate mycelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+
single spores	+	+	+	+	+	+	+	+	+	–	+	+	+	–
heat sensitive sp.	+	+	+	+	+	+	+	+	+	a	+	+	+	a
pigment color	cl	cl	cl	cl	cl	b	b	cl	y	cl	y	cl	cl	o
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from D-glucose	–	+	–	+	+	–	–	–	–	–	–	–	–	–
Growth at														
27 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	+
37 °C	+	+	+	+	–	+	+	+	+	–	+	+	+	+
44 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50 °C	+	+	+	+	+	+	+	+	+	+	+	–	–	+
55 °C	+	+	+	+	+	+	+	+	+	+	+	–	–	–
61 °C	–	+	+	+	+	+	+	+	–	–	–	–	–	–
63 °C	–	+	+	+	+	+	–	+	–	–	–	–	–	–
66 °C	–	+	+	+	+	+	–	+	–	–	–	–	–	–
69 °C	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Degradation of														
agar-agar	–	+	–	–	–	+	–	–	–	–	–	–	–	–
CMC	+	+	+	+	+	+	+	+	+	+	+	+	+	+
filter paper	+	+	+	+	+	+	+	+	+	+	+	+	+	+
chitin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
xylan	+	+	+	+	+	+	+	+	+	+	+	+	+	+
amilose	+	+	+	+	+	+	+	+	+	+	–	+	+	–
pectin	–	+	+	+	+	–	–	+	–	–	–	+	+	–
Tween 80	+	+	+	+	+	+	+	+	+	+	+	+	+	+
gelatine	+	+	+	+	+	+	+	+	+	+	+	+	+	+

T. c., *Thermomonospora curvata* ATCC 19995; T. f., *Thermomonospora fusca* ATCC 27730; cl, colorless; y, pale yellow; b, brown; o, orange; a, absent; +, positive; –, negative

decrease was observed during the next 23 month period; at the end of the experiment no significant difference was found between the efficiency of the two techniques, the number of CFU/ampoules became stable around $0.95–1.3 \times 10^6$. The rapid decrease observed in the first months of storage was probably due to the death of the more sensitive mycelial cells present in the starting material.

Morphological characteristics, basic biochemical traits, temperature ranges required for growth, and polymer degrading abilities of the actinomycetes isolated by us as well as of the two reference strains are summarized in Table 1. Data on carbon source

Table 2

Carbon source utilization test of the isolated actinomycetes

Strains	T. c	T. f.	TM 514	TM 513	TM 51	TB 110	TB 108	TB 107	TB 105	TB 102	TB 100	K 52	K 51	K 21
Growth on														
adonitol	+	–	+	–	+	–	–	–	+	+	+	+	–	+
L-arabinose	–	–	–	–	–	–	–	–	–	+	+	–	–	–
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	–	+	+	+	+	+	–	+	+	+	+	–	–	–
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	–	+	+	+	+	+	–	+	–	+	–	–	–	+
L-rhamnose	–	–	–	+	–	–	–	–	–	–	+	–	+	–
D-ribose	+	–	–	–	–	+	+	+	+	+	+	+	+	–
D-xylose	–	–	–	–	–	+	+	–	+	+	+	+	+	+
L-sorbose	–	–	–	–	–	–	–	–	+	+	–	–	–	–
cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
lactose	–	+	+	+	+	+	–	+	–	+	+	–	+	+
maltose	+	+	+	+	+	+	–	+	+	+	–	+	–	–
melibiose	–	–	+	+	+	–	–	–	–	+	–	–	+	+
saccharose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
trehalose	+	+	+	+	+	+	+	+	+	–	+	+	+	–
cellotriose	nt	+	nt	nt	+	nt	nt	nt	nt	nt	nt	nt	nt	nt
melesitose	+	+	+	+	+	+	+	+	+	+	+	–	+	+
salicin	–	+	+	+	+	+	+	+	+	+	+	+	+	–
dulcitol	–	–	–	–	–	–	–	–	–	+	–	+	+	–
erithritol	–	–	–	–	–	–	–	–	+	+	–	–	–	+
glycerol	–	+	–	+	+	–	+	–	–	–	–	–	+	–
inositol	+	–	–	–	–	–	–	–	+	–	–	–	–	–
D-sorbitol	–	–	–	–	–	+	–	+	–	+		+	–	+
gluconic acid	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Na-citrate	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Na-malonate	–	–	+	+	–	+	+	+	+	–	+	–	–	–+

T. c., *Thermonospora curvata* ATCC 19995; T. f., *Thermomonospora fusca* ATCC 27730; nt, not tested; +, positive; –, negative

utilization are given in Table 2. All strains proved to be Gram-positive, they were aerobic, formed branched, nonfragmented vegetative mycelium, and all but two strains (TB102 and K21) produced single, heat sensitive nonmotile aleuriospores on aerial hyphae. Temperature ranges required for growth were between 27 and 69 °C; the lowest limit (T_{min}) was observed for strain K21, while the highest temperature (T_{max} = 69 °C) was tolerated by strain TM51. Altogether six strains (*T. fusca* ATCC 27730, K51, K52, TB107, TM51, TM513 and TM514) were able to grow at 66 °C, a temperature known to be tolerated by real thermophilic microorganisms.

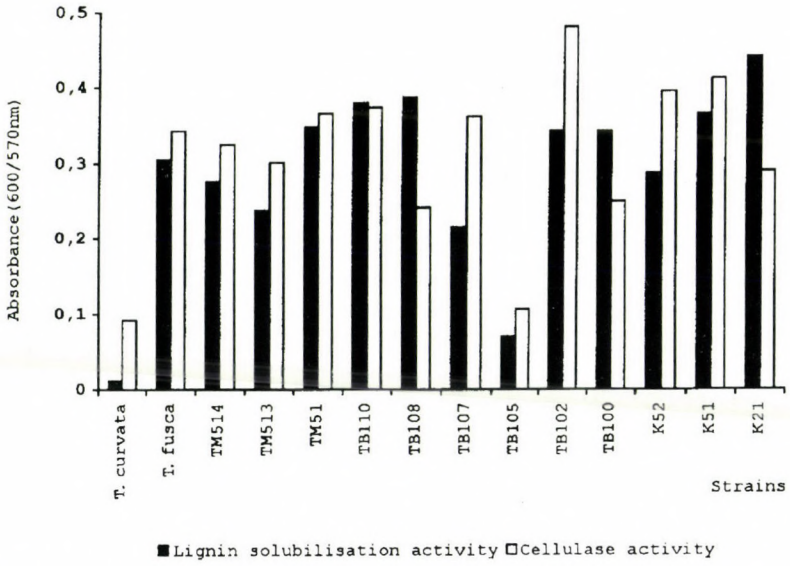

Fig. 2. Lignin solubilization and total cellulase activity of *Thermomonospora* and *Micromonospora* strains

Based on their temperature requirements the 12 actinomycete strains isolated by us, could be classified as thermotolerants and thermophiles according to the terms of Cross (1968). Representatives of the thermotolerant group (TB108, TB105, TB102, TB100, K52, K51, K21, as well as the reference strain, *T. curvata* ATCC 19995) tolerated a narrow range of temperature, only one of them (K21) was able to grow under 30 °C and none of them showed any activity above 55 °C. The group of thermophiles (TM514, TM513, TM51, TB110, TB107, as well as the reference strain, *T. fusca* ATCC 27730) could grow in a broader range of temperature (37–66 °C), the highest temperature (69 °C) was tolerated by strain TM 51. The heat tolerance of these thermophiles corresponds to the average temperature generated during the thermophilic stage of the aerobic composting technologies (Finstein, 1986).

All strains were able to hydrolyse soluble-, microcrystalline- and crystalline-cellulose, as well as xylan, gelatine and Tween 80. Two strains (*T. fusca* ATCC 27730 and TB110) could decompose agar-agar, but none of them were able to degrade chitin. Six strains (*T. fusca* ATCC 27730, TM514, TM513, TM51, TB107, K52, K51) showed pectinolytic activity. Furthermore, all fourteen strains were able to grow on straw, however their lignin solubilization activity, presented in Fig. 2 varied greatly; a 40-fold difference was observed between the most active strain, K21 (0.444 APPL/$OD_{.600}$) and the least active one, *T. curvata* ATCC 19995 (0.012 APPL).

The levels of total cellulase activity showed a fivefold variation among the twelve strains, TB102 proved to be the most active isolate in this respect. Activity levels of the

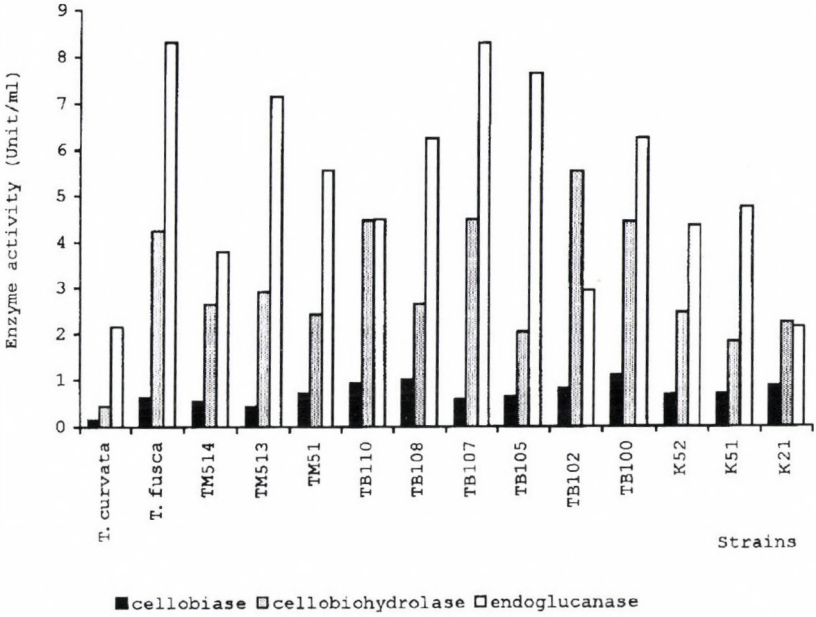

Fig. 3. Cellulase enzyme activities of *Thermomonospora* and *Micromonospora* strains

three cellulase components are presented in Fig. 3. All strains exhibited low cellobiase activity relative to the cellobiohydrolase activity. Cellobiase levels ranged between 0.015 U/ml (*T. curvata* ATCC 19995) and 1.11 U/ml (TB100). The most efficient cellobiohydrolase producers were strains *T. fusca* ATCC 27730, TB110, TB107, TB102, TB100, their activities exceeded 4 U/ml. The maximum level of cellobiohydrolase activity was 5.53 U/ml, expressed by strain TB102. All strains showed outstanding endoglucanase production ranging from 8.3 to 2.15 U/ml, the most active strains were TB107 and *T. fusca* ATCC 27730.

Based on their morphology, temperature requirements, decomposing ability and carbon source utilization 10 strains out of the 12 isolated by us could be placed in the genus *Thermomonospora*, among them TB107, TM51, TM513, and TM514 belong to the species *T. fusca*, K51 and K52 are *Thermomonospora alba*, while two further strains (K21 and TB102) are members of the genus *Micromonospora*.

The varied and extensive lytic activity of the strains characterized in this study corresponds to former data on the enzyme production of thermophilic actinomycetes found in composted organic materials (Stutzenberger, 1977; Kroppenstedt and Goodfellow, 1992; Walker and Wilson, 1991; McCarty and Williams, 1992). This large variety of hydrolytic enzymes and the high temperature generated during the biodegradation process would efficiently eradicate the phytopathogenic microbes in composted plant residues.

In the biosphere, lignocellulose is decomposed by fungi and actinomycetes (McCarty and Williams, 1992); *Thermomonospora* species are among the most potent lignin-degrading microbes (Trigo and Ball, 1994). Some strains of our collection, namely TB108, TB110, TM51 and K21 exerted outstanding lignin-solubilization values, exceeding those known from the literature. A general correlation was observed between lignin degrading ability and cellulase activity of the actinomycetes characterized in this study. Strains able for an efficient lignin solubilization expressed generally high levels of cellulase activity and conversely, the low cellulase activity was parallelled with a weak lignin degrading ability, like in strains *T. curvata* ATCC 19995 and TB105. The two systems coevolved in the most active strains ensuring an economic degradation of the lignocellulose complex.

β-glucosidase activity of the actinomycetes tested by us were relatively low. This was the weakest point of the complete cellulase chain, that could be explained the thermolabile nature of β-glucosidases (Stutzenberger, 1991).

Lignolytic *Thermomonospora* strains are potent agents for the biological bleaching of pulps and the ability of some strains to attack simultaneously all the major components of lignocellulose – like lignin, cellulose, hemicellulose – may be utilized to develop a direct conversion of lignocellulosic wastes into valuable organic fertilizers that became at the same time free from plant pathogenic microbes due to the concerted action of the lytic enzymes and the high temperature generated during the composting process.

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Superoxide Dismutases and Amino Acid Compositions of Dismutases from Leaves of Different Wheat Cultivars

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Second leaves of broad spectra of wheat cultivars contain three electrophoretically-distinct superoxide dismutases (SODs). One of these resembled the Mn–SOD, becaure it was not inhibited by cyanide ion, but was inactivated with chloroform: ethanol tratment (Tsuchihashi-reagent). The remaining SODs of wheat leaves resembled the Cu, Zn–SODs, which has already been identified from a wide range of eukaryotes. These two Cu, Zn–SODs of wheat leaves were isolated, purified and characterized by amino acid analysis. The amino acid composition of Cu, Zn–SOD was similar from all wheat cultivars, with high Lys content. Results obtained confirm the suggestion that Cu, Zn–SODs production in wheat cultivars is under the control of similar genes. The young leaves of wheat seems to be convenient source of SOD for comparison.

Superoxide dismutases (EC 1.15.1.1.) are a group of enzymes which catalyzes the dismutation of superoxide radicals (O^{-}_{2}) (Štajner et al., 1993, 1995) and occurs in three forms as Cu, Zn, Mn- and Fe-enzymes depending on the prosthetic metals (Bowler et al., 1992). In higher plants Cu, Zn–SOD is a major isoenzyme (Asada et al., 1980) whereas Mn–SOD is present at lower activities (Rabinowitch and Fridovich, 1983). Fe–SOD isoenzyme was found in the plant families *Gingkoaceae*, *Cruciferae*, recently also in leaves of lemon trees (Sevilla et al., 1987) in different cultivars of citrus species (Almansa et al., 1989) and in glyoxysomes of waterlemon. Localization of Cu, Zn–SOD had been demonstrated in spinach, pea chloroplasts (Asada et al., 1973, and Duke and Salin, 1983), as well as in wheat germ, but in maize and tomato Cu, Zn–SOD is localised in chloroplasts and also in cytosol (Baum et al., 1983; Kwiatowski and Kaniuga, 1986). Thus it appears that two classes of Cu, Zn–SOD isoenzyme chloroplastic and cytosolic occur in plants, but relationship between each isoenzyme from various plants have not been demonstrated.

SODs from different plant sources differ from each other in their amino acid composition, therefore suppose that the isoenzymes are products of different genes (Kanematsu and Asada, 1989).

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Materials and Methods

Plant materials

The Yugoslav wheat cultivars (*Triticum aestivum* L.) NS Rana 2 and NS-732, the Hungarian cultivar Bánkuti 1205, the American cultivar Atlas 66 and Siete Cerros from Mexico were chosen for the experiment. Surface sterilized seeds were placed in Petri dishes with qualitative filter paper soaked in feeding Hoagland solution (per l:l.18 g $Ca(NO_3)_2.4H_2O$; 0.306 g KNO_3; 0.136 g KH_2PO_4 0,25 g $MgSO_4.7H_2O$) and grown in a greenhouse for 21 days. Second leaf was used for the further study.

Preparation of the superoxide dismutase extract

All the operations were performed at 0–4 °C. Green leaves where previously washed with deionized water, homogenised with 0.1 mol/l Na_2HPO_4 pH 7.3, followed by amonium sulfate saturations, and DEAE cellulose fractionation ad described by Sawada et al. (1971). The result is shown in Fig. 1. Fractions having higher absorbance at 280 nm were pooled and used for further examinations (see Fig. 1).

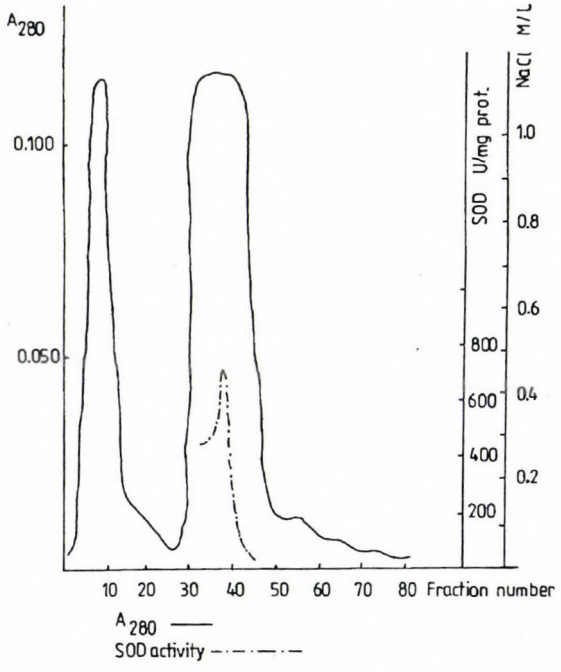

Fig. 1. Column chromatography of SOD on DEAE cellulose with a linear gradient of NaCl

Enzyme assay

The SOD activity was determined by the method of Misra and Fridovich (1972) based on the inhibition of autoxidation of adrenaline–adrenochrome at pH 10.2.

Polyacrylamide gel electrophoresis (PAGE)

Leaves were homogenized with see-sand in a mortar and suspended in 4 ml 0.05 mol/l potassium phosphate buffer pH 7.8. Homogenates were centrifuged at 15000 g for 30 min in cold room. SOD isoenzymes were separated by discontinuous PAGE of crude extracts using an anodic TRIS/GLY system pH 8.4 according to Ranieri et al. (1992). For activity staining the gels were incubated in 20 mM pyrophosphate buffer pH 8.3 containing 0.5 mM nitro blue tetrazolium chlorie and 6.25 μM phenazine methosulfate for 30 min at 27 °C followed by soaking in 0.15 mM NADH in the same buffer for 20 min, too. The colourless zones correspond to SOD activity SOD activity in gels was quantited by linear scaning densitometry. To distinguish Cu, Zn, Fe and Mn–SOD 2 mM KCN or 2 mM H_2O_2 were added to staining solutions Bridges and Salin (1981), (Štajner et al., 1994).

Amino acid composition

Fractions with high SOD activities were hydrolyzed in duplicate in 6 mol/l HCl in evacuated sealed tubes for 24, 48 and 72 h at 110 °C and determined the amino acid composition by the method of Moore (1963).

Results and Discussion

By partial purification of superoxide dismutase from different wheat cultivars did not results show notable differences. As a representative cultivar NS Rana 2 was chosen and results of its purification procedure are summarized in Table 1.

Electrophoretic studies

Electrophoretic studies of purified fractions having high SOD activites (Fig. 1) were conducted for all examined cultivars and results are presented in Figs 2 and 3.

In general leaves of NS Rana 2, Bánkuti 1205, NS 732 and Atlas 66 contained four electrophoretically distinct SOD which were identified by using 2 mM KCN and 2 mM od H_2O_2 as two manganese (bands 3 and 4) SOD and two copper-zinc-containing (bands 1 and 2) SOD (Fig. 2), what is in agreement with results obtained by investigations of other plants species such as watermelon Sandalio and Del Rio (1986), lemon (Sevilla et al., 1986). Rice leaves contained also two Mn–SOD but four different Cu, Zn–SODs Kanematsu and Asada (1989). Cultivar Siete Corros contained three electropho-

Table 1

Purification of SOD from the wheat cultivar NS Rana 2

	Purification stage	Protein, mg/l	SOD activity, U/mg protein	Increase of SOD activity, %	Volume, ml
1.	0.1 mol/l disodium phosphate extraction	18.32	16.67	12.42	157
2.	Amonium sulfate fractionation (35% saturation)	16.70	27.61	65.63	150
3.	Amonium sulfate fractionation (55% saturation)	16.50	56.90	241.33	120
4.	Dialyze the fractions	11.75	119.82	618.78	22
5.	Acetone treatment the fraction	4.72	397.82	2286.44	15
6.	DEAE cellulose chromatography	2.64	814.79	4787.46	3

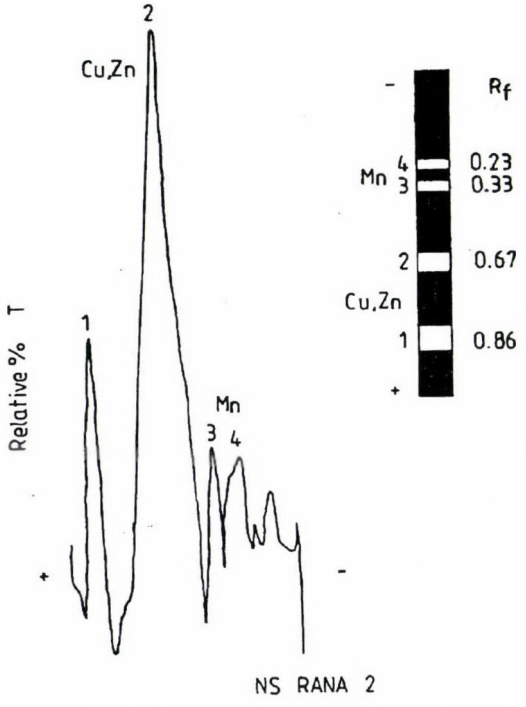

Fig. 2. Densitometric scan of SOD activity-stained gel electrophoretogram and R_f values of cultivar NS Rana 2

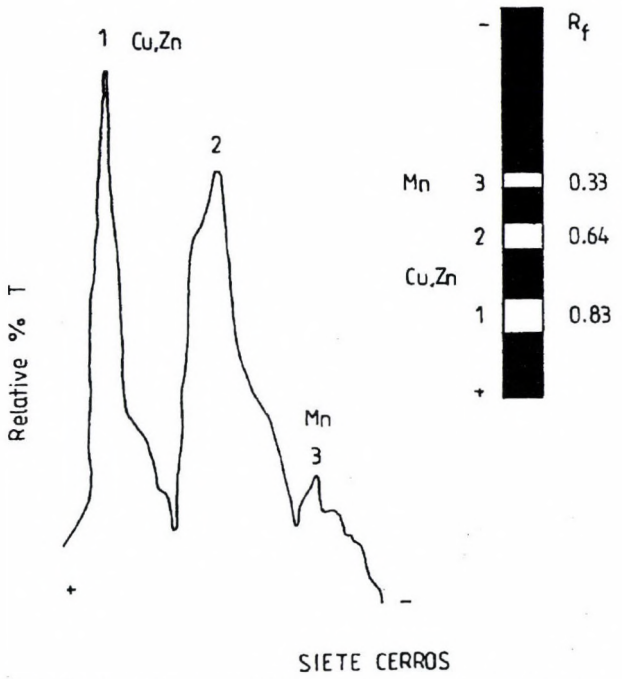

Fig. 3. Densitometric scan of SOD activity stained gel electrophoretogram and R_f values of cultivar Siete Cerros

retically distinct SODs which were identified by using KCN and H_2O_2 as one manganese (band 3) and two copper-zinc (bands 1 and 2) SOD. Spring cultivar Siete Cerros had a similar isoenzyme pattern as other wheat cultivars (Ranieri et al., 1992) (Fig. 3).

Amino acid composition

Examined leaves showed prominent differencies in amino acid composition, so as a representative sample cultivar NS-Rana 2 was taken. Obtained results are presented in Table 2.

Wheat leaves SOD was characterized by high content of serine, valine, aspartic and glutamic acids such as SOD from rice leaves Kanematsu and Asada (1989) but Lys and Arg content was much higher than in rice leaves SOD. High content of Asp acid was also observed in green pea SOD, where Lys conent was wery low as in rice leaves. The wheat SOD had no Trp and Tys such as the enzymes from rice leaves and green pea. Relevant difference to rice and pea SOD was observed only in Lys content. It was high in rice and similar to bovine and human erythrocyte SOD (Sawada et al., 1971).

Table 2

Amino acid compositions of wheat Cu, Zn–SOD

Amino acid	No. of amino acid
Asp	4
Thr	2
Ser	3
Glu	4
Pro	4
Gly	5
Ala + Cys	3
Val	4
Met	0
Ile	1
Leu	2
Tyr	0
Phe	1
Lys	3
His	1
Arg	2
Trp	0

Our results clearly indicate that production of SOD in examined wheat cultivars from different parts of Earth are under control of similar genes because the amino acid composition was similar and relations between isoenzymes most of examined cultivars too. Comparing to other plant species wheat cultivars differ in amino acid composition especially in Lys content. Our results confirm that SODs production in different plant species is under control of different genes. According to our experience, young leaves of wheat seem to be convenient and a profitable source of this enzyme in order to get a large quantity of partly purified enzyme for pharmaceutical and cosmetic industry.

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Effect of Sowbane Mosaic *Sobemovirus* (SoMV) on the Germination Biology of Some *Chenopodium* Species

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The aim of the study was to examine the effect of autecological factors and sowbane mosaic *Sobemovirus* (SoMV) infection on the germination of *Chenopodium album* L., *Chenopodium murale* L. and *Chenopodium quinoa* Willd. Temperature, light and different storage conditions significantly influenced the germination of the seeds. Germination of seeds from the virus infected plants was reduced by 2–84%, depending on ecological factors, although higher germination rate index (GRI) was obtained in some cases. The phytochrome activity of the seeds was also altered by virus infection.

The genus *Chenopodium* which involves more than 200 species has not only economical but virological importance as well. *Chenopodium album* L. is among the worst weeds in the world (Holm et al., 1977) and is a common annual weed in Hungary, too. In order of the importance of the weeds, *C. album* was on the third place in 1987 in Hungary (Tóth et al., 1989). *Chenopodium murale* L. is a less important weed. *Chenopodium quinoa* Willd. is a cultivated plant in South-America (Hegi, 1979). In the series CMI/AAB Descriptions of Plant Viruses nine *Chenopodium* species are described as diagnostic, propagation, assay and purification hosts. The most important are *C. quinoa* and *C. amaranticolor* Coste et Reyn. in this respect (Horváth, 1993). *Chenopodium murale* and *C. album* are important weed hosts of tomato spotted wilt *Tospovirus* and host plants for the vector of western flower thrips (*Frankliniella occidentalis* Pergande) (Cho et al., 1987, 1989). *Chenopodium murale* is known to be suitable for separating the often jointly occuring potato X *Potexvirus* and potato Y *Potyvirus* (Horváth, 1969, 1983). *Chenopodium* species are the best hosts of sowbane mosaic *Sobemovirus* (SoMV). Since its discovery (Silva et al., 1958) and description (Bennett and Costa, 1961) SoMV is often in the centre of virologists. This fact can be explained with its easy transmission by pollen, seed and mechanical way (Kado, 1971; Horváth, 1972; Schuster et al., 1973; Seghal, 1981; Francki and Miles, 1985; Francki et al., 1985; Lehoczky and Salamon, 1989). SoMV is transmissible up to 70% in seeds of *C. album*, *C. murale*, *C. quinoa* and *Atriplex pacifica* Nels. (Bennett and Costa, 1961; Bancroft and Tolin, 1967). Natural occurrence of SoMV on *C. hybridum* L. in Hungary was first reported by Horváth et al. (1993). The host range of SoMV includes 14 natural and 59 artificial hosts from 16 families (Teakle, 1968; Bercks and Querfurt, 1969; Quacquarelli, 1971; Šarić, 1971; Šutić et al., 1971; Juretić, 1976; Šutić and Juretić, 1976; Buturović and Juretić, 1980; Šarić and Juretić, 1980; Horváth et al., 1993; Teakle, 1996). Bos and Huijberts (1996)

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reported the natural infection of spinach (*Spinacia oleracea* L.) by grapevine isolate of SoMV and its natural seed transmission in spinach. It seems that the host range of SoMV increases in future. Only few data is available abouth the germination biology of the seeds of the virus infected plants. Horváth (1980) reported that the kernel weight and the germination of the seeds of rape (*Brassica napus* L.) may reduce by 40 and 20%, respectively due to cucumber mosaic *Cucumovirus* (CMV) infection. Therefore the aim of our study was to examine the germination ecology of the seeds derived from the *Chenopodium* species infected with SoMV.

Materials and Methods

The seeds of *C. album*, *C. murale* and *C. quinoa* were sown in sterilized boxes in our virological glasshouse free of vectors. The seedlings were planted in plastic pots (12 cm in diameter), containing standard soil mixture (pH: 7.2, humus: 55%). Plants were inoculated at 8–10 leaves phenological stage using carborundum-spatula technique with plant tissue sap containing virus diluted with 0.02 M phosphate buffer (pH: 7.2) in the ratio 1:2. Three weeks before inoculation H isolate of SoMV (Horváth et al., 1993) was propagated on *C. quinoa*. Ripened seeds were collected from the healthy and the diseased plants infected by SoMV as well. Some batches of the freshly harvested seeds were stored dry in paper bags at room temperature (25 °C), some were stored in moist sand at 4 °C for two months. Germination tests under laboratory conditions were made in Petri dishes containing two filter papers moistened with distilled water. There were four replicates of 100 seeds and tests continued for 10 days. The seedlings were counted and removed every day. Germination rate index (GRI) was counted after Burgert and Burnside (1972). The seeds were germinated at 15 and 25 °C. The incubators were illuminated for 16 hours a day. To determine germination in darkness the dishes were wrapped in a double thickness of black linen bags and the assessment was made under weak green illumination. To examine the presence of phytochrome system in seeds (Borthwick et al., 1952) the response to light of different wavelenght will be studied in a future experiment. Seeds were imbibed at 22 °C for 1 and 3 days and then subjected to red (R) and far-red (FR) light for one and three minutes. After the treatments the seeds were placed at 22 °C in darkness.

Result and Discussion

The different storage conditions influenced the germination of *C. album* to a greater extent, than did the virus infection, although the germination rate of the seeds derived from the diseased plant were reduced by 2–84%, depending on ecological factors. Walkey et al. (1985) found that the viability of seeds of *Nicotiana* and *Chenopodium* species from virus infected plants were slightly reduced. Germination was en-

Table 1

Effect of ecological factors and virus infection on the germination of *Chenopodium* species

Chenopodium species	Storage conditions								
	Freshly harvested				25 °C dry				4 °C wet
	Germination %								
	15 °C		25 °C		15 °C		25 °C		25 °C
	light	dark	light	dark	light	dark	light	dark	dark
*C. album**	47.5	5	64.25	11	87.5	43.5	87.5	55.3	59.5
*C. album***	38.2	4.75	63	12.3	58	6.75	82.5	13	45.3
	LSD(P = 0.05) = 2.50								
*C. quinoa**	96.3	97.3	100	100	99	98	98	100	***
*C. quinoa***	96	94.8	97	99	81.5	92.3	92.3	93.5	***
	LSD(P = 0.05) = 1.37								
*C. murale**	49.75	7.5	49	3	***	***	***	***	23
*C. murale***	28.5	1.25	40.5	2.25	***	***	***	***	15
	LSD(P = 0.05) = 3.19								

* Seeds derived from the healthy plants
** Seeds derived from the plants infected with SoMV
*** Not investigated

hanced when the seeds were stored both in wet sand at 4 °C and at 25 °C under dry condition as compared to germination of freshly harvested seeds. Baskin and Baskin (1987) reported, that seeds of *C. album* require exposure to low temperature to afterripen completely, but prolonged periods of chilling could result the induction of secondary dormancy (Roberts and Benjamin, 1979). Light seemed to promote germination, similar to observation of other authors (Roberts and Benjamin, 1979; Caussanel, 1980; Solymosi, 1981; Bouwmeester and Karssen, 1989; Béres, 1993). Higher germination rates were obtained at 25 °C, than at 15 °C. Sauerborn et al. (1988) reported that seeds of *C. album* germinate between 5 and 40 °C, the optimum temperature is between 15 and 25 °C. It is very interesting – when the seeds were previously stored under dry conditions at 25 °C – that the germination of seeds of the diseased plants was much more lower in dark, than that of seeds derived from the healthy plants. Light treatment eliminated the difference between the germination rates. It is concluded that virus infection and dark together induced the dormancy of the seeds, while light helped the seeds to overcome dormancy (Table 1). The values of GRI were significantly influenced by storage conditions, temperature, light and virus infection. Seed samples stored at 4 °C in wet sand and derived from the plants infected with SoMV germinated more rapidly, than those of seeds derived from healthy plants (Table 2).

Table 2

Effect of ecological factors and virus infection on the germination rate index (GRI) of *Chenopodium* species

Chenopodium species	Storage conditions								
	Freshly harvested				25 °C dry				4 °C wet
	Germination %								
	15 °C		25 °C		15 °C		25 °C		25 °C
	light	dark	light	dark	light	dark	light	dark	dark
*C. album**	17.3	18.6	25.9	36.7	18.4	19.2	32.1	29.6	39.7
*C. album***	16	14.9	26.4	31.8	14.5	14.9	22.7	23.4	50.4
	LSD(P = 0.05) = 1.46								
*C. quinoa**	41.3	42.4	76.1	78	48.7	50	83.5	89.7	***
*C. quinoa***	49.2	49.6	92.6	94.8	41.2	43.5	62	69.1	***
	LSD(P = 0.05) = 1.26								
*C. murale**	17.2	12.7	27.5	24.5	***	***	***	***	55.4
*C. murale***	13.6	16.2	24.3	18.8	***	***	***	***	68.4
	LSD(P = 0.05) = 6.10								

* Seeds derived from the healthy plants
** Seeds derived from the plants infected with SoMV
*** Not investigated

Seeds from the virus infected *C. album* plants showed an increased phytochrome activity as compared to seeds of the healthy plants. After one day of imbibition and irradiated with 1 min. R light the germination was more than four times larger as compared to dark control. After three days of imbibition and 3 min. R light the phytochrome activity was the maximum. This fact suggests that the virus infection influenced the dormancy of the seeds. The germination of the seeds of the healthy plants was significantly promoted when the imbibition period had taken 3 days and the seeds were subjected to 3 min R light. Promoting effect of FR light was observed, too. FR light yields approximately 2% active form of phytochrome which is enough for high sensitivity seeds to germinate (Taylorson, 1972), (Table 3).

The seeds of *C. quinoa* were less dormant. The germination rates approached 100% in all cases. This fact can be explained with the fact that *C. quinoa* originally is a cultivated plant (Hegi, 1979) therefore dormancy as a survival strategy is not important for the species, as in case of the most weeds (Taylorson, 1987).Virus infection has resulted av. 10% reduction in germination rates. These seeds freshly harvested from the infected plant reached the maximum germination earlier than did those of the healthy plants (see Tables 1 and 2).

The lowest germination in dark was obtained in case of *C. murale*. The maximum value was 50%. The germination in dark was negligible, suggesting the presence of

Table 3

Changing of phytochrome activity in imbibition time of *C. album* and *C. murale*

Chenopodium species	Imbibition time (days)								
	1	1	1	1	3	3	3	3	
	Light								
	1'R	3'R	1'FR	3'FR	1'R	3'R	1'FR	3'Fr	dark
	Germination %								
*C. album**	48	57	52	32	46	66	36	43	55
*C. album***	57	57	41	18	33	74	19	34	13
	LSD(P = 0.05) = 3.74								
*C. murale**	16	49	9	7	28	40	3	1	3
*C. murale***	4	28	1	2	9	14	2	0	1
	LSD(P = 0.05) = 1.89								

* Seeds derived from the healthy plants
** Seeds derived from the plants infected with SoMV

phytochrome system, as in many other species (Frankland and Taylorson, 1983; Kendrick, 1984; Taylorson, 1982, 1987). Virus infection reduced the germination by 18–63%, depending on ecological factors (see Table 1). Higher GRI was reached in case of seeds derived from the diseased plants when they were stored in wet sand at 4 °C as compared the seeds derived from the healthy plants (see Table 2).

The phytochrome activity of the seeds from both the healthy and the virus infected plants was the maximum when the seeds were irradiated with 3 min. R light after three days of imbibition period (see Table 3).

It is concluded that the germination of seeds from the virus infected plants was generally lower than the germination of seeds of the healthy plants, although the effect of environmental factors was greater in some cases. Future investigations are necessary to determine the extent of seed transmission and to clarify whether the virus infection of the plants altered only the dormancy of the seeds or seed viability as well.

Our results call attention to the fact that virus infection may influence germination characteristics of weed seeds, therefore – in inderect way – it contributes to the reduction of a weed population.

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SPECIAL ISSUE: THE ROLE OF CLIMATE CHANGE AND POLLUTION ON INSECTS

Papers presented at the Workshops

"Effect of the Possible Global Warming on the Insect Diversity and Distribution"
(Organized by F. Kozár and G. Pellizzari)
and

"The Role of Air and Soil Pollution in Development of Forest Insect Outbreaks"
(Organized by P. Nuorteva),

XX. INTERNATIONAL CONGRESS OF ENTOMOLOGY,

25–31 August, 1996, Firenze, Italy

Acta Phytopathologica et Entomologica Hungarica 32 (1–2), pp. 127–128 (1997)

The Role of Air Pollution and Climate Change in Development of Forest Insect Outbreaks – Guest Editorial

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Most entomologists think that environmental damage (including air pollution and climate change), and insect damage in forests are two quite different things. Consequently, it is considered that it will be the task of environmentalists to protect the forests against pollution and climate warming; and it will be the task of forest entomologists to protect the forests against insect pests. This kind of division of labour appears, however, to be impossible in the situation that has recently developed in the forests of Europe and Northern America. A new type of forest decline has appeared there. It is confined to pollution stressed areas, and historically the disease has followed the development of industrialization, but insects will finally kill the trees. The disease begins with pollution related symptoms, which include growth anomalies and general weakening, but later it culminates to fatal injury caused by insects or plant pathogenic fungi. Cases where the forests are dying without pest assistance are rare.

This new type of forest decline may cause calamities where really extensive forest areas are loosing all trees. Such cases have occurred in the Czech Republic, in lesser extent also in Poland and Germany. The disease may, however, cause forest decline also through a hidden mode by attacking small groups of trees or even single trees groving among healthy ones. In these cases it is possible to realize the occurrence of the disease only through the fact that more trees are dying in the forest than normally. Originally, the Germans observed the disease through statistical increase of summer fellings performed in order to eliminate sick trees from forests.

The disease has appeared to be a multistress syndrome, where no single factor is killing the trees, but the total effect of several stresses. In the spectrum of stresses, pollution dominates in the initial stage, insect pests in the fatally ending final stage. That is to say that pollution is not alone able to kill the trees, and insects are not able to invade the trees, if the pollutants have not weakened their pest resistance.

Attempts to control this disease brings forest entomologists and environmentalists to close collaboration. This is very important, because in the field of environmental protection the entomologists have too often been outsiders. This is an absurd situation because insects play a very essential role in the function of the global ecosystem. So because their biomass is high and their species diversity overwhelming. It is surely impossible to understand the environmental problems correctly or to find permanent environ-

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mental adjustments, if one does not consider in full the influence of insects. Therefore it is very important to support by all available means the collaboration of environmentalists and entomologists. The organization of these two sessions has been a measure to support this collaboration.

When forest entomologists adjoin their efforts to environmental protection, they will support a very important thing. The task of environmentalists is to protect the life supporting machinery of nature against damage caused by man, when he is taking benefits from nature with the force of his knowledge.

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Insects in a Changing World (Introductory lecture)

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In the recent years several insect species have shown substantial northward expansion in Europe, of an average speed of 20–50 km/year. In some cases we found unexpected, abrupt movement for 200–700 km northward, over short period of time. The warmer winters could promote the northward expansion of insects.

The very mild winters in Central Europe did not cause earlier spring development of the insects studied. The change in the phenology of the insects is very small and requires a much longer time for a clear trend to emerge. The population dynamics of local insects in general is not affected, and each species follows its own "usual" trend, although some insect species showed different densities after mild winter. New pests (without efficient natural regulation) could show great outbreaks. There is an increased danger of invasions of southern migrant pests during hot summers. The hot summers can cause some variation in the number of generations of insects, but the consequences for populations in the short-term are unfavourable. There is also an increased danger of new pest introductions from other warmer parts of the World, because of the better possibilities for overwintering. The northward expansion, the new outbreaks, the introductions and northward migrations of several termophilous insect species could be explained partly by the long period of years with mild winters, and somtimes with hot summers.

The study of the possible effects of global warming is of considerable interest to entomologists. It was the subject of several workshop in recent years (Anonymous, 1993; 1996; Zombori and Peregovits, 1992, etc.). The basis for this interest was that the average temperature of the earth showed a continuous increase (it was +0.5 °C over the last ten years) (Aldhous, 1991). Long term mathematical models demonstrate that the climate of Europe will be dominated by mild winters and colder summers in future (Gavin and Kuhla, 1989). In Hungary over 110 years 1.1 °C increase of the winter temperatures was measured, although with considerable regional variation (Stollár et al., 1993). This warming trend could change the present distribution of animals and plants. In Europe, if the forecasted extremes are realised the major vegetation zones could shift for more than 1000 km towards the nort over the next 100 years (Groot, 1988). According to the opinion of other authors this process would not be very quick and would not be catastrophic for the vegetation. Some of these changes could even be favourable for some regions (Kobak and Kondrasheva, 1992; Schneider and Root, 1996). The shift of vegetation will be followed by herbivorous insects and the latter by their parasitoids, increasing the biodiversity of some regions. The changes in the distribution will effect the biodiversity of regions. With the increase in temperature a northward shift of termophilous species will

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occur, increasing the species number, but in parallel the number of northern species will decrease. From the biological properties of insect species it would be possible to forecast which species have the potential for changing their geographic range. In the case of *Lepidoptera* the species overwintering in the pupal stage would be the first to spread northward (Sviridov, 1989).

Any change in the climate could be reflected very quickly in the distribution and density of a mobile group such as the insects. Thus Kozár and Nagy Dávid (1986) detected a stable northward spread of some southern insects. Similarly different groups of southern insects appeared in northern parts of Europe in recent years (Barbier, 1991; Buleza, 1989; Hluchy, 1990; Hreblay et al., 1991; Krcmar and Merdic, 1991; Leskó, 1991; Marek and Gregor, 1989; Marcuzzi, 1990; Merdic, 1991; Merkl, 1991; Nash and Agassiz, 1991; Tremblay, 1991; etc.). The plants show a similar trend. In Hungary, a high number of new plant species appeared during this century (Solymosi, 1992). There is also a danger of an increase in the invasion, migration and introduction of southern insect species.

The climate changes could also act on the biological potential of insects. In some cases the decrease in winter mortality, can result in outbreak (Bale, 1991), or after mild winters, the development of insects could start earlier in spring (Bale, 1991; Collier et al., 1991; Sparks and Carey, 1995). The number of generations could increase (Collier et al., 1991; Farrow, 1991; Porter et al., 1991). Sutherst and Maywald (1985) proposed a mathematical model to forecast the potential changes.

In order to study the possible effect of global warming it is very important to find good indicator species and groups: 1. with well-documented distribution records over long periods of time (50–100 years), and from many large geographic regions (Anonymous, 1988; Farrow, 1991; Kozár and Nagy Dávid, 1986; Kozár and Konstantinova, 1981; Pollard and Yates, 1992; Williams and Liebhold, 1995); 2. with long series of density data collected by using different trapping methods, such as light or suction traps (Harrington et al., 1991); 3. with various numbers of generation per year. For the selection of the indicator species or groups, several parameters were proposed by Groot et al. (1995).

These indicator species and groups are possible candidates for studying: 1. the changes in distribution and the speed of expansion; 2. the changes of density in space and time, and the frequency of outbreaks; 3. the changes in the mortality factors; 4. the changes in the seasonal development of insects; 4. the changes of the biodiversity of certain regions. Additionally it would also be necessary to study the changes in species composition of different communities. Some of these points will be discussed in more detail later in this paper.

Result and Discussion

I. Recent changes in the distribution of some insect species

1. Distribution. In the recent years several southern insect species were observed to move significantly northward in Europe, at an average speed of 20–50 km/year. In some cases insects could reach 700 km over a very short time, as a result of "jumping dispersion". To a lesser degree some species extended their distribution in a south eastern direction. This could be explained by the relatively cold summers, observed in this region during the years with mild winters (Kozár, 1992).

One of the best examples of the northward expansion of insects in Central Europe is of *Pseudaulacaspis pentagona*. This termophilous insect has a wide Mediterranean distribution, and for a long time lived only in the southern part of Europe. However, after a so-called "resting" period it started moving to the northern part of Europe and was established in different localities and continues to occupy new territories (B. Celada Grouard-personal communication; Jansen, 1995; Kozár 1990; Kozár et al., 1994; Kozár et al., 1995; Kreiter, 1996) (Fig. 1). This process accelerated, especially after 1970. Dur-

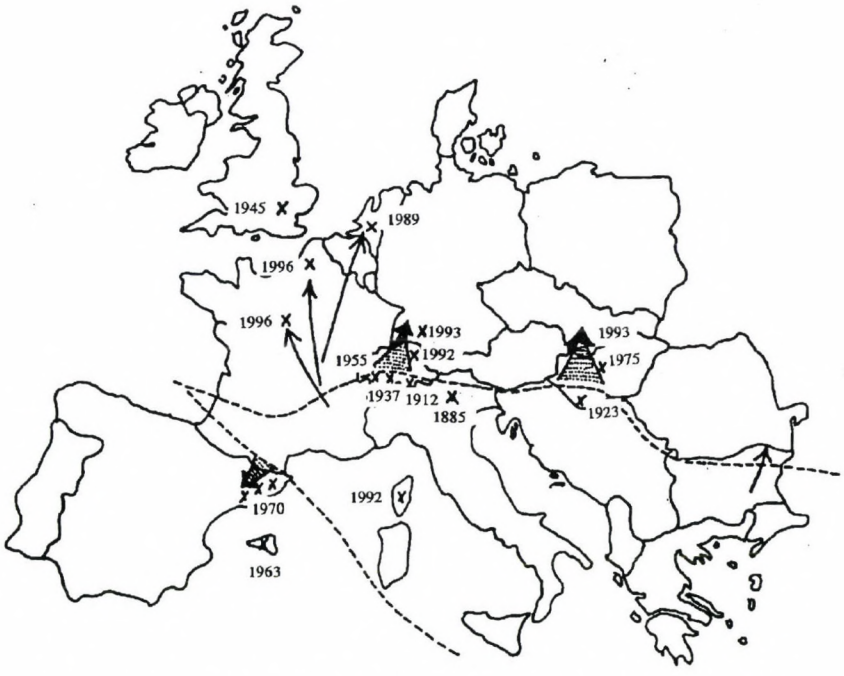

Fig. 1. Northward spreading of *Pseudaulacaspis pentagona* in Europe (---before 1975)

ing the last 20–25 years this species spread northward by about 200 km. As Fig. 1 shows the speed of movement is not a stable character, neither in time nor space.

Corytuca ciliata, an American bug species, expanded its range by about 800 km northward in Southern Europe over 20 years. In Hungary this species spread about 300 km northward in a 500 km wide zone over the last 12 years.

Over the last 20–25 years a similar trend was observed among certain other American species. Thus *Trialeurodes packardi* spread in Hungary by about 200 km, and *Parectopa robiniella* expanded northward in Southern Europe by about 1000 km.

After a 25 year "rest" the Mediterranean *Bulgaleurodes cotesi* moved northward from Southern Europe to Hungary (about 700 km) and started to spread. Because of the abrupt movement the real speed of expansion is not easy to calculate.

Apart from the fact that the tropical *Bemisia tabaci* spread all over Europe in greenhouses during few years, in the Mediterranean Region this species also tends to expand northward in the fields. As in other parts of Central Europe this species is in Hungary only present in greenhouses (Kozár et al., 1991). If the winters will be mild in future *B. tabaci* could expand into Hungary from the south in outdoor conditions, or individuals escaping from greenhouses could overwinter in the field. In such cases it would not be easy to separate the northward expansion of the natural populations from the locally surviving ones that excaped from greenhouses. There are many similar examples in the entomological literature. Data could also be collected from collections of museums, or extracted from different computerised long-term databases from different parts of the world.

2. Migrations, invasions. New outbreaks of some pest species such as *Ceratitis capitata* and *Helicoverpa armigera* in South Europe, were followed by northward migrations in Europe (Kozár, 1996). *C. capitata* appeared on a number of occasions in Hungary during this century, but overwintering was not observed (Biber, 1992). Analyses of its Hungarian appearances show that it happened in years with higher than average summer temperatures (Fig. 2), the winter temperature not affecting the migrations. According to data from the literature (Fimiani, 1989) the years of migrations into Hungary were parallelled by outbreaks in the Mediterranean Region. In these years the migrations not only concerned Hungary, but several Central European countries as well. Similar results were found in the case of *H. armigera* (Fig. 3), but this species was capable of overwintering in Hungary during years when winters were warmer than average (Szabóky and Szentkirályi, 1995).

3. New introductions. In Europe several important newly introduced pest species were observed in recent years, such as *Diabrotica virgifera virgifera* in Serbia, two *Rhagoletis* species in Italy and Switzerland, etc. (Kozár, 1996). Analysing the establishment of important new pests in Central Europe during this century, we found that it was strongly correlated with winter temperatures, and independent of summer temperatures (Fig. 4).

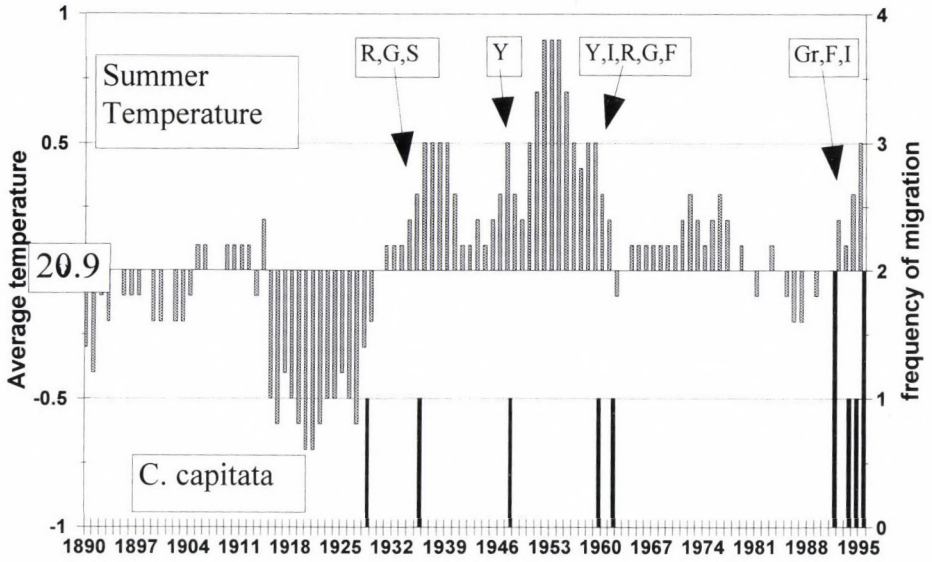

Fig. 2. Appearance of *Ceratitis capitata* in Hungary in relation to summer temperatures, with additions of main outbreak periods in different countries. Abbreviations: F–France, G–Germany, Gr–Greece, I–Italy, R–Russia, S–Switzerland, Y–Yugoslavia

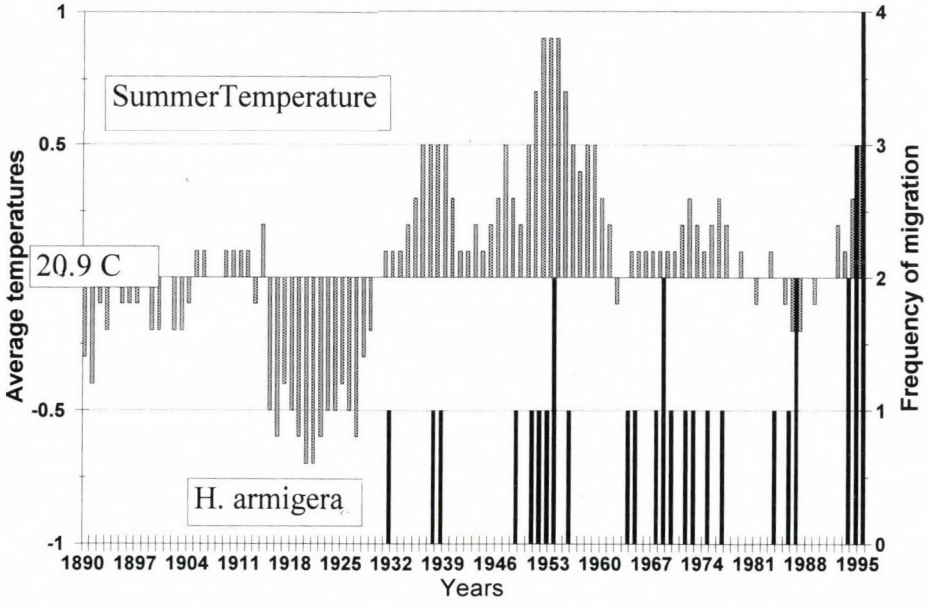

Fig. 3. Appearance of *Helicoverpa armigera* in Hungary in relation to summer temperatures

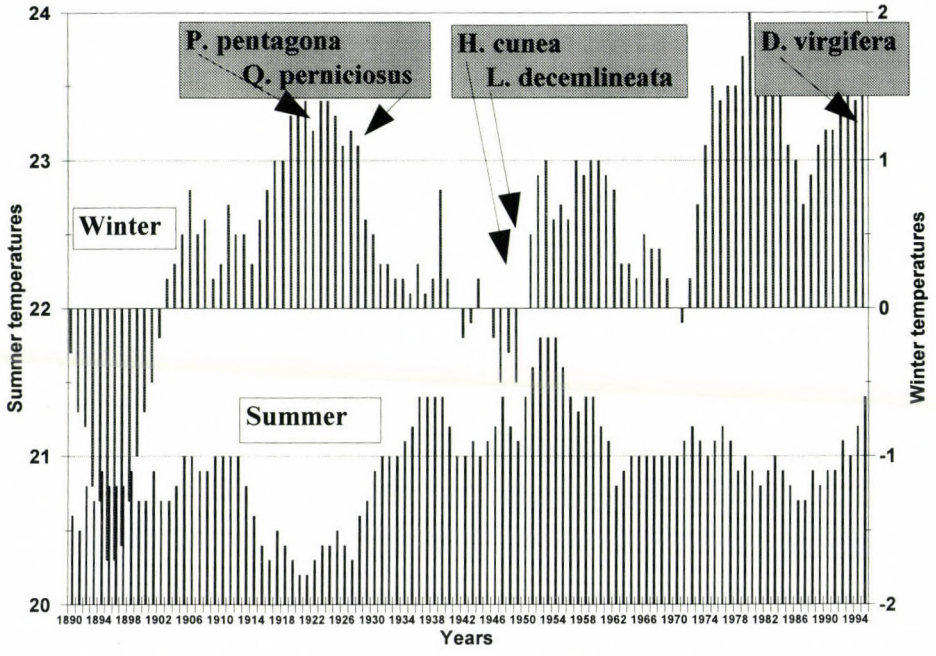

Fig. 4. Appearance of new pests in Hungary in relation to winter and summer temperatures

II. Population dynamics

New outbreaks of termophilous insects were observed in different countries, such as *Dociostaurus maroccanus* in Hungary (Nagy, 1990, 1994).

However, studies on the effect of mild winters on the density of *R. cerasi* and *A. pomorum* gave different results. After mild winters a smaller number of *R. cerasi* (P-10%) and a higher number of *A. pomorum* (P-10%) was found. Szentkirályi (1992) found higher numbers of hemerobiids after mild winters. The numbers of *R. cerasi* were significantly (P-5%) higher as a result of warmer summer temperatures. Williams and Liebhold (1995) found opposite reactions to climatic changes in different pest outbreaks in different localities in the USA. These results show that mild winters did not necessary lead to an increase on the density of insects. Farrow (1991) came to a similar conclusion. The reaction of different species could be quite different and every case requires special study.

III. Seasonal development

In some countries a warmer spring was observed after mild winters, with a subsequent earlier beginning in the phenological changes of the insects. The changes occur very slowly, about 7–10 days/100 years (Sparks and Carey, 1995). In contrast, in Central

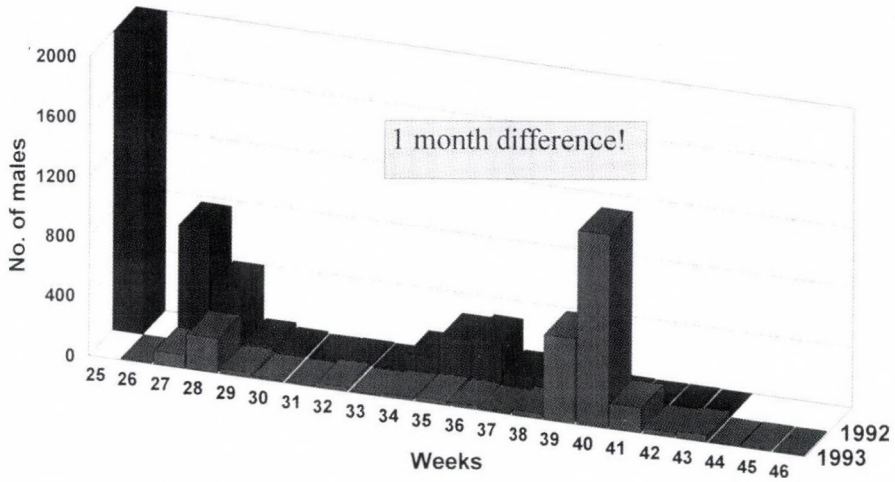

Fig. 5. Flight patterns of males of *Pseudaulacaspis pentagona* in Hungary (1992–1993)

Europe colder springs were observed after milder winters, with the insects appearing later.

Between 1978–1991 the effect of mild winters on the flowering phenology of Malus, Cerasus, Syringa, Hyacinthus and Galanthus species was studied in Hungary. During these years no significant correlation between winter temperatures and the flowering time and no consequent changes in flowering times were observed. It showed only the usual yearly variation determined by spring weather. However, the beginning of flowering of *Robinia pseudoacacia*, studied by Walkovszky and Dunay (1990) in Hungary, started one week earlier than 100 years ago.

In these years we studied the flying phenology of *Rhagoletis cerasi* and *Anthonomus pomorum* by using yellow sticky traps and tree branch beating techniques. There was no significant correlation between the average winter temperatures and the commencement of swarming of these insects, contrary to results from England, where after a mild winter earlier swarming of other insects was found (Bale et al., 1991). Perhaps, this difference could be explained by different spring weather in these two countries.

In a very small number of cases some changes in the generation number were observed. In recent years the second flight of *Ostrinia nubilalis* even appeared in the northern part of Hungary (Nagy, 1961), in agreement with the model predictions by Porter et al. (1991). After hot summers a third flight of males of *Quadraspidiotus perniciosus* was observed in Hungary (Kozár and Konczné Benedicty, 1996). This partial third generation could have negative consequences for the species, because these insects can-not overwinter, and subsequently the density will decrease in the next year.

The new insect species are not well adapted to the conditions of that region, and show great variation in phenology. The male flight of *Pseudaulacaspis pentagona* shows one month differences in different years in Hungary (Fig. 5). This variation can cause

different problems: in synchronisation of the parasitoids with the host (resulting in failure of biological control projects?), and in timing pest control applications.

Studying the relationship between plant and insect phenology, no significant correlation was found in Hungary. However, Worner et al. (1995) found good correlation between host plant and insect phenology in some localities in England. It is likely that the temperature requirements of the host plants and the insects are different.

IV. Biodiversity

The insect fauna of Hungary could be characterised by the overwhelming number of termo- and xerophilic insects (Mahunka, 1991). Consequently any warming quickly and substantially will increase the number of southern insect species, especially in the southern parts of the country, as was shown in previous parts. In parallel, the cold-tolerant and higrophilous species will disappear, but this process will be slower (Sviridov, 1989). As a result of these two opposite processes the total number of insect species will increase. Among new insects the less specialised pest species will appear first. A reliable calculation would not be easy to make, because the new appearances would be more easy to detect than those that disappear and we would only can be sure after several decades. The appearance of new insect species will be accelerated by the invasion of new Mediterranean plant species of the region. Each plant species will attract specialised herbivorous insects and the latter their natural enemies.

In the following we present a preliminary calculation of the changes in biodiversity in Hungary. According to Strong, Lawton and Southwood (1984) each plant species on average has 10 insect species (herbivorous and beneficial) associated with it. Using this ratio, combined with the fact that over 200 years about 100 (5.2%) plant species appeared in Hungary (personal communication T. Simon), we can calculate that 1000 new insect species could appear. By calculation with the proportion of increase of plants (5.2%), and from the known insect species number in the country (40.000), the counted increase will be 2000 new insects species for that time. The known examples of new insect species appearing in the country over the last 100 years show great variation in the different insect groups. The number of scale insects increased by 3.4%, while in the case of the whiteflies is reached 29%. Over the same period 41 plant species (1.9%) disappeared from the Hungarian flora (personal communication T. Simon), this could result the disappearance of 400 insect species. If we will deduct from the total number of insect species the disappearance of about 2%, we get 800 species. The difference between appearance and disappearance will be a total increase over 200 years of 600–1200 insect species. This increasing trend could be substantially accelerated by global warming.

Conclusion

The northward expansion of insects can be explained by mild winters during that period. The relatively cold summers during that period could also have cause a southward expansion of northern, boreal, or Atlantic species.

The species originating from America are not the best examples for studying the effect of climate changes on insect distribution, but it is remarkable that all these species were established in Southern Europe for the first time, and only than did they start their northward expansion. Alien species have higher rate of expansion than the indigenous ones, so they could be good indicators, even of short-term changes in the climate.

Mild winters were found not to be an obligatory cause of earlier spring development and higher density of insects.

The warmer climate and the consequent northward expansion of Mediterranean insect species can create better conditons for some National Parks in Hungary with xerophilous flora and fauna (Kiskunság, Szársomlyó, Aggtelek) to enrich their biodiversity. Some others with boreal and montane flora and fauna, like Bükk and Bátorliget can lose species, as was shown by Dennis and Shreeve (1991) in England. The fauna of small National Parks (Jablonski, 1991), like Bátorliget, is especially endangered.

Because of the absence of their natural enemies, new invader insects can cause serious problems in agriculture through long term outbreaks.

Although faunistic maps containing distribution records could help to follow the expansion of the insects, for a better understanding of the process. It seems necessary to organise well planned European regional surveys from time to time based on standardised methods using different groups of insects, as was done by Kozár and Konstantinova (1981) on fruit infesting scale insects in a continent-wide survey. The European light and suction trap networks could give much help in the study of changes in the distribution and density of different groups of insects, under different climatic conditions.

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The Occurrence of *Panonychus citri* (McGregor) (Acari: Tetranychidae) in Northern Italy: Distribution, Host Range and Phenology

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In the late 80s, symptoms linked to spider mite feeding were observed on ornamental plants, in particular *Prunus laurocerasus*, in Padua (Venetia, Northern Italy). Surprisingly, symptoms were caused by *Panonychus citri* (McGregor) a tetranychid well-known as one of the major pests of citrus in the world. Previously, *P. citri* had been recorded in southern Italy, especially on citrus. In subsequent years the species has been recorded on different ornamental plants.

In the laboratory, mites collected on *P. laurocerasus* in Padua developed successfully on *Citrus aurantium*, *Prunus laurocerasus*, *Poncirus trifoliata* and *Pyrus serotina*.

Field observations, carried out in different sites and on *P. laurocerasus*, showed that *P. citri* populations reached the highest densities in spring-early summer in correspondence with the development of the new vegetation. Some weeks later population densities dramatically decreased. The impact of predators did not appear to be important. A new population increase in late summer was seldom observed.

In northern Italy, climatic conditions are quite different from those recorded in the Mediterranean area, especially as regards winter temperatures. However, *P. citri* infestations on ornamentals have been recorded for a number of years both in urban and non-urban areas, typically on hedges along roads. The factors involved in this phenomenon and the implications are discussed.

Symptoms apparently caused by spider mites, i.e. leaf bronzing and stunted shoot growth, were observed in July of 1988 on hedges of *Prunus laurocerasus* L., an ornamental evergreen, in some towns of northern Italy. Surprisingly, the symptoms were associated with the occurrence of *Panonychus citri* (McGregor), a tetranychid mite well known as a serious pest of citrus in southern Italy and in several citrus growing areas of the world. In subsequent years *P. citri* was found in several sites of northern Italy not exclusively on *P. laurocerasus* but also on other evergreen ornamentals (e.g. *Jasminum officinale* L.).

Prior to these records, the geographic distribution of *P. citri* in Italy was consistent with that of citrus, including Campania, Apulia, Calabria, Sicily and Sardinia with Latium as the northern limit (Ciampolini and Rota, 1973; Barbagallo and Perrotta, 1977; Ciampolini and Foti, 1979; Lanza et al., 1980; Russo, 1991). The biology and ecology of *P. citri* and the role of natural enemies on citrus in Italy were also studied (Ciampolini and Rota, 1973; Vacante et al., 1980; Ragusa et al., 1983; Delrio and Monagheddu, 1986; Benfatto and Vacante, 1986).

The occurrence of *P. citri* on *P. laurocerasus* has been reported in southern France where citrus is also cultivated (Fauvel and Cotton, 1986). Severe bronzing was

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observed in late summer but the interactions between *P. citri* and this host were not analyzed. Southern France represented the northern limit of the species' distribution in western Europe until the presentation of the present work.

Detailed studies on *P. citri* have been carried out in Japan. In particular, a number of publications have dealt with two strains, called diapausing and non-diapausing, characterized by different morphology, geographic distribution, biology and behaviour (e.g. Shinkaji, 1979; Takafuji and Morimoto, 1983; Takafuji and Fujimoto, 1985; Osakabe, 1987; Uchida and Shinkaji, 1989; Kunimoto et al., 1991).

Initially, the strain found on *P. laurocerasus* in Padua was suspected to belong to the diapausing strain due to its geographic distribution and overwintering. More recently, the rank of species was assigned to the diapausing strain, i.e. *P. mori* Yokoyama (Ehara and Goth, 1992). Some mites were reexamined in accordance with this description and their identity as *P. citri* was confirmed.

Research carried out in Japan emphasizes the importance of studying the characteristics (e.g. host range and esterase isozymes) of *P. citri* populations occurring on different plants, including ornamentals. A new species living on *Osmanthus* spp. has recently been distinguished from *P. citri* and described as *Panonychus osmanthi* Ehara and Gotoh (Kitashima and Gotoh, 1995; Ehara and Gotoh, 1996; Osakabe and Komazaki, 1996a, 1996b). The different hosts of *P. citri* in northern Italy and souther Italy (mainly citrus) and the differences in climatic conditions between the former region and citrus growing areas, have promoted this study on the behaviour of the strain found on *P. laurocerasus*.

Materials and Methods

Laboratory studies

A study on the developmental success on various host plants of the mites belonging to the strain found on the cherry laurel in Padua was conducted in the laboratory. Adult females were reared on detached leaves placed in so-called arenas on a water-saturated cotton panel. A N-P-K solution (7–5–6) was used in order to improve leaf maintainance. The eggs obtained were used in experiments aiming to evaluate the development on four hosts: *P. laurocerasus*, *Pyrus serotina* Rehder var. *culta* Rehder, *Citrus aurantium* L., *Poncirus trifoliata* Raf. The eggs were placed on detached leaves of these plants at 27 ± 2 °C, 70 ± 10% R. H., 16:8 L.D. Observations were made every 24–48 h. A number of females that had developed on the different hosts were placed on fresh leaves for 14 days, at the same experimental conditions, to obtain preliminary data on oviposition. Data on development was analyzed by using analysis of variance and the means were separated using Duncan' test.

Field studies

Observations on the population dynamics of *P. citri* were carried out on *P. laurocerasus* hedges during 1992 and 1993. Three sites gave the most interesting data. The first two sites considered were situated along streets in two nearby squares in Padua (P. Salvemini, P. Stazione). In the latter, potted plants were considered. The third site was along the main road of a village (P. S. Nicolò), 10 kilometers from Padua. During 1992, samplings were carried out in two sites (P. Salvemini and P. S. Nicolò) while they were made on the three sites in 1993.

Observations were performed every week, usually from May to September, by collecting 50 leaves from each site. In the first sampling, 25 leaves that had developed in the previous year and 25 current-year leaves were collected. When the current-year leaves were fully developed, only the latter were sampled. The leaves were immediately transported to the laboratory in order to evaluate the densities of spider mites and of their potential predators. The different developmental stages of *P. citri* were divided into four classes (eggs, juveniles, males, females). Records of mites occurring on the two different leaf surfaces were also considered separately. The data was analyzed by using analysis of variance and the means were separated using Duncan' test.

Results

Geographic distribution

Records on the geographic distribution of *P. citri* were taken from 1988 to 1996. The species was recorded on *P. laurocerasus* in Piedmont (Turin and nearby areas), Lombardy (Milan), Venetia (several towns and small localities), Trent province (Borgo Valsugana, above the 46th parallel, is the northern limit), Friuli-Venezia Giulia (Udine, Pordenone), Emilia-Romagna (Bologna). The mite was frequently found in touristic seaside localities (e.g. Jesolo, Lignano, Bibione). In some sites *P. citri* occurred on other evergreen ornamentals such as *Jasminum officianale* L., *Elaeagnus pungens* Thunb. var. *maculata* Hort., *Elaeagnus ebbingei* Thunb. The symptoms varied from more or less severe leaf bronzing to stunted shoot growth, to leaf fall (especially on potted plants). In some cases, infestations were observed in several successive years.

Laboratory studies

Development was successfully completed on the four host plants. The data indirectly confirmed that this strain belonged to *P. citri* since *P. mori* is not able to develop on citrus (Shinkaji, 1961; Morimoto and Takafuji, 1983) with the exception of *P. trifoliata* (Osakabe, 1987). Developmental success varied from 51.59% (*P. trifoliata*) to 78.86% (*P. laurocerasus*). Values obtained on *C. aurantium* and *P. serotina* were intermediate (respectively 70.89% and 69.94%). Females developed faster ($P < 0.01$) on *P.*

laurocerasus and *P. serotina* (respectively 11.81 and 11.78 days) than on *C. aurantium* and *P. trifoliata* (respectively 13.06 and 13.23 days). The development of males was also significantly faster ($P < 0.01$) on *P. laurocerasus* and *P. serotina* (respectively 11.44 and 11.60 days) than on *P. trifoliata* (12.16 days) and *C. aurantium* (12.81 days). Preliminary data on oviposition showed values of 1–2 eggs/female/day on all plants during the period considered.

Field studies

P. Salvemini

Four different hedges were monitored but *P. citri* was constantly found on only one of them. During 1992 *P. citri* densities peaked in July reaching moderate levels. Females were much more abundant than males (Fig. 1). Predators (coccinellids, chrysopids and phytoseiids) were seldom collected, suggesting that the tetranychid decline observed in late July was related to other factors (e.g. leaf quality). Observations on leaves of different ages showed that tetranychids were more abundant on the current-year leaves (Figs 2, 3). Concerning eggs, means were significantly different ($P < 0.05$, on several dates (27 May, 4, 15, 25 June, 3 July) while differences in motile forms were found in the last four samplings. A higher number of eggs was layed on the leaf upper surface (Fig. 4), especially along the midrib, while juveniles and adult stages were some-

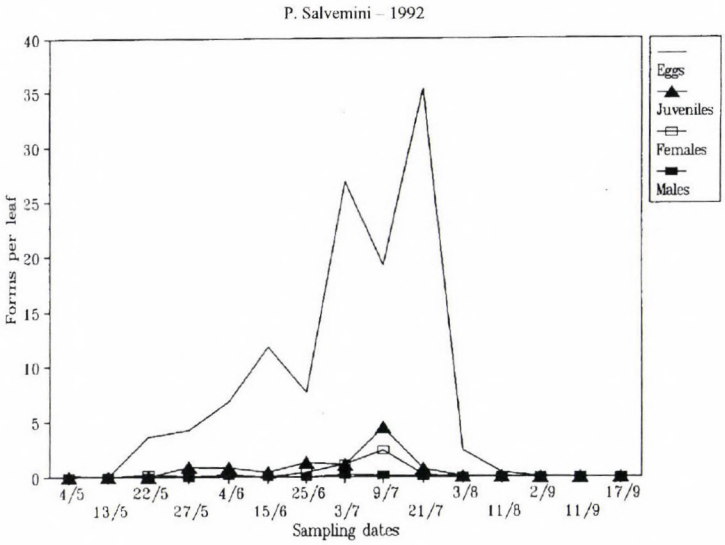

Fig. 1. Population dynamics of *P. citri* at P. Salvemini during 1992

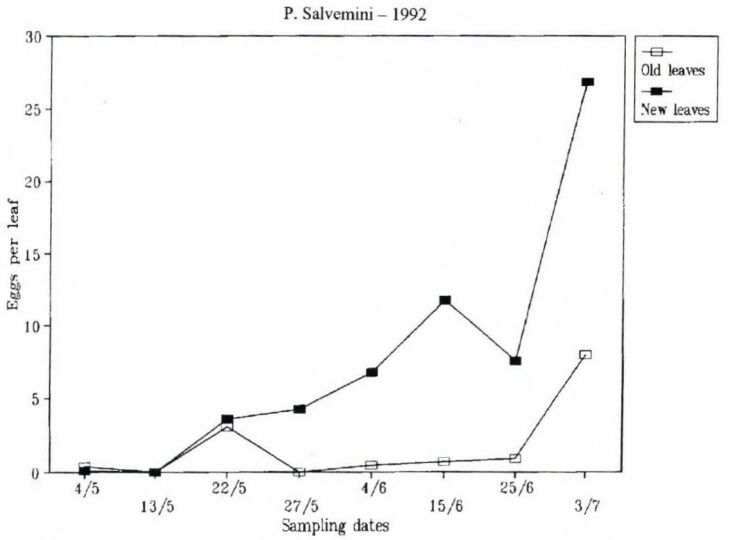

Fig. 2. *P. citri* eggs recorded on current-year (new) leaves or on old leaves at P. Salvemini during May–July 1992

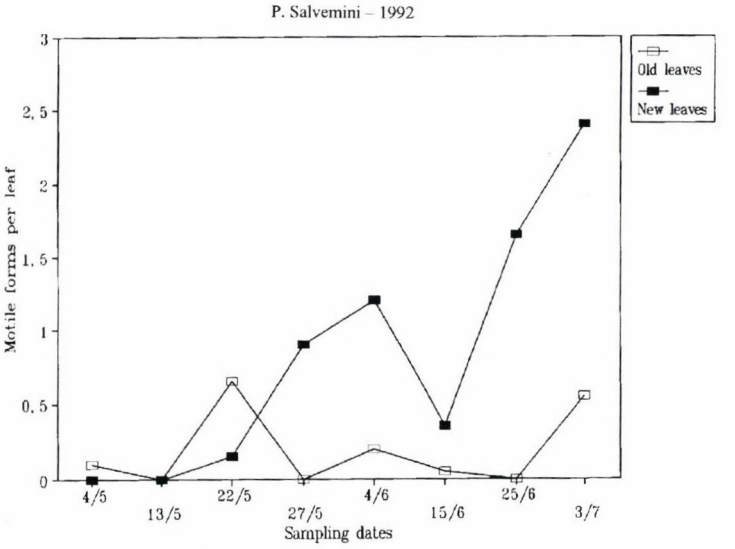

Fig. 3. *P. citri* motile forms recorded on current-year (new) leaves or on old leaves at P. Salvemini during May–July 1992

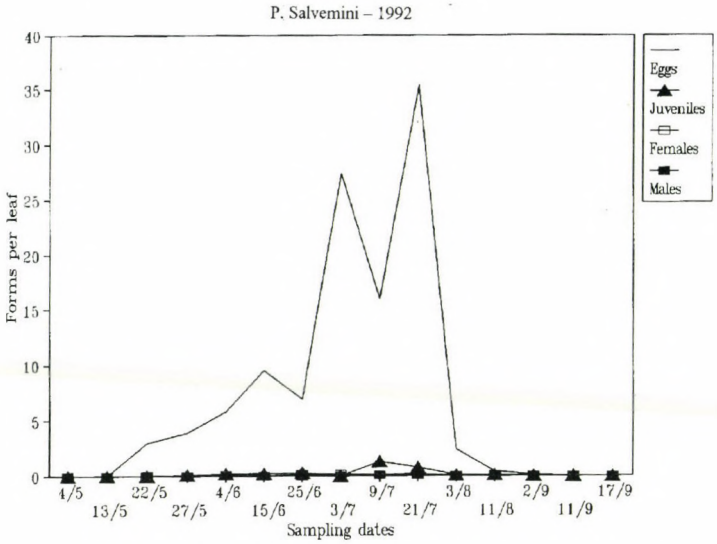

Fig. 4. Population dynamics of *P. citri* on the upper leaf surface at P. Salvemini during 1992

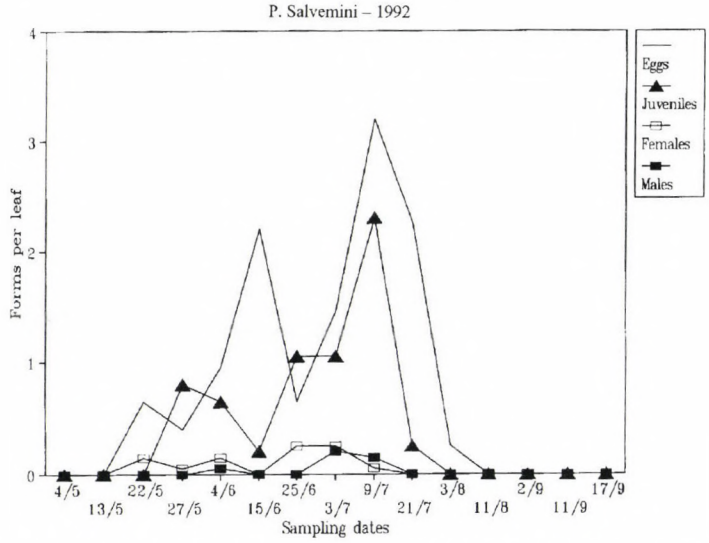

Fig. 5. Population dynamics of *P. citri* on the leaf undersurface at P. Salvemini during 1992

times more abundant on the leaf undersurface (Fig. 5). Concerning eggs, differences were significant ($P < 0.05$) on several dates (25 June, 3, 9, 21 July and 3 August) while, among motile forms, differences were recorded on 3 July. Low numbers of mites overwintered, and mostly in the egg stage.

One year later, *P. citri* populations increased in late May, reaching a peak during the first few days of June (Fig. 6). Some weeks later, mite densities dramatically declined but this phenomenon did not appear to be associated with predator activity. Sex-ratio was seen to be different than in the previous year, males being more abundant. A higher number of spider mites was recorded on the current-year leaves (Fig. 7). Differences in eggs or motile forms were significant ($P < 0.01$) on several occasions (18 and 25 May, 1, 8, 15 and 22 June). The higher abundance of eggs in the upper leaf surface and of juveniles and adults on the leaf undersurface was confirmed (Figs 8, 9). Concerning eggs and motile forms, means were significantly different ($P < 0.01$) on 25 May, 1, 8, 15 June. Egg values were also different on 29 June.

P. Stazione

Observations were carried out on potted plants during 1993. *P. citri* densities increased during the second half of May, peaking at the beginning of June (Fig. 10). Populations dramatically decreased, soon reaching low levels. The role of predators in controlling spider mites appeared to be negligible. In August a new increase of *P. citri* densities was observed but spider mites reached lower densities than in spring. Sex-ratio

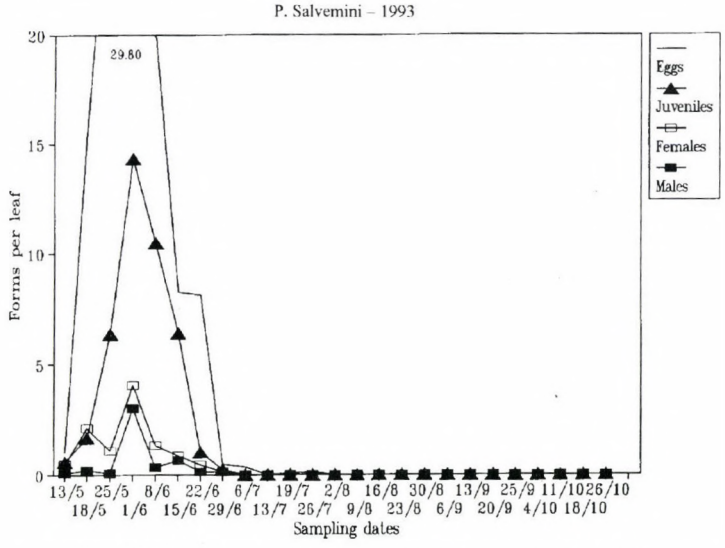

Fig. 6. Population dynamics of *P. citri* at P. Salvemini during 1993

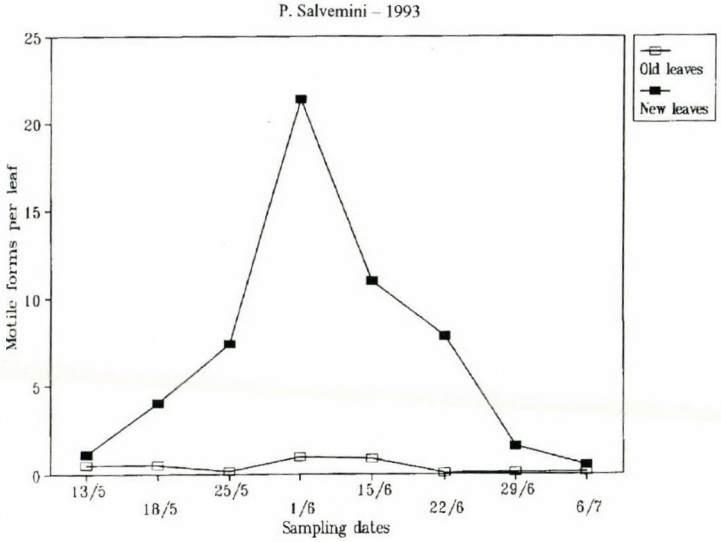

Fig. 7. *P. citri* motile forms recorded on current-year (new) leaves or on old leaves at P. Salvemini during May–July 1993

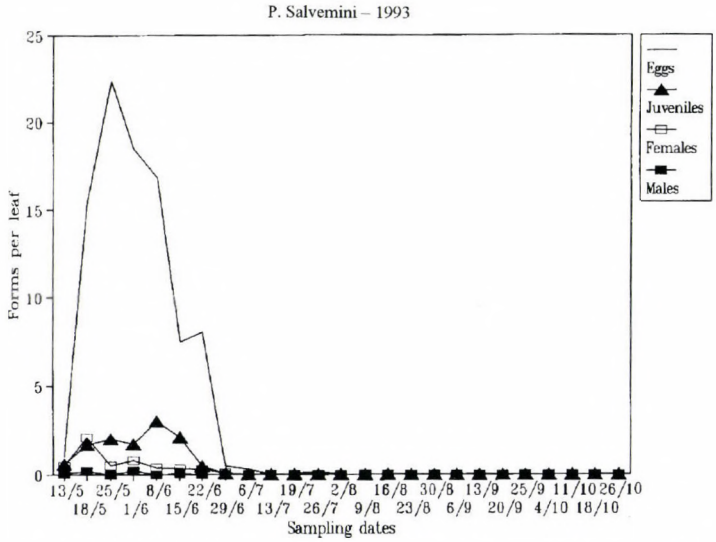

Fig. 8. Population dynamics of *P. citri* on the upper leaf surface at P. Salvemini during 1993

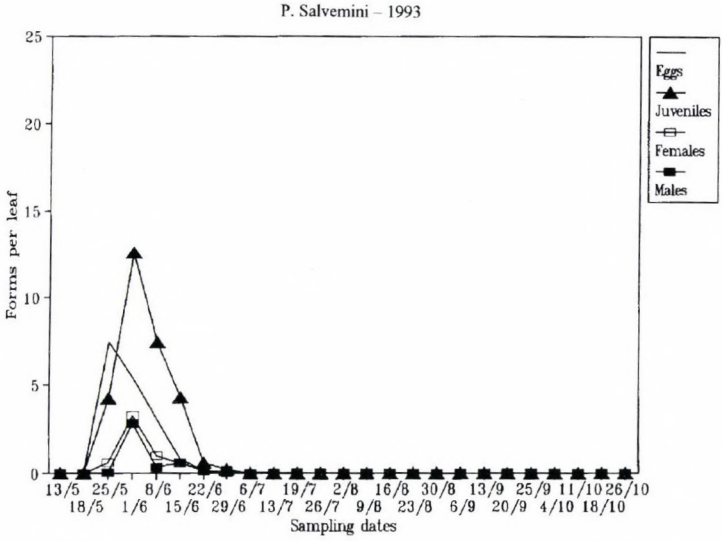

Fig. 9. Population dynamics of *P. citri* on the leaf undersurface at P. Salvemini during 1993

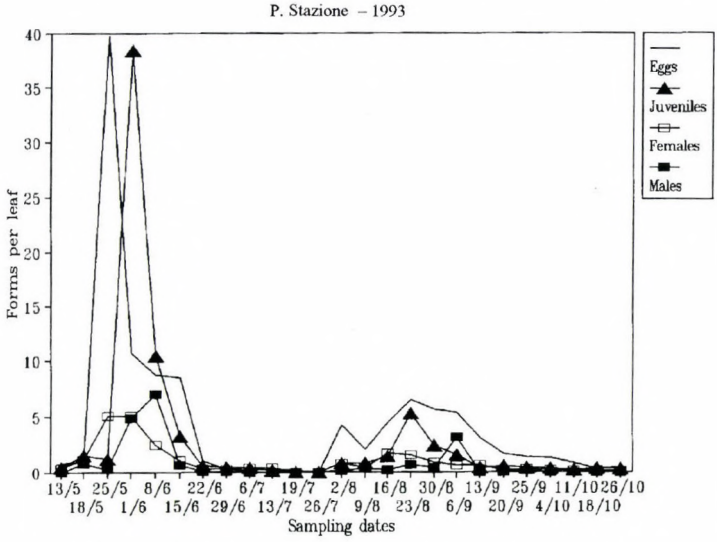

Fig. 10. Population dynamics of *P. citri* at P. Stazione during 1993

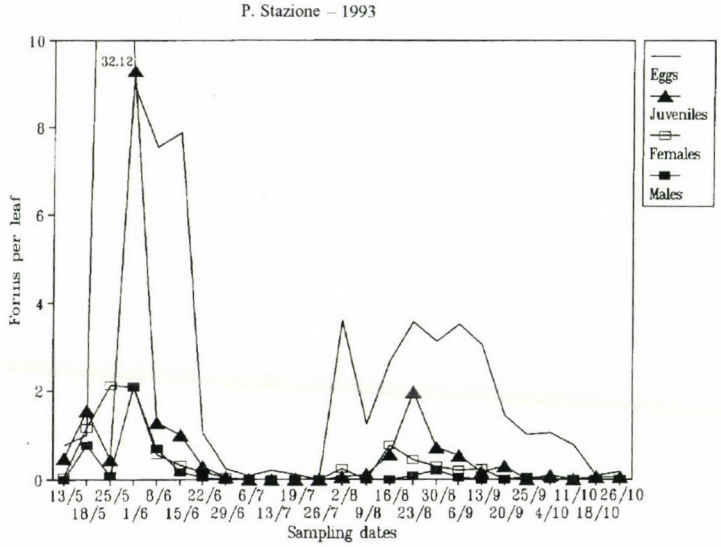

Fig. 11. Population dynamics of *P. citri* on the upper leaf surface at P. Stazione during 1993

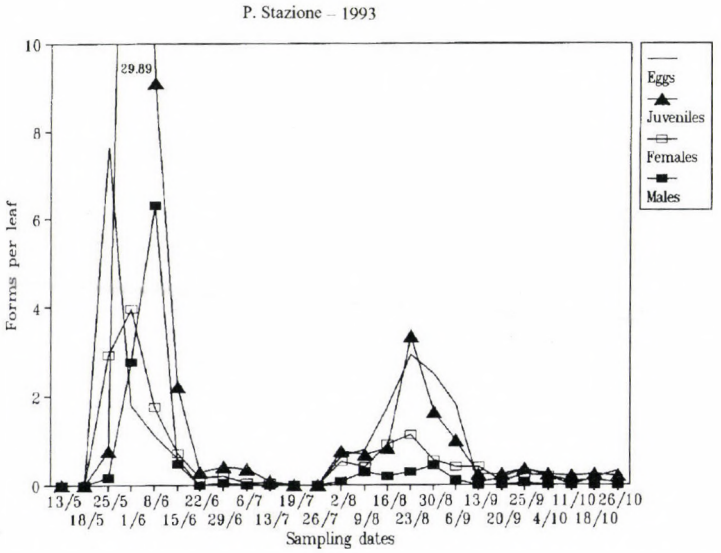

Fig. 12. Population dynamics of *P. citri* on the leaf undersurface at P. Stazione during 1993

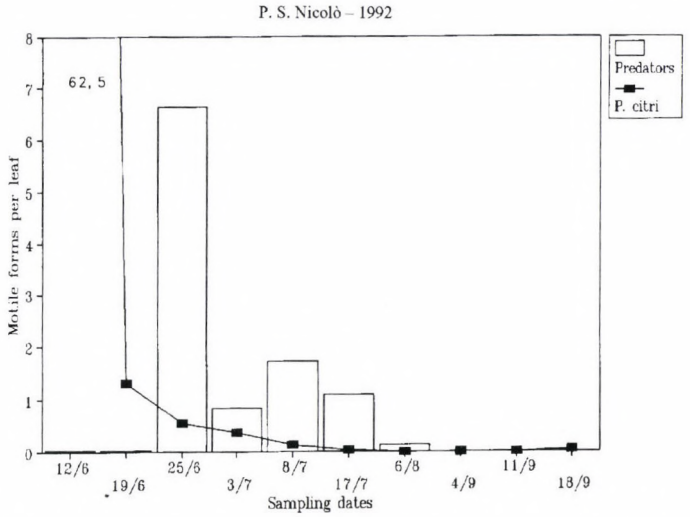

Fig. 13. Population dynamics of *P. citri* and of its predators at P. S. Nicolò during 1992

was higher than in other sites. A higher amount of eggs was layed on the upper leaf surface while motile forms were more abundant on the leaf undersurface (Figs 11, 12). Concerning eggs, means were significantly different ($P < 0.05$) on several dates (from 25 May to 22 June, 13 September, 4 and 11 October). Differences in the number of motile forms were frequently found (1 and 8 June, 6 July, 2 and 9 August).

P. S. Nicolò

In 1992 observations began in June, in correspondence with a high spider mite density on the leaves of one of the two hedges monitored (Fig. 13). Mites were rarely found in the second hedge, situated close to the first one. Some weeks later *P. citri* populations declined. The occurrence of a relevant number of predators was recorded during this phase, suggesting their potential role. The most frequent predators were a thrips, i.e. *Aelothrips* sp., and a phytoseiid, i.e. *Amblyseius finlandicus* (Oudemans). Some chrysopids and coccinellids were also observed preying upon spider mites. In the subsequent year *P. citri* populations reached negligible levels on both hedges.

Discussion

The developmental success of mites on *P. laurocerasus* was comparable with that recorded on *C. aurantium*. Preliminary data also showed that developmental times on cherry laurel were better than those observed on sour orange. These results could be

affected by strain history. However, the data supports the suitability of *P. laurocerasus* as a host and partly explain the widespread distribution of the pest in northern Italy where this plant is very frequent, while *C. aurantium* (and other citrus) cannot be cultivated, except in a few mild areas (e.g. Liguria), because of low winter temperatures.

The phenology of *P. citri* on *P. laurocerasus* showed a typical increase in late spring–early summer during the first vegetation growth while a second population increase was observed on potted plants only during mid-summer. The role of leaf quality (i.e. new vegetation) on population increases appeared to be clear. Field studies also indicated that *P. citri* densities decreased rapidly after peaks. The impact of natural enemies did not explain the population decline, except in one case. In contrast, in southern Italy as well as in various citrus growing areas, the complex of natural enemies is very effective in controlling *P. citri* especially when non-selective pesticides are not used (Viggiani, 1982; McMurtry, 1985; Benfatto and Vacante, 1986; Delrio, 1986).

Among factors affecting population dynamics on cherry laurel, the increase of summer temperatures, and probably the deterioration of leaf quality due to mite overcrowding, appeared to be more relevant than the impact of natural enemies.

On citrus, mite densities increase in spring, late summer and fall (Boyce, 1936; Ciampolini and Rota, 1973; Keetch, 1971a; Jeppson et al., 1975; Garcia-Mari et al., 1984). Climatic conditions play a significant role on the seasonal dynamics (Jeppson et al., 1957; Keetch, 1971b; Yasuda, 1980). In the laboratory, high mortality of immature stages occurs at temperatures above 30 °C (Delrio and Monagheddu, 1986). The pest prefers newly developed leaves to old ones and lay more eggs on uninfested leaves (Henderson and Holloway, 1942; Mijuskovic, 1974). Mite persistence is strongly affected by the degree of infestation as females disperse rapidly from severely infested leaves (Jeppson et al., 1957, 1961; Munger, 1963).

Therefore, the mite's seasonal dynamics on citrus and on cherry laurel shows common traits as regard its relation with leaf quality and vegetation growth phases.

The different ratio between eggs and motile forms on the two leaf undersurfaces, as observed on *P. laurocerasus*, might be explained by a different impact on the juveniles' survival by some environmental factors (e.g. temperature and R. H.). However, mite migration from one leaf surface to another should be monitored in order to understand this phenomenon.

Sex-ratio attained different values in the two first sites monitored and in particular the female proportion was higher for populations occurring in a garden (P. Salvemini) than for those living on isolated potted plants (P. Stazione). A detailed study carried out in Japan on citrus reported a higher sex-ratio in field populations than in those growing in a greenhouse. These differences may be attributed to environmental factors, plant physiology or mite population density (Yasuda, 1980). Results showed that, within a garden, certain plants were infested during successive years while the pest was virtually absent in others. Potted plants are frequently infested, probably because the impact of limiting factors is lower.

Prior to this contribution, the northern limit of *P. citri* in Italy was the Latina area, situated between the 41st and the 42nd parallels (Ciampolini and Rota, 1973) and in

western Europe it appeared to be the Montpellier area, below the 44th parallel (Fauvel and Cotton, 1986). Therefore, the records obtained in the present study (in particular Borgo Valsugana, above the 46th parallel) represent the northern limit of the species in western Europe. In eastern European countries, the pest distribution is not well documented (Anonymous, 1965).

The widespread distribution of *P. citri* in northern Italy is currently restricted to evergreen ornamentals but the species is well known as a polyphagous pest (Ashihara, 1987; Osakabe, 1987). In Japan, *P. citri* populations can survive on *Ilex crenata* Thunb. and migrate on pears where mite densities peak is late summer (Kunimoto et al., 1993). The occurrence of *P. citri* in northern Italy should be carefully monitored as the cultivation of some of preferred hosts of *P. citri*, e.g. japanese pear, in increasing in some areas.

Among the different factors affecting the spread of the pest in northern Italy, climate – *P. citri* interactions should be investigated more thoroughly. Mite occurrence in the towns would be allowed by higher winter temperatures in urban areas but severe infestations were also recorded in the country, especially along the main roads. Apparently, the winter temperatures occurring in the last decade in northern Italy did not affect pest survival. It is well known that *P. citri* motile forms can tolerate sudden temperature drops (Simanton, 1965). In southern France, infestations on *P. laurocerasus* were observed in a season following very low winter temperatures, i.e. minimal values attained –9 °C (Fauvel and Cotton, 1986). In January 1997 a number of juveniles, males and females was found to be still active on *J. officinale* potted plants despite the fact that the temperatures had reached minimal values of –7 °C for some days, three weeks before.

Pollution appears to be a common trait giving an advantage to the pest in urban areas and in the country, as infestations are commonly observed on plants with dusty leaves.

Recently, *Dialeurodes citri* (Ashm.), a pest of citrus in southern Italy and in various growing areas, has been commonly found on some evergreen ornamentals (e.g. *Ligustrum* spp.) in northern Italy (Arzone and Vidano, 1990; Duso, unpublished data). The increasing winter temperatures of the last decades may be considered one of the most important factors to allow the establishement of *P. citri* and *D. citri* in northern Italy. However, their spreading is certainly favoured by the fact that they feed on evergreen ornamentals that are more resistant to cold conditions than cirus.

The repeated infestations recorded in several sites and the wide pest distribution suggest that the spread of *P. citri* (and probably *D. citri*) to northern Italy is not recent. In accordance with Fauvel and Cotton (1986), nurseries may have an important role in pest dispersion.

Acknowledgements

The identity of *P. citri* was confirmed by Prof. V. Vacante (Università della Tuscia, Viterbo, Italy) and Dr. T. Gotoh (Ibaraki University, Japan). The former author and Dr. M. Osakabe (Persimmon and Grape Research Center, Hiroshima, Japan) have substantially improved the manuscript. I wish to thank Drs. R. Dal Gallo, M. Pasini and P. Camporese for their assistance. Research financially supported by the National Research Council of Italy (C. N. R.), Research Group "Integrated Control of Plant Pests". Paper N. 353.

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Native Insects Expanding their Range in South Africa

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Two conspicuous species of indigenous insects, the stink bug *Calidea dregii* (Hemiptera: Scutelleridae) and the leaf mimicking katydid *Zabalius aridus* (Orthoptera: Tettigoniidae) have recently expanded their range from the north-eastern regions of South Africa to the Western Cape, as did the butterfly *Mylothris chloris agathina* (Lepidoptera: Pieridae) earlier. Similar expansion of the ranges on insects in the northern hemiphere have been ascribed to milder winters, but it is unknown which factors enabled insects in South Africa to invade an area characterized by cooler, drier summers and wetter winters than what they were used to.

During the past few years a number of conspicuous and striking insects have arrived in the Western Cape. They are all indigenous to South Africa, but was previously known only from the northern and eastern parts of the country, a direct distance of some 1200 km away. It is considered worth documenting these new locality records as the expansion of the ranges of these insects might be in response to subtle changes in climatological or other environmental conditions. They could therefore serve as biological indicators of these changes.

Geertsema (1985) recorded the arrival in 1980 of the butterfly *Mylothris chloris agathina* (Cramer) (Lepidoptera: Pieridae) in the Western Cape, where it spread rapidly. It is a common species in the eastern and north eastern parts of South Africa and occurs in Swaziland, Mozambique, Zimbabwe and Botswana (Dickson and Kroon, 1978). It probably followed a route along the south coast to extend its range, as host plants are continuously available (Geertsema, 1985). Geertsema states that the introduction of this butterfly is apparently due to its familial tendency for migration, but regards it as intriguing that it only took place during the previous few years.

During April 1995 and 1996 the iridescent stink bug *Calidea dregii* Gen. (Hemiptera: Scutelleridae) was found at Piketberg and Porterville in such large numbers that they caused damage to garden plants and were submitted for identification. These brightly coloured insects with purple-black spots on a metallic blue-green background dorsally and an orange ventrum, have not yet been seen anywhere else in the Western Cape. The only specimens in insect collections of the Western Cape (SAM* and US**)

* Insect collection of the South African Museum, Cape Town.
** Insect collection of the University of Stellenbosch.

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derive from the northern and eastern provinces. According to Enlitz et al., (1989) it is widely distributed, especially in the eastern parts of Africa, Madagascar and Arabia where it is a pest of various crops, including sunflower and cotton.

Similarly a very conspicuous insect, the large leaf mimicking katydid *Zabalius aridus* Wrk. (Orthoptera: Tettigoniidae) was first seen in Stellenbosch during 1992, when a single specimen was collected. Another specimen was noticed in Stellenbosch during April 1996 by the author. The SAM only has one specimen from the Western Cape, collected at Bishopscourt, Cape Town during August 1992. The only other specimen in the collection is from Durban, Kwazulu/Natal in the east.

Some insect species have become much more abundant in recent years. The large, fruit and flower eating beetle *Pachnoda sinuata flaviventris* (Gory and Percheron) (Coleoptera: Scarabaeidae) was considered to be absent in the Western Cape by Donaldson (1979), giving Péringuey (1904) as a reference. However, single specimens have been collected at various times in Stellenbosch since 1941 (US). It was taken up in the AcUS collection of pests from Worcester in 1959 and 1960 and from Welgemoed in 1980. Today it is a common pest in home gardens of the Western Cape.

Similarly, the diurnal hawk moth *Macroglossum trochilus* (Hübner), the so-called African Humming Bird (Pinhey, 1962, 1975), is now frequently seen in Stellenbosch home gardens where it was absent before. I first saw and collected it in 1989 (US). It was not represented in the University of Stellenbosch collection at the time, but SAM has a specimen collected in Cape Town in 1903 and Pinhey (1975) states that it occurs in the Western Cape. These two species have therefore been present in the Western Cape in very low numbers for quite some time, but for unknown reasons have become abundant. It is the type of response one sometimes sees when insects are favoured by the monocultures of agricultural production and inconspicuous or rare insects become agricultural pests, but these two insects are not associated with agriculture.

What do we make of these phenomena? It is common for insects to appear in new localities, especially those that feed on agricultural crops. By various means, particularly transportation by humans, they find their way to areas where their host plants are cultivated. Thus the Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), has since 1980 expanded its geographical range from western North America to all the continents (Giliomee, 1989). However, it is certainly unusual for a number of insect species to expand their range within one country from a sub-tropical to a temperate region over a short period, as has happened with the insects described here. None of them are linked to a particular host plant that might have expanded its range as they are all polyphagous. For various reasons human transportation can also be ruled out as the general agent of their spread. With the exception of *C. dregii* their juvenile stages do not occur on commercial plants, while the adults are active and free living. In fact, if they had been conducive to human transportation they would probably have spread a long time ago.

One can be certain that these insects would not have been overlooked in the Western Cape previously since their appearance is such that entomologists would have noticed them, entomology students would have collected them and the interested public would

have enquired about them. The question should now be asked: are these insects just the tip of the iceberg, i.e. are many less conspicuous insects also expanding their range?

In the northern hemisphere indications were also found that insects tend to expand their ranges, but in the opposite direction. Kozár and Nagy Dávid (1986) and Kozár (1992) found that several insect species substantially increased their northward distribution in Central Europe during the last 20 years. The authors suggest that this may be due to milder winters in the region. Likewise warmer, drier conditions are considered as the possible cause for the range expansions of several British butterfly species since 1960 (Dennis, 1993).

It is not clear which, if any, environmental changes could have caused the local insects to expand their range or increase their numbers. The most important climatological factors affecting insects are moisture and temperature (Dennis, 1993). The climate of the Western Cape differs radically from that of the north-eastern regions where the insects originated in that more than 80% of the annual rainfall occurs respectively during the winter and summer months (Tyson, 1986). The Western Cape is cooler than the north-eastern regions with about 30 days per annum having maximum temperatures exceeding 30 °C compared to 30–120 days, but the winters are mild – only about five days have minimum temperatures falling below 5 °C (Tyson, 1986). Insects expanding their range from north-east to south-west would therefore have to adapt to the cooler, arid summers and wetter winter conditions. The dry summers or wet winters could both be severe limiting factors in preventing insects to expand their range.

The Western Cape is not known to have experienced wetter summers or drier winter conditions than normal during the last two decades. The reason for the range expansion of the insects therefore remains uncertain.

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Ecological Effects of Introduced Species in North America

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The rate of invasion of North America by exotic organisms has increased considerably over the last 100 years. Among the species of biological invaders, there is considerable variation both in their taxonomic identity and their ecological role. Among taxonomic groups, plants, insects, and fungi have probably gained most notoriety for spectacular effects. Probably the most common ecological roles of invaders have been primary producers and herbivores but other groups such as parasitoids, pathogens, predators, and pollinators may also have profound ecological effects. For each of these ecological roles, it is possible to identify the most common direct ecological effects. These direct effects include competition with native species and direct trophic interactions (e.g. predation). Many species may cause more complex ecological effects that involve species at multiple trophic levels. While these complex effects may have the greatest effect on ecosystem properties, they are difficult to predict.

For millions of years the distribution of the world's biota has been restricted by oceans and other natural barriers. However, the paleontological record indicates that over the past several million years, species ranges have been in constant flux within continents due to temporal variations in climate (Huntley and Birks, 1983; Davis, 1976). During the last 100 years, human activities, especially international travel and trade, have circumvented these barriers and species are invading new continents at a rate that vastly exceeds the natural movement. As early as 900 A.D., the range of several European animal and plant species had expanded as a result of human movement (Crosby, 1986). The consequences of increased human mobility and subsequent redistribution of the world's biota has significantly contributed to global changes (Vitousek et al., 1966) and represent a substantial environmental challenge for the next century.

Prior to 1800, few exotic arthropod species became established in North America but the establishment rate has accelerated considerably over the last 150 years. Presumably this temporal changes reflects the increases in intercontinental mobility that also began around 1800. Plant species introductions into North America have been occurring since the first wave of colonists. There have been steady increases of plant species into Europe from other parts of Europe, America, and Asia since at least 1500 (Kowarik, 1995).

Many introduced species cause little ecological or economic impact and may remain unnoticed for many years. An increasing number of species, however, may cause

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substantial ecological impacts. These impacts often result in considerable economic impacts to intensely managed systems such as in agricultural crops (Pimentel, 1986). As a result, extensive effort is sometimes applied to reducing the impacts of introduced pests via quarantine, inspection, eradication, and biological control. Natural ecosystems or preserves are as vulnerable as intensively manipulated areas. There has been growing recognition that introduced organisms represent a major threat to management of natural areas (Bratton, 1982; Harty, 1986; Hester, 1991).

Most of the studies of the effects of introduced organisms in natural ecosystems has focused on plants. While many introduced plants have had spectacular and obvious impacts in North America, other types of organisms, especially insects and fungi, can also cause substantial disruption to natural process in any ecological system. Taxonomic association does not limit the likelihood of invasion or impact of non-indigenous organisms. Table 1 demonstrates that there are invaders from virtually every taxonomic group, although the most noted, disruptive, and visible invaders to date have been plants, insects and fungi.

An introduced organism's ecological role determines the type and extent of effect it will have on an ecosystem. The ecological niches exploited by introduced species are as varied as their taxonomic associations (Table 2). Most of the literature on the ecology of introduced species has focused on primary producers (e.g. Diamond and Davis, 1981; Evans, 1983; Apfelbaum and Sams, 1987). Most of the attention given to organisms other than plants has focused on insect pests (mostly herbivores) and plant pathogens (Liebhold et al., 1995). However, there are a variety of other ecological roles played by exotics that exert disruptive forces on ecosystems.

Aside from characterizing invasive organisms based solely on their ecological role, it is important to consider ecological roles of resident species. For example, the extent to which an invasive plant has substantial competitive interactions depends both on the composition of the native flora and the biology of the invader. If the exotic plant species most successfully competes with a native species that is of little overall important to an ecosystem, then the invasive plant may also assume little importance. Vitousek (1984) pointed out that comparison of the effects of biological invasion on ecosystem processes represents a unique method for comparing the importance of individual species to ecosystem function and that some invading species may act as "keystone species" and their presence may cause manifest ecological change in collective ecosystem properties such as productivity, decomposition, and nutrient cycling.

In this paper, we intend to contrast the ecological impacts of introduced organism of varying ecological roles. Currently there is a poor understanding of why certain alien species, in certain habitats have such dramatic effects. We show that in all groups, generalizations can be made about the predominance of direct ecological effects (e.g. competition, predation), but indirect effects are complex. This comparison of the varying ecological roles of invasive organisms provides insight into predicting the impacts of future invasions by alien species.

Table 1

Examples of introduced organisms into North America, organized by taxonomic order

Kingdom	Species	Description	Reference
Plantae	*Melaleuca quinqueneriva*	Melaleuca tree	Diamond and Davis ,1991
	Lythrum salicara	Purple loostrife	Stucky, 1980
Animalia	*Lymantria dispar*	foliage-feeding insect	Liebhold et al., 1995
	Trichosurus vulpecula	foliage-feeding mammal	Rose et al., 1992
Protista	*Nosema locustae*	Grasshopper pathogen	Lockwood and DeBrey, 1990
	Plasmodium relictum	bird parasite (avian malaria)	Warner, 1968
Monera	*Staphylococcus*, spp.	avian pathogen	van Riper and van Riper, 1985
Fungi	*Cronartium ribicola*	plant pathogen	Kendall and Arno, 1990
	Entomaphaga maimaiga	insect pathogen	Hajek et al., 1995

Table 2

Ecological roles exploited by selected introduced organisms affecting natural and managed areas

Ecological role	Species	Reference
Primary producer	*Melaleuca quinqueneriva*	Diamond and Davis, 1991
	Rosa multiflora	Evans ,1983
Herbivore	*Lymantria dispar*	Liebhold et al., 1995
	Adelges picea	Witter and Ragenovich, 1986
Plant pathogen	*Cronartium ribicola*	Kendall and Arno, 1990
	Fusarium subglutinans	Storer et al., 1994
Animal pathogen	*Entomophaga maimaiga*	Hajek et al., 1995
	Plasmodium relictum	Warner, 1968
Predator	*Paratrechina fulva*	Zenner-Polania, 1994
	Calosoma sycophanta	Burges, 1911
Parasitoid	*Compsilura concinnata*	Culver, 1919
	Agrypon flaveolatum	Roland, 1988
Parasite	*Ixodes laysanensis*	van Riper and van Riper, 1985
Pollinator	*Apis mellifera*	Roubik, 1983
	Xylocopa sonorina	Gerling, 1983
Detritivore	*Linepithema humile*	Ward, 1987
	Lumbricus terrestris	Kalisz and Dotson, 1989

Producers

In North America, much of the attention on nonindigenous species has focused on vegetation (mostly primary producers), by virtue of the great number of introduced plant species. The principal ecological impact of these species is typically competition and the subsequent threat to endemic species (Bratton, 1982). This competition can result in

decreased floral diversity, displacement of native species, and in extreme cases, can cause extinction of endemic species. There is great concern about consequences of non-indigenous plant species, particularly in unique habitats, nature preserves, and areas with species of special concern where the threat of species eradication is real.

Lythrum salicaria is a good example of an exotic plant that has caused profound ecological effects because of its competitive interactions. It invades wetlands throughout North America and forms the dominant herbaceous cover in areas that previously supported a diverse flora. This species out-competes native vegetation both by overtopping plants, preventing undergrowth, and by prodigous production of viable seeds. *Lythrum* currently threatens two native plant species as well as a species of turtle and populations of a bird species, the black tern (Thompson et al., 1987).

Introductions of primary producers can also affect ecosystem processes indirectly via other effects such as changes in soil stability, soil moisture, and changes in fire regimes. Well-known examples from North America include *Tamarix* spp. which successfully alters the hydrologic regime through its ability to exploit aquifers, and occurs primarily in the southwestern United States where water resources issues reign as significant environmental concerns. *Shinus terebinthifolius* and *Causuaria* spp. also alter the hydrology in the Southeastern United States through increased evapotranspiration. Such modifications of the ecological system may consequently unbalance the disturbance regime (e.g. increase drought stress). As an example, in Florida, *Melaleuca quinquenervia* has created an increased fire frequency in what was formerly wet habitats (Ewel, 1986). *M. quinqueneriva* is perfectly adapted to fire with serotinous capsule as a fruit and with a deep, spongy bark that insulates the cambium. However, the outer bark is flaky and burns readily as do the volatile oils in the foliage. Consequently, *Melaleuca* abundance has replaced native cypress and pine hammocks in important wetland habitats as well as altering the long-term dynamics of these ecosystems.

In addition to affecting native plant species, induced changes in plant community structure can alter the abundance and structure at other trophic levels, such as herbivores, pollinators, and predators. For example, Lockwood and DeBrey (1990b) hypothesize that the introduction of plains cottonwood, *Populus deltoides* var. *occidentalis*, through out the western great plains may have contributed to the extinction of the Rocky Mountain grasshopper, *Melanoplus spretus* via destruction of habitat.

Herbivores

The effects of introduced herbivores are often spectacular. Outbreaks of phytophagous insects, such as *Lymantria dispar* L., *Adelges picea* and *A. tsugae* can be spectacular, causing heavy mortality to most of the overstory trees (Langdon and Johnson, 1992; Liebhold et al., 1995). These insects are significant in ecological systems in North America primarily because their preferred host tree species *Quercus* spp. *Abies*, spp. and *Tsuga canadensis* respectively, have dominant roles in forests of the eastern United States. When intense plant mortality occurs in relict stands, there is considerable potential for indirect extinction of plants and animals associated with these isolated communities.

An example of the concern over potential loss of endangered ecosystems as a result of an introduced herbivore is provided by *Adelges picea* in isolated stands of *Abies fraseri* in the southern Appalachians (Hain and Arthur, 1985; Witter and Ragenovich, 1986).

Relatively little is known abouth the competitive interactions between introduced herbivores and the native species that occur sympatrically. This may be in part due to the relatively low importance of competition in regulation of herbivore populations.

Plant pathogens

In forests of North America, plant pathogens rank as the most significant group of organisms as agents of changes in the forest (Liebhold et al., 1995), and introductions of plant pathogens have resulted in substantial alteration of communities. The best example of this is the chestnut blight fungus, *Cryphonectria parasitica*, which has resulted in the virtual elimination of *Castanea dentata*, throughout the eastern U.S. and has forever changed important forest ecosystem processes (Kuhlman, 1978). *C. parasitica* impact was due to the significant and dominant role that *C. dentata* assumed in forests of the eastern U. S. Introduced plant pathogens may also result in the elimination of other significant forest tree species, such as beech bark disease, *Nectria coccinea* var. *faginata*, and its potential to reduce the abundance of *Fagus grandifolia*, or Dutch elm disease, *Ophiostoma ulmi*, which has caused widespread mortality of *Ulmus* spp. in North America. The pitch canker fungus, *Fusarium subglutinans* f. sp. *Pini* has recently been discovered to infect *Pinus radiata* in California (Storer et al., 1994). Although this pine species has been widely planted globally, its native range is restricted to a limited area in southern California. Mortality due to *Fusarium* could result in the elimination of native gene pool of a very localized species.

The dramatic declines of native plant species caused by introduced pathogens can result in a series of indirect effects on other species. The complexity of these impacts is illustrated by the interaction between grizzly bears (*Ursus arctos horriblilis*), *Pinus albicaulis*, human habituation, and *Cronartium ribicola*, an invasive plant pathogen. The grizzly bear, like many other mammals, uses *P. albicaulis* seeds because of their high fat content and abundance during pre-hibernation feeding activities. In years of prolific seed crops, bears tend to remain and feed in high elevation areas until hibernation. However, when seed crops are small, they expand their feeding activities with a tendency to range closer to human facilities, which usually results in increased bear mortality and conflicts with humans (Kendall and Arno, 1990). White pine blister rust is currently epidemic over most of the range of whitebark pine and is considered major cause of its reduced seed production, damage, and mortality (Hoff and Hagle, 1990). As a result, this disease will undoubtedly greatly reduce densities of several wildlife species, especially the grizzly bear.

Animal pathogens

Entomophaga praxibuli originated in Australia and has been released in North America for the purpose of controlling the grasshopper *Melanoplus* spp. in grasslands of western United States (Lockwood, 1993). Many non-pest species of grasshopper co-occur with the target grasshopper and they may be rare or have a limited distribution, so there exists the potential for extinction of a non-target grasshopper. Furthermore, the reduction in abundance of grasshoppers, may result in increases in undesirable plant species.

In Hawaii, introduced diseases have caused dramatic declines in the indigenous avifauna (Warner, 1968). Several bird species exist in specific habitats and have limited distributions, therefore the possibility of extirpation or extinction of bird species exists. Such diseases were spread by mosquitoes, another non-indigenous species. Although some the blood parasites causing the avian diseases likely existed long ago, without an adequate vector, there was little cause for concern.

Predator

The mongoose, *Herpestes auropunctatus*, was introduced in the Caribbean islands in the 1880s to reduce populations of rats in sugar cane fields. It has since contributed to the elimination of seven species of reptiles and preys upon important bird species. Similarly, three predatory land snails, *Euglandina rosea*, *Gonaxis kibweziensis*, and *G. quadrilateralis*, were introduced to control the giant African snail, *Achatina fluica*, but all have been implicated in reducing populations of indigenous tree snails (Hadfield, 1981).

Parasitoids

In classical biological control efforts, parasitoids are often introduced with the objective of limiting the abundance of a non-indigenous pest. In North America biological control has been shown to be a relatively effective strategy (Dahlsten, 1986). However, biological control agents may also pose a threat to non-target indigenous species, if the agent is a generalist (Howarth, 1991). There is actually very little evidence of harmful effects by biological control agents but more information about these problems in needed (Funasaki et al., 1988).

Although not a North American problem, successful parasite introduction in Fiji provides a good example of the ecological effects of non-native parasitoids. In 1925 a tachinid from Malaysia, *Bessa remota*, was introduced to reduce populations of the cocoanut moth, *Levuana iridescences*. This parasitoid was so effective that *L. iridescences* was entirely eliminated from Fiji and may have contributed to the extinction of a non-pest species, *Heteropan dolens* (Howarth, 1991).

Parasites

There are few examples describing introduced parasites in North America. One of note that has accounted for destabilizing fish populations in the Great Lakes is *Glugea hertwigi*, established in 1960s (Mills et al., 1994). The dramatic number of introduced fish in North America provides ample opportunity for introductions of parasites and diseases (Krueger and May, 1991).

Pollinator

The European honeybee, *Apis mellifera*, provides a good example of the competitive effects of an exotic pollinator. It was introduced to North America by the first colonists, and therefore the full impact of this introduction will never be known. Research has demonstrated that *Apis mellifera* is both a more efficient and more active forager than native bees and therefore may displace native pollinators (Paton, 1993).

Also, preferential foraging and displacement of native pollinators may potentially alter the plant species composition through disruption of pollination and reduction of seed production. In a study of Africanized honey bess (feral hybrids of African and European *A. mellifera*) and native pollinators, Roubik (1983) found that introductions of the Africanized honey bee resulted in decreased abundance of native pollinators. Similarly, when non-native bees are introduced in a flora-rich area, native bees tend to pursue less valuable resources, are less capable of competing for nectar, and foraging is not adequate for recruitment of nest mates (Schaffer et al., 1983).

Seed dispersal, though obviously different from pollination, is also an important component of plant reproduction and demography, and can be modified by organisms that are not involved in such mutualistic relationships. For example, Bond and Slingsby (1984) demonstrated the importance of native ants in the dispersal of seeds of native vegetation, and how the introduction of Argentine ant (*Iridomyrmex humilis*) has disrupted the mutualism. *I. humilis* fail to adequately disperse the seeds of myrmechorous species, thereby reducing the successful regeneration of the plant species.

Detritivores

Little attention has been directed to the role of detritivores in ecological systems, and even less to the potential of changing composition due to introduced organisms. Most taxonomists agree that there are only 5 families, 5 genera and > 60 species of earthworms endemic to the eastern United States, but there are possibly 18 species, 10 genera, and 2 families of non-native earthworms in the eastern United States (Kalisz and Dotson, 1989). Stebbins (1962) was first to note that exotic earthworms were displacing native earthworms. His assertion that non-native earthworms were more abundant in highly disturbed land was supported by research conducted by Kalisz and Dotson (1989) who found that many indigenous species could exist only in undisturbed and non-fragmented landscapes. Because of their sensitivity to disturbance, native earthworm

assemblages may serve as indicators of a area's relative disturbance. As yet, implications of the species shift from native to non-native are not well understand. Considering the role of detritivores, however, it is possible that ecosystem processes, such as nutrient cycling, may be substantially modified by non-native taxa.

Summary

Ecosystems are composed of many guilds of species that play specific ecological roles. For each of these ecological roles there are at least some representatives of biological invaders in North America. Probably the most common ecological roles of invaders have been primary producers and herbivores but other groups such as parasitoids, pathogens, predators, and pollinators may also have profound ecological effects. For each of these ecological roles, the most common direct ecological effects usually include competition with native species and direct trophic interactions (e.g. predation). In worst-case scenarios, these effects have resulted in extinction of species and/or loss of diversity. Often, introduced species may cause more complex indirect ecological effects that involve species at multiple trophic levels. These complex effects may influence ecosystem properties such as productivity and nutrient cycling but they are difficult to predict.

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1945–1995: Fifty Years of Incidental Insect Pest Introduction to Italy

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More than one hundred exotic insect pests have been introduced to Italy in the years between 1945–95. The new introduced species (115) are ordered in chronological lists according to the main host plants. They are mainly pests of ornamentals, woody plants and Citrus. The three Orders accounting for most of the introductions are the Homoptera (76% of the total number of introduced species), Coleoptera (10%), Lepidoptera (7%). The majority of the introduced species have come from America (36%), Asia (25%), Africa (17%), Australia (7%). In some cases Italy has been the first noticed focus of an exotic pest in Europe. From this first focus the pest has expanded throughout Italy and towards neighbouring countries (i.e. *Corythucha ciliata* Say, *Metcalfa pruinosa* Say and *Parectopa robiniella* Clemens).

During the last fifty years there has been an ever increasing movement of plants and animals from one part of the world to another, leading to a change in the composition of native fauna and flora. Among animals, the insects are the most common immigrant species.

The position of the Italian peninsula, in the center of the Mediterranean basin, enables intensive commercial exchange and the transport of plants and goods. This is why the incidental introduction of exotic insect species has became quite a common event. Furthermore, the range of climatic parameters allows the establishment of tropical and subtropical species in the southern regions and of more northern species in the temperate climate of north and central Italy.

In this paper, a period of fifty years, from 1945 till 1995, has been checked to evaluate how many exotic species have been incidentally introduced to Italy and how many have succeded in acclimatizing. Only the insects of agricultural importance have been considered. We have not taken into account the insects of stored products or timber even if there are numerous records of the incidental introduction of exotic species especially in the main Italian harbours. The infested products are usually destroyed by quarantine services at their arrival. Insects of medical importance have not been considered either: in fact only *Aedes albopictus* (Skuse) (Culicidae), incidentally introduced in 1990, has reached a pest status and is currently widely distributed in northern Italy, central Italy and Sardinia.

On the basis of bibliographic sources the new introduced species have been listed in chronological order. We have considered the date of the first published record as the

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date of introduction of a species to Italy, even if we are aware that in several cases the species had been present in our territory for some time, but had not yet been recorded. The species are grouped in lists according to their main host plants (woody plants, fruit trees and grape, ornamentals, horticultural and agronomic crops, Citrus). Ancillary information is given: Family or Superfamily of pertinence, host plant, region or continent of origin, date of first recording. The species whose origin is uncertain or unknown are marked with a question mark (Table 1).

During the last fifty years more than one hundred phytophagous species (namely 115) have been incidentally introducted to Italy. Most of the introduced species (89) were able to acclimatize in the new territory. Of these, 35 are at the moment widely distributed throughout Italy or in southern regions only, depending on their climatic requirements. In some cases the newly introduced pest survives only in greenhouses in north Italy while it becomes an outdoor pest in south Italy (e.g. *Frankliniella occidentalis*). Other 28 species have succeded in acclimatizing and are distributed in the Italian territory even if the invasion of neighbouring territories seems a slow process; usually they have low population levels (e.g. *Diaspidiotus osborni*, *Opogona sacchari*, *Tekallis arundicolens*).

The acclimatized localized species are 28 in number. In some cases they are pests of crops or ornamentals whose cultivation is restricted to some disjunct areas so that it does in fact become difficult for them to spread. This is the case of *Epithrix hirtipennis* on tobacco and of some species (e.g. *Nipaecoccus nipae*, *Pseudaonidia paeoniae*, *Aulacaspis tubercolaris*, *Odonaspis greeni*) living outdoors on exotic plants in botanic gardens or nurseries. In other cases this situation is due to the fact that they were at first introduced to small islands where they could persist without the possibility of spreading (e.g. *Marchallina hellenica* on the island of Ischia, *Acizzia hollisi* on the island of Lampedusa).

The greenhouse species are 19 in number. Several of them are mentioned in rare records (e.g. *Gymnaspis aechmeae*, *Pseudococcus microcirculus*), others appear more common (e.g. *Hypogeococcus pungens*). Some greenhouse species have been introduced with the trade of bonsai trees, that has greatly increased in the last few years (e.g. *Tinocallis ulmiparvifoliae*, *Neophyllaphis podocarpi*).

Quarantine services are apparently unable to stop incidental introduction or to prevent the spread of new introduced species. In fact we know only two cases of the destruction of new foci, namely *Demyrsus meleoides* and *Limacoccus brasiliensis*.

With regard to the host plants, the majority (57) of the introduced species are pests of ornamentals, linked with the import and trade of ornamental plants: many are now widely distributed pests in gardens and parks (e.g. *Ceroplastes japonicus*, *Dasineura gleditchiae*, *Acizzia uncatoides*).

The insects of woody plants make up 25 species. Several of them (e.g. *Hyphantria cunea*, *Eupulvinaria hydrangeae* and the coniferous trees aphids) have spread throughout Italy causing severe damage. This group includes many North American species, some of which started colonizing Europe from a first Italian focus (e.g. *Corythucha ciliata*, *Parectopa robiniella*, *Metcalfa pruinosa*).

Table 1

List of the new introduced insect species in Italy

Status of insects (x)	Insect species	Family or Superfamily	Host plant	Place of the origin	First publication
	WOODY PLANTS				
aw	*Eopineus strobus* (Hartig)	Aphidoidea	Pinus	North America	Martelli, 1960
aw	*Corythucha ciliata* Say	Tingidae	Platanus	North America	Servadei, 1966
aw	*Gilletteella coweni* (Gillette)	Aphidoidea	Pseudotsuga	North America	Cantiani, 1968
aw	*Parectopa robiniella* Clemens	Gracillariidae	Robinia	North America	Vidano, 1970
aw	*Cedrobium laportei* Remaudiere	Aphidoidea	Cedrus	North Africa	Covassi, 1971
aw	*Phoracanta semipunctata* (F.)	Cerambicidae	Eucalyptus	Australia	Tassi, 1970
aw	*Cinara cedri* Mimeur	Aphidoidea	Cedrus	Morocco, Turkey	Covassi and Binazzi, 1974
aw	*Gonipterus scutellatus* Gyllenhal	Curculionidae	Eucalyptus	Australia	Arzone, 1976; Sampò, 1976
aw	*Eupulvinaria hydrangeae* (Steinweden)	Coccoidea	Tilia	?	Pellizzari Scaltriti, 1976
aw	*Appendiseta robiniae* (Gillette)	Aphidoidea	Robinia	North America	Micieli de Biase and Calambuca, 1979
aw	*Metcalfa pruinosa* Say	Flatidae	Polyphagous	North America	Zangheri and Donadini, 1980
aw	*Gilletteella cooley* (Gillette)	Aphidoidea	Pseudotsuga	North America	Covassi and Binazzi, 1981
al	*Pineus orientalis* (Dreyfus)	Aphidoidea	Pinus	Eastern Europe, Asia	Covassi and Binazzi, 1981
a	*Matsucoccus feytaudi* Ducasse	Coccoidea	Pinus	France	Arzone and Vidano, 1981
aw	*Hyphantria cunea* Drury	Arctidae	Polyphagous	North America	Ippolito and Parenzan, 1981
al	*Ctenarytaina eucalypti* Maskell	Psylloidea	Eucalyptus	Australia	Cavalcaselle, 1982
al	*Marchallina hellenica* Gennadius	Coccoidea	Pinus	Turkey, Greece	Tranfaglia and Tremblay, 1984
aw	*Tinocallis kahwaluokalani* (Kirkaldy)	Aphidoidea	Lagerstroemia	East Asia	Patti, 1984
a	*Diaspidiotus osborni* (Newell and Cockerell)	Coccoidea	Platanus	North America	Kozar et al., 1984
al	*Callidiellum rufipenne* Motschulsky	Cerambicidae	Juniperus	Japan	Campadelli and Sama, 1989
aw	*Phyllonorycter robiniellus* (Clemens)	Gracillariidae	Robinia	North America	Bolchi Serini and Trematerra, 1989
al	*Japananus hyalinus* (Osborn)	Cicadellidae	Acer	Asia	Arzone and Vidano, 1990
al	*Drepanaphis acerifoliae* (Thomas)	Aphidoidea	Acer	North America	Lozzia and Binaghi, 1992
al	*Monellia caryae* (Monell)	Aphidoidea	Juglans	North America	Lozzia and Binaghi, 1992
a	*Myzocallis walshii* (Monell)	Aphidoidea	Quercus	North America	Patti and Lozzia, 1994

Table 1

Status of insects (x)	Insect species	Family or Superfamily	Host plant	Place of the origin	First publication
	FRUIT TREES and GRAPE				
aw	*Stictocephala bisonia* Kopp and Yonke	Membracidae	Vitis	North America	Goidanich, 1946
aw	*Scaphoideus titanus* Ball	Cicadellidae	Vitis	North America	Vidano, 1964
al	*Pseudodendrothrips mori* (Niwa)	Thripidae	Morus	East Asia	Cappellozza and Miotto, 1975
a	*Pterochloroides persicae* (Cholodkovsky)	Aphidoidea	Prunus	Asia	Roberti, 1975
al	*Peliococcus serratus* (Ferris)	Coccoidea	Corylus	North America	Tranfaglia, 1976
al	*Pulvinaria innumerabilis* Rathvon	Coccoidea	Vitis	North America	Pellizzari Scaltriti, 1977
al	*Stomaphis mordvilkoi* Hille Ris Lambers	Aphidoidea	Juglans	Asia	Colombo, 1981
f	*Paramyelois transitella*	Phycitidae	Juglans	North America	Trematerra, 1988
a	*Epuraea luteola* Erichson	Nitidulidae	Prunus	Tropics	Audisio and Scaramozzino, 1989
aw	*Xylotrechus stebbingi* Gahan	Cerambicidae	Morus	Himalaya	Dioli and Viganó, 1990
a	*Rhagoletis completa* Cresson	Tephritidae	Juglans	North America	Duso, 1991
	ORNAMENTALS				
al	*Pseudaonidia paeoniae* Cockerell	Coccoidea	Camellia	Asia	Pegazzano, 1949
al	*Nipaecoccus nipae* (Maskell)	Coccoidea	Polyphagous	Central America?	Costantino, 1950
g	*Gymnaspis aechmeae* Newstead	Coccoidea	Bromeliaceae	Tropics	Balachowsky, 1953
f	*Aspidiotus destructor* (Signoret)	Coccoidea	Musa	Africa	Jannone and Binaghi, 1962
a	*Opogona sacchari* (Bojer)	Tineidae	Polyphagous	Africa	Jannone, 1966
a	*Melanaphis bambusae* (Fullaway)	Aphidoidea	Bambusa	East Asia	Hille Ris Lambers, 1966/67
a	*Tekallis arundicolens* (Clarcke)	Aphidoidea	Bambusa	East Asia	Hille Ris Lambers, 1966/67
aw	*Epichoristodes acerbella* (Walker)	Tortricidae	Polyphagous	South Africa	Zangheri and Cavalloro, 1971
aw	*Illinoia azaleae* (Mason)	Aphidoidea	Rhododendrum	North America	Süss, 1972/73
g	*Pentalonia nigronervosa* Coquerel	Aphidoidea	Polyphagous	Tropics	Süss, 1972/73
d	*Demyrsus meleoides* Pascoe	Curculionidae	Cycas	Australia	Covassi, 1974
a	*Dactylotrypes longicollis* (Wollaston)	Scolytidae	Palms	Canary Islands	Sampò and Olmi, 1975
a	*Ovaticoccus agavium* Douglas	Coccoidea	Agave	?	Tranfaglia, 1976
aw	*Liriomyza trifolii* Burgess	Agromyzidae	Polyphagous	North America	Arzone, 1979
f	*Dysmicoccus brevipes* (Cockerell)	Coccoidea	Ananas	America?	Tranfaglia, 1981

Table 1

Status of insects (x)	Insect species	Family or Superfamily	Host plant	Place of the origin	First publication
g	*Vryburgia rimariae* Tranfaglia	Coccoidea	Rimaria	South Africa	Tranfaglia, 1981
a	*Phenacoccus madeirensis* Green	Coccoidea	Polyphagous	Central America?	Tranfaglia, 1981
g	*Rhizoecus cacticans* (Hambleton)	Coccoidea	Cactaceae	?	Tranfaglia, 1981
a	*Delottococcus euphorbiae* (Ezz. and McConn.)	Coccoidea	Polyphagous	Africa	Tranfaglia, 1981
a	*Inglisia lounsburyi* (Cockerell)	Coccoidea	Pelargonium	Africa	Tranfaglia and Marotta, 1982
g	*Dysmicoccus neobrevipes* Bearsdley	Coccoidea	Musa	Central America	Tranfaglia, 1983
aw	*Acizzia uncatoides* (Ferris and Klyver)	Psylloidea	Acacia	Australia	Arzone and Vidano, 1983
aw	*Dasineura gleditchiae* (Osten Sacken)	Cecidomyiidae	Gleditschia	North America	Bolchi Serini and Volonté, 1984–85
aw	*Ceroplastes japonicus* Green	Coccoidea	Polyphagous	East Asia	Kozár et al., 1984
al	*Acizzia acaciaebaileyanae* (Frogatt)	Psylloidea	Acacia	Australia	Rapisarda, 1985
al	*Dialeurodes (?) formosensis* Takahashi	Aleyrodidae	Viburnum	East Asia	Iaccarino, 1985
g	*Hypogeococcus pungens* Granara de Will.	Coccoidea	Cactaceae	South America	Süss and Trematerra, 1986
a	*Graphocephala fennahi* Young	Cicadellidae	Rhododendrum	North America	Vidano et al., 1987
g	*Pinnaspis strachani* (Cooley)	Coccoidea	Poliphagous	Africa	Tranfaglia and Viggiani, 1988
a	*Aloephagus myersi* Essig	Aphidoidea	Aloe, Gasteria	Africa	Micieli De Biase, 1988
a	*Tekallis arundinariae* (Essig)	Aphidoidea	Bambusa	Asia	Patti and Tornatore, 1988
al	*Acizzia hollisi (*Burckhardt)	Psylloidea	Acacia	Australia	Conci and Tamanini, 1989
aw	*Frankliniella occidentalis* (Pergande)	Thripidae	Polyphagous	America	Arzone et al., 1989
g	*Chaetococcus bambusae* (Maskell)	Coccoidea	Bambusa	Asia	Porcelli, 1990
a	*Phenicococcus marlatti* (Cockerell)	Coccoidea	Phoenix	Africa	Marotta and Tranfaglia, 1990
al	*Odonaspis greeni* (Cockerell)	Coccoidea	Bambusa	Asia	Porcelli, 1990
al	*Aulacaspis tubercularis* Newstead	Coccoidea	Mangifera	Tropics	Porcelli, 1990
al	*Dialeurodes chittendeni* Laing	Aleyrodidae	Rhododendrum	Asia	Arzone and Vidano, 1990
g	*Neophyllaphis podocarpi* Takahashi	Aphidoidea	Podocarpus	East Asia	Limonta, 1990
aw	*Takecallis taiwanus* (Takahashi)	Aphidoidea	Phyllostachis	East Asia	Limonta, 1990
a	*Pealius azaleae* (Baker and Moles)	Aleyrodidae	Rhododendrum	Asia	Del Bene et al., 1991
g	*Pseudococcus microcirculus* McKenzie	Coccoidea	Orchidaceae	South America	Camporese and Pellizzari, 1991
g	*Eriococcus coccineus* Cockerell	Coccoidea	Cactaceae	Mexico	Marotta and Garonna, 1991
g	*Selenaspidus albus* McKenzie	Coccoidea	Euphorbia	South Africa	Marotta and Garonna, 1991

Table 1

Status of insects (x)	Insect species	Family or Superfamily	Host plant	Place of the origin	First publication
g	*Dysmicoccus mackenziei* Bearsdley	Coccoidea	Tillandsia	Mexico	Marotta, 1992
a	*Planococcus halli* Ezzat and McConell	Coccoidea	Nerium	?	Marotta, 1992
g	*Xyleborus affinis* Eichhof	Scolytidae	Dracaena	Central America	Carrai, 1992
g	*Rhizoecus americanus* (Hambleton)	Coccoidea	Saintpaulia	America	Russo and Mazzeo, 1992
al	*Pseudaulacaspis cockerelli* (Cooley)	Coccoidea	Polyphagous	East Asia	Russo and Mazzeo, 1992
d	*Limacoccus brasiliensis* (Hempel)	Coccoidea	Arecastrum	South America	Garonna and Carrai, 1993
al	*Protopulvinaria pyriformis* (Cockerell)	Coccoidea	Polyphagous	South America	Pellizzari Scaltriti, 1993
al	*Umbaspis regularis* (Newstead)	Coccoidea	Polyphagous	Africa	Pellizzari Scaltriti, 1993
g	*Rhizoecus saintpauliae* Williams	Coccoidea	Polyphagous	East Asia	Pellizzari and Pavan, 1994
g	*Rhizoecus dianthi* Green	Coccoidea	Polyphagous	?	Marotta, 1995
g	*Rhizoecus latus* (Hambleton)	Coccoidea	Polyphagous	South America	Marotta, 1995
g	*Tinocallis ulmiparvifoliae* Matsumura	Aphidoidea	Ulmus	East Asia	Lucchi and Pollini, 1995
	HORTICULTURAL and AGRONOMIC CROPS				
aw	*Leptinotarsa decemlineata* Say	Chrysomelidae	Solanum	North America	Della Beffa, 1945
al	*Bactericera tremblayi* (Wagner)	Psylloidea	Alliumcepa	ex USSR	Tremblay, 1958
a	*Aspidomorpha cincta* F.	Chrysomelidae	Beta vulgaris	Africa	Zangheri, 1960
a	*Allocontarinia sorghicola* (Coquillet)	Cecidomyiidae	Sorghum	Africa	Mariani and Beccari, 1964
a	*Choristoneura lafauryana* Ragonot	Tortricidae	Polyphagous	Europe	Ivancich Gambaro, 1964
a	*Nearctaphis bakeri* (Cowen)	Aphidoidea	Trifolium	North America	Patti and Ricci, 1982
al	*Epithrix hirtipennis* (Melsheimer)	Chrysomelidae	Nicotiana tabacum	America	Sannino et al., 1984
al	*Brachycorinella asparagi* Mordvilko	Aphidoidae	Asparagus	Eastern Europe	Coceano, 1989
al	*Glischrochilus quadrisignatus* (Say)	Nitidulidae	Polyphagous	North America	Audisio, 1990
al	*Cicadulina bipunctata* (Melichar)	Cicadellidae	Gramineae	North Africa	Arzone and Vidano, 1990
aw	*Liriomyza huidobrensis* (Blanchard)	Agromyzidae	Polyphagous	America	Süss, 1991
a	*Antonina graminis* (Maskell)	Coccoidea	Gramineae	?	Marotta, 1992

Table 1

Status of insects (x)	Insect species	Family or Superfamily	Host plant	Place of the origin	First publication
	CITRUS				
a	*Contarinia citri* Barnes	Cecidomyiidae	Citrus	Africa?	Genduso, 1963–64
aw	*Aphis spyraecola* Patch	Aphidoidea	Citrus	?	Barbagallo, 1965
aw	*Dialeurodes citri* (Ashmead)	Aleyrodidae	Citrus	Asia	Genduso, 1967–69
aw	*Aleurothrixus floccosus* Maskell	Aleyrodidae	Citrus	Central-South America	Onillon, 1969
aw	*Pseudococcus calceolariae* (Maskell)	Coccoidea	Citrus	Australia	Viggiani, 1970
aw	*Coccus pseudomagnoliarum* (Kuwana)	Coccoidea	Citrus	Japan	Barbagallo, 1974, Tranfaglia, 1974
a	*Bemisia afer* (Priesner and Hosny)	Aleyrodidae	Citrus	Africa	Mineo and Viggiani, 1975
al	*Unaspis yanonensis* (Kuwana)	Coccoidea	Citrus	East Asia	Arzone and Alma, 1987
aw	*Parabemisia myricae* (Kuwana)	Aleyrodidae	Citrus	East Asia	Rapisarda et al., 1990
a	*Aonidiella citrina* (Coquillet)	Coccoidea	Citrus	Asia	Longo et al., 1994
aw	*Phyllocnistis citrella* Stainton	Gracillaridae	Citrus	Asia	Ortu et al., 1995

x Abbreviations:

a: acclimatized species
aw: acclimatized, widespread species
al: acclimatized localized species
g: greenhouse species
f: species recorded on imported fruits
d: destroyed focus

We consider it that interesting to present the case histories of these species, following, step by step, their invasion of the European countries.

Corythucha ciliata, a pest of the plane tree, was first noticed in north-east Italy in 1966 (Servadei, 1966) and in a few years spread throughout Italy, reaching Sardinia in 1979 and Sicily in 1984. It expanded towards other countries and invaded Slovenia and Croatia in 1970, France in 1975, Hungary in 1976, Spain in 1981 and Corsica in 1983 (De Battisti et al., 1984–85). It was noticed in Austria in 1982 (Höpoltseder, 1984), in Switzerland in 1983 (Wicki, 1983), in Germany in 1987 (Klausnitzer, 1988), in Bulgaria in 1987 (Iusifov, 1990).

Parectopa robiniella, monophagous on the black locust, a North American tree, was detected in Italy in 1970 (Vidano, 1970) and is now widespread throughout the Italian peninsula. It reached Switzerland in 1971, Slovenia in 1982, Hungary in 1983, and western France in 1987 (Bolchi Serini, 1990). From Hungary it penetrated southern Slovakia in 1989 (Kulfan, 1989). In Austria it was first recorded in 1992 (Hümer, 1992).

Metcalfa pruinosa, a polyphagous species, was detected in north eastern Italy in 1979 (Zangheri and Donadini, 1980) and, to date, it has colonized north Italy, central Italy and Sardinia and has become a pest of trees, ornamentals, crops and wild plants. It was recorded in France in 1986, in Switzerland and Slovenia in 1993 (Jermini et al., 1995).

The insects of fruit trees and grapes are 11 species in number, of which only four, namely *Stictocephala bisonia*, *Scaphoideus titanus*, *Pulvinaria innumerabilis*, *Rhagoletis completa* have attained a pest status. The other persist locally, generally at low population levels.

The insects of horticultural and agronomic crops include 12 species, that appear to be localized pests, with the exception of the well-known *Leptinotarsa decemlineata* and of *Liriomyza huidobrensis* that is still spreading in Italy.

The insects of Citrus number 11 species, distributed in the Liguria region (north Italy), southern Italy, Sardinia and Sicily. The Citrus cultivation is characterized by continuous incidental introductions of pests that quickly spread throughout the Citrus cultivation areas of the Mediterranean basin. The last incidental introduction of a Citrus pest (*Phyllocnistis citrella*) occurred in 1995.

Over a period of fifty years, 115 exotic species have arrived in Italy, meaning more than 2 species/year. If we consider the first twenty years (1945–1964), we notice that incidental introductions were a rare event (12 records, i.e. 0.6 species/year). Starting from the decade 1965–1974 we observe a remarkable increase in the number of introduced species: in fact they pass from 6 in the previous decade to 18. During the last twenty years (1975–1995) we notice that 84 exotic species were recorded, that is an average of 4 species/year and 73% of the species introduced in fifty years. This is undoubtedly due to a great increase in commercial exchange and traffic but also, in part, to a deeper study of our fauna composition that has led to the discovery of exotic species in our territory which have probably been present for several years.

With regard to the continent or region of origin, 42 (36%) of the introduced

species are from America, 29 (25%) from Asia, 19 (17%) from Africa, 8 (7%) from Australia and 13 are of unknown origin or cosmopolitan.

The orders accounting for most of the introduction are Homoptera (87 species, 76% of the introduced species) followed by Coleoptera (12 species, 10%), Lepidoptera (8 species, 7%), Diptera (6 species, 5%) and Tysanoptera (2 species, 2%). The Coccoidea (Homoptera) are the most numerous (43 species, that means 37% of the introduced species). They are followed by Aphidoidea with 25 species (22%).

This list is intended as a conservative list: it is possible that published records of newly introduced species have escaped our check; furthermore, we are acquainted with the incidental introduction of two other exotic insects to Italy in 1996: *Vinsonia stellifera* (Westwood) (Coccoidea) on ornamentals (Paparatti, in press) and *Phyllocnistis vitegenella* Clemens (Gracillariidae) on *Vitis vinifera* (Girolami, personal communication). For these reasons this list will certainly increase in a short time.

While the incidental introduction of exotic pests appears a common event, the incidental introduction of beneficial insects is less frequent. It is encouraging to report that recently *Trichopoda pennipes* F. (Diptera, Tachinidae), a North American parasitoid of *Nezara viridula* (L.), has arrived in Italy, has established and is currently an effective antagonist of the southern stink bug (Colazza et al., 1996).

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Effects of Climatic Variations on Long-term Fluctuation Patterns of Ground Beetles (Coleoptera, Carabidae) Collected by Light Trapping in Hungary

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The authors analyse long-term data series of three ground beetle species in order to detect the possible effects of annual climatic changes on the flight activity fluctuation patterns. The carabid species were the hygrophilous *Clivina fossor*, the xerophilous *Harpalus griseus* and *Harpalus froelichii*.

The species were collected by the Hungarian light trap network during the period of 1982–1992. The mean yearly total number of captured individuals was implemented for analyses. The characteristics of seasonal and long-term fluctuation patterns were investigated by the methods of time series analysis. For the analysis of effects of climate fluctuation, aridity indices, seasonal amounts of precipitation and mean summer temperature were taken into accounts.

By the increasing drought the flight activity of *C. fossor* significantly increased because of the adaptive emigration from drying wet habitats. Contrary, the flight response was declining when the winter and summer amount of precipitation and soil moisture increased. The *H. griseus* reacted inversly to the climatic variation as *C. fossor*, while *H. froelichii* responded less consistently to aridity changes. The authors draw the conclusion that two *Harpalus* species' humidity preference range is rather mesophilous than xerophilous in Hungary.

Ground beetles as generalist polyphagous predators play important role in agricultural and forest habitats, as they are natural enemies of many insects pests (Lövei and Sunderland, 1996). Carabids have very diverse types of life history, environmental requirements and they are generally sensitive to the different biotic and abiotic factors. The numerous species are relatively well known ecologically and taxonomically so they are very applicable for bioindication of environmental changes (Turin et al., 1991; Luff et al., 1992). Besides source of food, the population dynamics of ground beetles are considerably influenced by other factors, like habitat structure and fragmentation, vegetation cover type, agricultural intensification, air pollution, soil moisture, and the microclimate of the habitat. For this reason they are potential indicators of land use-change which can be, according to several authors, responsible for the extinction of many rare species and it could have caused the generally detected area contraction and decrease of abundance of ground beetles in northern and western regions of Europe (Andersen, 1987; Turin and Den Boer, 1988; Desender and Turin, 1989; Den Boer and Van Dijk, 1994; Desender et al., 1994; Luff and Woiwod, 1995).

Another environmental factor that has a wide-range effect is the climate. The regional and/or global level of climatic change and fluctuation can considerably influence

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carabids. In order to analyse how climatic change affects insects, we need data-series collected in uniform way, from large-scale and long-term trapping network (Harrington and Stork, 1995). The most general collecting method of ground beetles is pitfall trapping, which is used to survey the pattern of active individuals on the surface. A lot of analyses were published regarding the long-term carabid time-series produced by this method, which were done in northern and western areas of Europe. Another important quantitative collecting method is the light trapping in order to create patterns of adult carabids. One reason that there are only a few articles published about it is that this method is suitable to collect just the macropterous individuals of species, furthermore, mass night flight needs appropriate temperature and this is successful only in the warmer places of Europe. Matalin (1996), applying several collecting methods, found that light trapping is properly applicable for sampling of ground beetles. Meanwhile the light trapping collects mainly predatory species, contrary of pitfall trapping. There were several authors who proved the usefulness of light trapping in practice, too (e.g. Honěk and Pulpán, 1983; Kádár and Szentkirályi, 1984; Kádár and Lövei, 1987; Basedow et al., 1990; Matalin, 1994). Still, the long-term analysis of carabid species with this method was reported only by Honěk and Pulpán (1983). The analyses up to this point cast light to the fact that predominantly newly hatched young individuals of full-winged adults fly in the prereproductive period (Meijer, 1974; Matalin, 1994). This dispersion by flight of carabids is often triggered by the disadvantageous effects of the unstable habitat (Den Boer et al., 1980). As a rule of thumb, it is assumed that the patterns of long-term time series of light trapping are able to indicate the effects of climatic fluctuations: the catch reflects the change of the size of the population, because the number of individuals of young adults is proportional to the abundance, and the measure of flight is greatly influenced by the climatic change.

Despite of the great number of long-term time series produced by pitfall trapping, the potential climatic effects on carabids were discussed by only a few articles until now (Horion, 1938; Lindroth, 1972; Hengeveld, 1985; Den Boer and Van Dijk, 1994; Desender et al., 1994; Müller-Motzfeld, 1995), and among them only Hengeveld (1985) and Desender et al. (1994) applied statistical analyses in order to indicate these effects.

These facts proved that it is necessary to examine the long-term time series produced by the determination of the material of ground species collected by the Hungarian agricultural light trap network, that works constantly since 1981, in order to indicate the effects of annual climatic fluctuations. These analyses are actual, because from the 1980s there was serious drought in several consecutive years, which had a considerable effect on the environment, too. In order to make the analyses, we selected carabid species such, that one of them prefers rather moist habitats (hygrophilous species) and the other two are known in literature as xerophilous species. The long-term data series (1982–1992) of the selected carabids were examined according to the following points: (1) description of the seasonality of each species, indication of the measure of interspecific overlap of seasonal flights, (2) detection of possible periodic fluctuations in long-term population patterns, description of the trends of changes, (3) analysis of measure of synchrony between long-term fluctuations patterns, (4) analysis of the effects of yearly fluctuations of cli-

matic variables on fluctuation patterns of ground beetle species. Our hypothesis was that the number of individuals captured and, consequently, the size of the population of the hygrophilous carabid species will decline in contrast with the xerophilous species, which is expected to increase in years characterised by droughty climate (drier and warmer seasons) and vice versa.

Materials and Methods

Collecting method and sampling sites

The Hungarian network consists of so-called Jermy-type light traps. Traps are without baffles, the light source is 2 m above ground surface, normal 100 W bulb with white light. Traps operated from 1st of March to 30th of October in each year.

The selection of the traps depends on the number of individuals collected from the ground beetle species. The data of the sites having a level of abundance too low to detect climatic effects, were excluded from further analysis. According to these criteria, the traps were set near the following towns or villages (the numbers within brackets are longitudinal geographical coordinates of light trap stations): Balassagyarmat (48° 04′, 19° 17′), Csopak (46° 58′, 17° 55′), Eger (47° 53′, 20° 23′), Hódmezővásárhely (46° 25′, 20° 20′), Kaposszerdahely (46° 22′, 17° 50′), Kenderes (47° 15′, 20° 41′), Kunszentmiklós (47° 02′, 19° 08′), Mikepércs (47° 22′, 21° 36′), Nadap (47° 16′, 18° 37′), Nyársapát (47° 10′, 19° 49′), Nyékládháza (48° 00′, 20° 51′), Pacsa (46° 44′, 17° 01′), Pápa (47° 19′, 17° 29′), Pécs (46° 03′, 18° 09′), Tanakajd (47° 16′, 16° 38′). The habitats of the surroundings of the traps were various, including orchards, vineyards, arable fields and parks.

The selected ground beetle species and their data

The requirement for the microclimate, especially the moisture of the habitat had a key role in the choice of the species, because climatic effects, or more specifically the possible influence of recent droughty years in Hungary, were to indicated in the first place. Based upon the Hungarian investigations and literature (e.g. Turin et al., 1977, 1991; Den Boer et al., 1980; Desender, 1983; Desender and Turin, 1989), the selected species, *Clivina fossor* (Linnaeus), *Harpalus griseus* (Panzer) and *Harpalus froelichii* (Sturm), can be characterised as follows. *C. fossor* is known as mesohygrophil-hygrophilous beetle, based upon its habitat preferences, that lives in wet and moist habitats (e.g. riparian places) but very eurytopic tolerant species to cultivation. It is mainly a predator carabid. *H. griseus* is a wide tolerant, eurytop and xerophilous species, living in xerotherm open habitats covered with grassy or weedy vegetation, or in arable fields. Although in northern areas of Europe it is a more or less stenotopic and rare species, which is seriously threatened, it can be frequently found anywhere in Hungary. *H. griseus* in known as an omnivorous carabid. In northern regions of Europe, *H. froelichii*

is a xerophilous, very stenotopic, rare seriously endangered species. It prefers dry and mesophil habitats with loose structure of soil (mainly sandy areas, forest edges, grasslands). Hungarian data on this species predominantly come from light traps. *H. froelichii* is also omnivorous species.

The most important point of view of the selection of carabid species was that their expected reaction to climatic changes had to be different, so species known as hygrophilous and xerophilous were analysed. Another point of view was to examine species that could be caught by light traps in greater number and the samples of traps had to given on overall picture of the population. This means that adults should be predominantly flying macropterous (full winged) individuals. The adult individuals of the two *Harpalus* species are totally and *C. fossor* are predominantly macropterous, with high flight capability. This is proved by catches of more significant total captures (*C. fossor*: 1938, *H. froelichii*: 8725 and *H. griseus*: 13871 individuals). Further requirement was in the data series selection that they must have been continuous and uniform. After determining the materials of ground beetles, we got yearly data series from 1982 to 1992 for analysis. To characterise the abundance level of ground beetles, we calculated the values of variables used in pattern-analyses from daily captures. For the seasonal flight-patterns we calculated the number of individuals caught per 10-day intervals. The three ground beetle species have high dispersal capability by flight, consequently, the catches are also in proportion with the number of individuals forced to emigrate by unfavourable environmental effects as yearly climatic changes. Since it is not known whether a given light trap catch consists of rather emigrating or immigrating individuals (or both of them), we used the mean of local data in the analyses to detect more successfully the general effects of climatic fluctuations. The usage of mean annual captures was supported by preliminary regression analysis, whereby the trends of population changes were identical in majority of the localities.

Climatic variables

The same variables, that have been used in former works to describe droughty periods, are presented here (Szentkirályi et al., 1995). The climatic characteristics were figured out from the monthly climate elements of the corresponding period and then the country-wide average for each year was calculated. More indices were used that had worked well in climatological practice before (Faragó et al., 1988). So we applied Selyaninov's hydrothermal coefficient (SHT = $P/\sum T/10$, where $T > 10$ °C, where P and T are monthly amount of precipitation and monthly mean temperature, respectively) and Pálfai's aridity index ($PAI = (T_{IV-VIII}/P_{X-VIII})\ k_T\ k_P\ k_S$, where T = mean temperature from April to August; P = sum of precipitation from October to August; k_T, k_P, k_S = corrections relating to number of heat days, length of period without precipitation, subsidence of water-level, respectively) (Pálfai, 1991) from those aridity indices that take into consideration the connection between the amount of precipitation and temperature. We received the temperature and precipitation time-series needed for the calculation of the indices from the stations of National Meteorological Service. Based upon the change in the values of

the indices, we managed to find which years had drought or water-deficit. In order to describe the annual intensity of drought, the measure of drought damage in forest was also taken into consideration (Szentkirályi et al., 1995).

In the analysis, some additional climatic variables were used: mean annual relative soil moisture content; mean summer temperature; mean winter precipitation (Oct.–Feb.); mean spring precipitation (March–May); mean summer precipitation (June–September).

Applied statistical analyses

To indicate any periodicity in fluctuation-patterns, we applied autocorrelation function method of time-series analysis and to show synchrony between patterns, we worked with serial cross-correlation (cross-correlation function, CCF) technics of time-series analysis, too (Szentkirályi, 1997). We considered the synchrony between fluctuation-patterns (a) high if there was significantly ($p < 5\%$) strong ($r > 0.6$) or medium ($0.3 < r < 0.6$) positive correlation for no larger lag than one time unit (1 decade or 1 year), while it was (b) weak or asynchronous if in more than two time units there was significant correlation. The aim of this study is to indicate the expected effects of climatic annual changes and to detect the increasing or decreasing population trends of ground beetles reacting to these effects. That is why linear regression analysis was satisfactory to investigate the correlation between data series.

Results

Seasonal patterns of flight activity

Figure 1 shows the seasonal percentage distribution of flight activity of the 3 carabid species calculated from the results of 15 light trap stations during 11 years.

The seasonal distribution of *C. fossor* is definitely bimodal. The first activity peak is in the last decade of May and the second one is in middle of July. The total length of the flight lasted from middle of May until first days of September, while the period of mass flight started in the second half of May and ended in July. The seasonal distribution of flight of the two other *Harpalus* species was unimodal. The mean flight of *H. froelichii* was from the end of June until the beginning of September. The mass flight came between the last decades of July and August. A definite peak of flight cannot be determined, because the number of captures, during the period of mass flight lasting one month, was evenly high.The flying activity of *H. griseus* was between the beginning of July and September. The mass flight took place in August indicated by an outstanding peak in the first decade of the month. In this short period of time, about 50% of the whole annual amount of captures was collected on average.

Comparing the 3 carabid species, the seasonal flight pattern shows that the flight of *C. fossor* is different from that of the two other species. On reason is the bimodality in

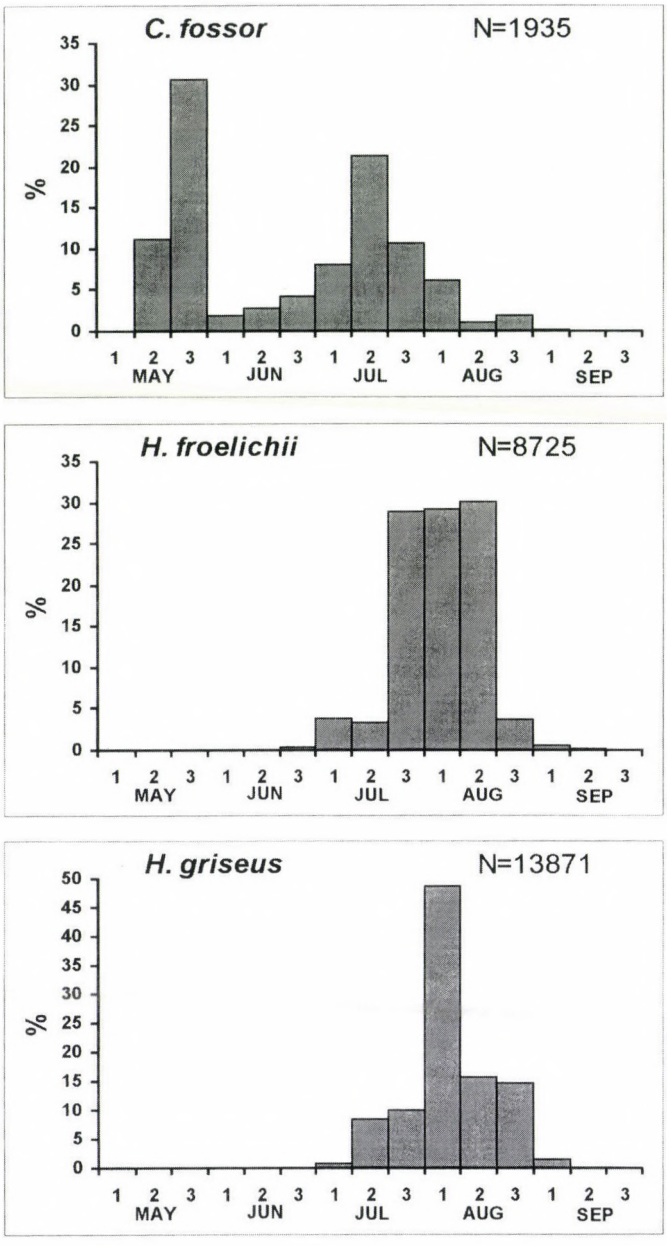

Fig. 1. Mean seasonal flight patterns of ground beetles from light trapping in agricultural lands between 1982 and 1992. (N: total number of individuals captured by all traps; y – axis: percentage of mean number of individuals caught in ten-day intervals)

flight, the other one is that its summer activity peak is two decades ahead of the other two *Harpalus* species. This was supported by the analysis of synchrony of flight patterns with use of cross-correlation function (CCF). Figure 2 A–C shows the CCF results relating to pairs of species in function of decadal lags. As it could have been expected according to the Fig. 1, the flight seasonality of the adults of the two *Harpalus* species indicates close overlap i.e. they are synchronised well (at 0 and ± 1 decadal lag, $r = 0.74$, $P < 5\%$). On the other hand, comparing *C. fossor* with the other two carabid species, the flight activity was not synchronised (at small lags there was no significant positive correlation).

Year-to-year long-term patterns of flight activity

Figure 3 presents the long-term yearly fluctuation patterns. The graphs on the left side are the mean of the yearly total captures and the graphs on the right side illustrate the annual proportion (%) of population (capture) peaks. The low mean yearly captures of *C. fossor* (below 40 individuals) suggest decreased field density contrary to the higher rates of the other *Harpalus* species. There was maximum in time series of mean captures of *C. fossor* in 1983, 1985, 1987 and in 1990, although only in 1983 and in 1987 was there extended regional level abundance growth relative to the proportion of yearly population peaks.

Patterns of *C. fossor* were inverse with relative to patterns of *H. griseus*: at points where the former carabid species had maximum, the other had minimum in the same years and vice versa. The mean number of individuals was maximum in 1982, 1984, 1989 and also in 1986 and 1992 according to the population peak pattern. *H. froelichii* was closer to *H. griseus* especially with regard to population peak pattern. At the same time in case of mean captures, *H. griseus* showed an eventual fall between 1982 and 1987, meanwhile *H. froelichii* was at a constantly low mean abudance level except for only the second half of the analysed period, at that time there was a considerable growth.

We could not detect any significant periodicity with either species in long-term patterns using ACF method of time-series analysis, so there is no further description about it. The results of analysis of interspecific synchrony between patterns are illustrated by Fig. 4 (A–C: CCF between annual mean number of individuals; D–F: CCF between population peak patterns). The results support the differences and partial similarities of patterns in Fig. 3. The inverse formation of patterns of *H. griseus* and *C. fossor* can be also proved by mean abundance and the coefficient values of significant negative correlation at 0 lag in case of population peak (C: $r = -0.78$, F: $r = -0.58$, $P = 5\%$). The patterns of the two *Harpalus* species were similar only partially. The positive value of CCF (see E: $r = 0.52$, NS) at 0 lag refers to the weak synchrony between the population peak patterns of *H. griseus* and *H. froelichii*, on the other hand the patterns of mean captures fluctuate significantly inversely (see B: at 0 lag $r = -0.46$, $P = 5\%$). At the same time the patterns of *H. froelichii* show a low tendency to overlap the patterns of *C. fossor* (A: see weak, not significant CCF values around 0 lag) and to fluctuate inversely (D: at 0 lag, $r = -0.35$, NS).

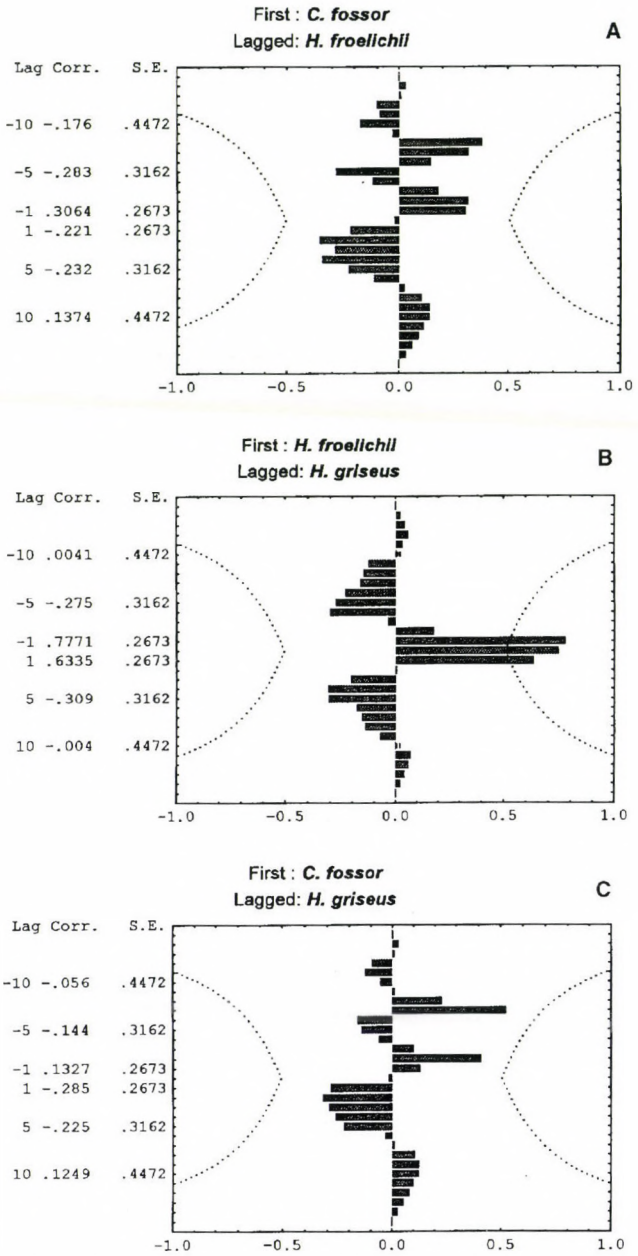

Fig. 2. Measure of synchrony between the mean seasonal flight patterns (see in Fig. 1) of ground beetles using cross-correlation function (unit of lag: ten-day interval; correlation coefficient, r: $-1 < r < 1$; dotted lines: confidence intervals at 95%)

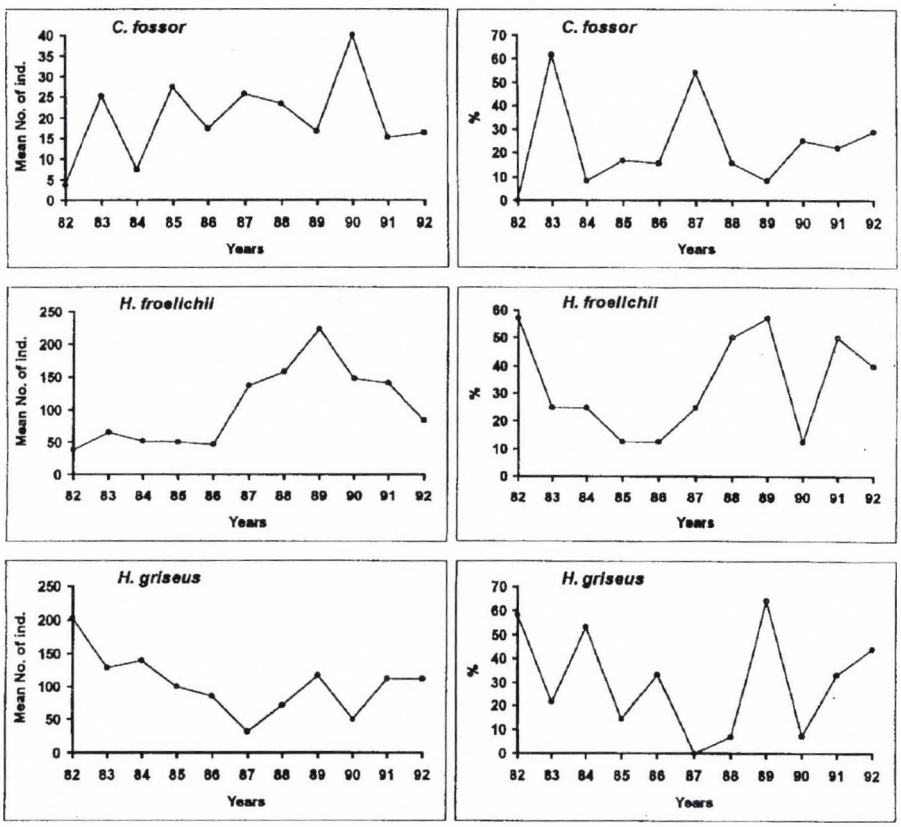

Fig. 3. Long-term yearly fluctuation patterns of carabids (y – axis: on the left: mean yearly number of individuals caught per traps; on the right: percentage of light trap sites where capture peaks were detected)

Effects of climatic variations

In order to indicate the tendencies of potential effects of climatic variables, Figs 5–6 summarise the results of applied regression analysis. On these figures only those regressions were displayed, that indicated some kind of tendency and the value of correlation coefficient (r) approached 0.3 or exceeded it. In majority of these cases, r was not significant, but the results are still capable to show increasing or decreasing tendencies of the expected effects characterising certain carabid species. Figure 5 illustrates the results calculated from different aridity and drought indices, using regression analysis. An obvious tendency can be observed in case of *C. fossor*: the larger the measure of increase in drought (PAI, DRD, SHT) and decrease of relative moisture of soil (RSM), the more the level of yearly mean captures increased (in case of SHT the lower values meant more

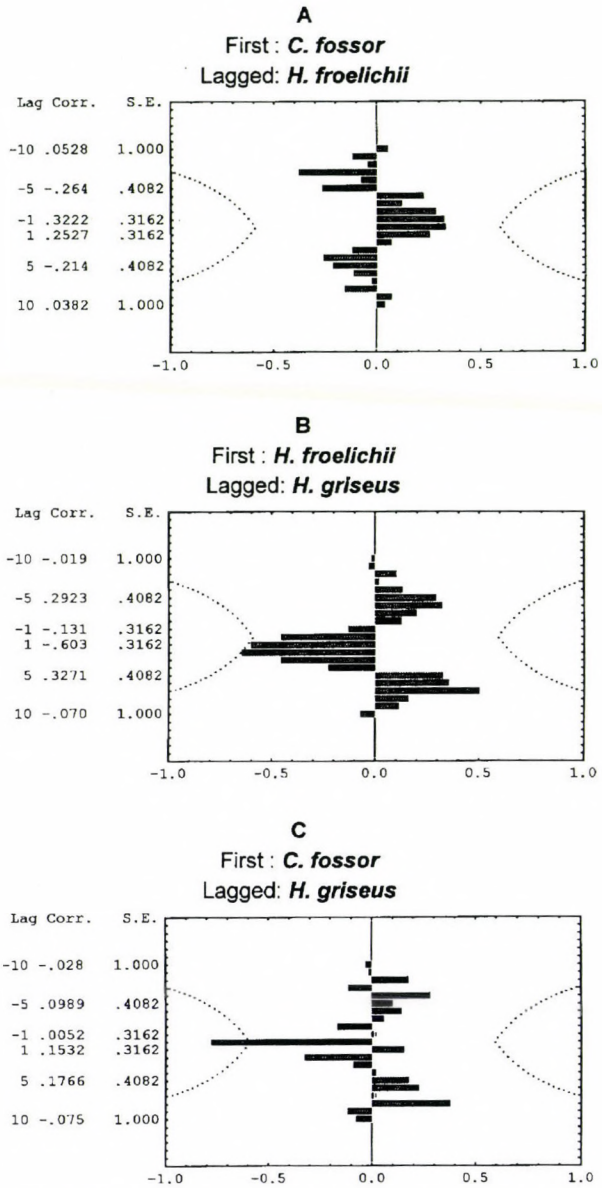

Fig. 4. Measure of synchrony level between long-term fluctuation patterns of ground beetle species using cross-correlation function (CCF). (A–C: CCF calculated between patterns of mean yearly number of individuals caught per trap)

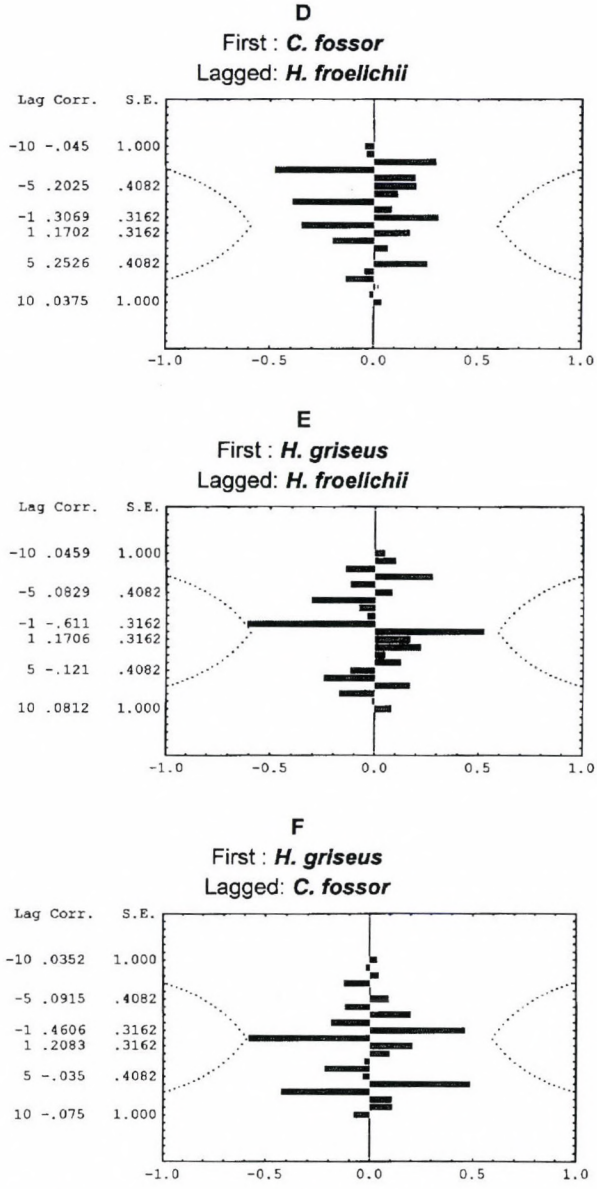

Fig. 4. Measure of synchrony level between long-term fluctuation patterns of ground beetle species using cross-correlation function (CCF). D–F: CCF calculated between patterns of percentage of light trap sites where capture peaks were detected)

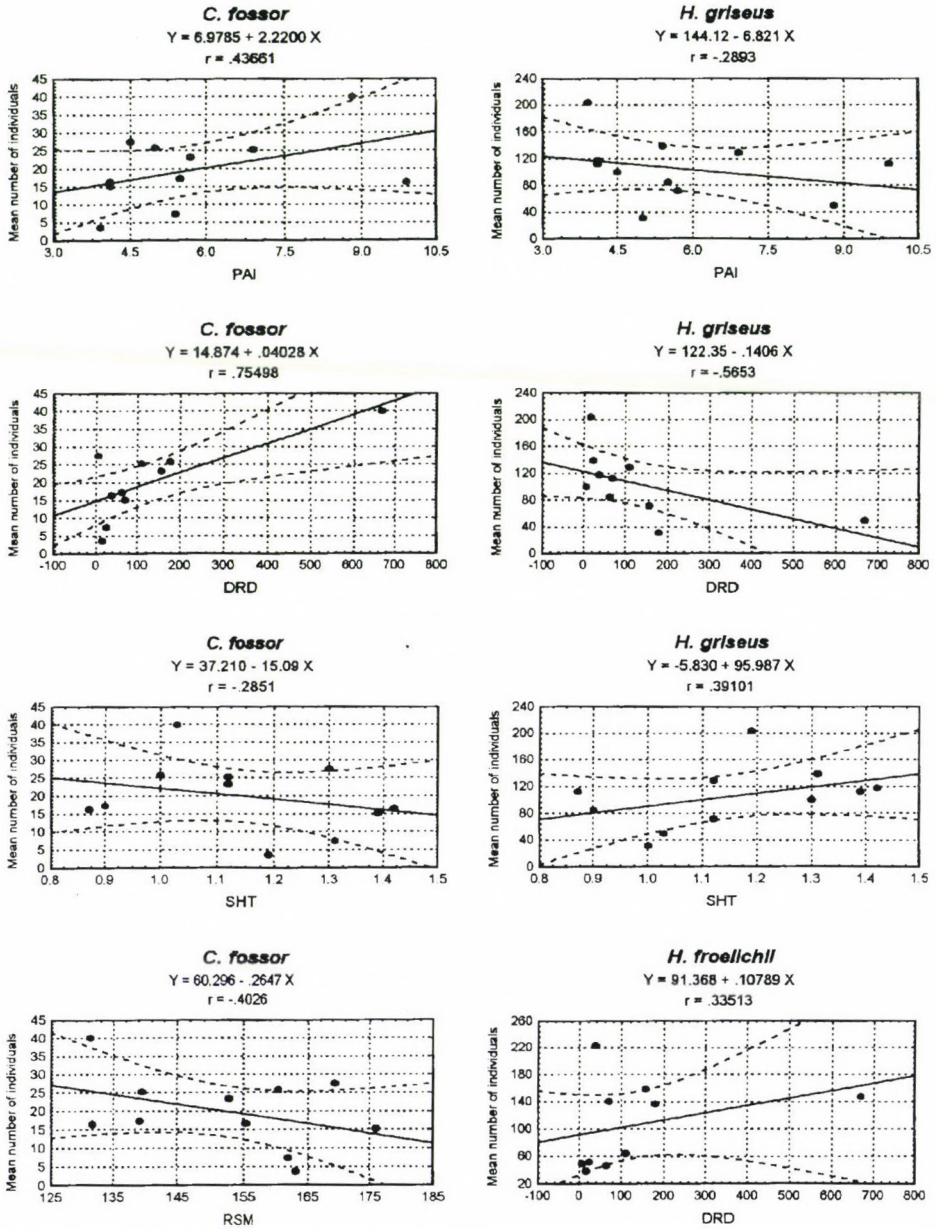

Fig. 5. Regression analysis for effects of climatic variables on yearly population fluctuations of ground beetles. (y – axis: mean yearly total number of individuals caught by light traps; x – axis: PAI = Pálfai's aridity index, SHT = Selyaninov's hydrothermal coefficient, DRD = degree of drought damage in forests per year, RSM = relative soil moisture content)

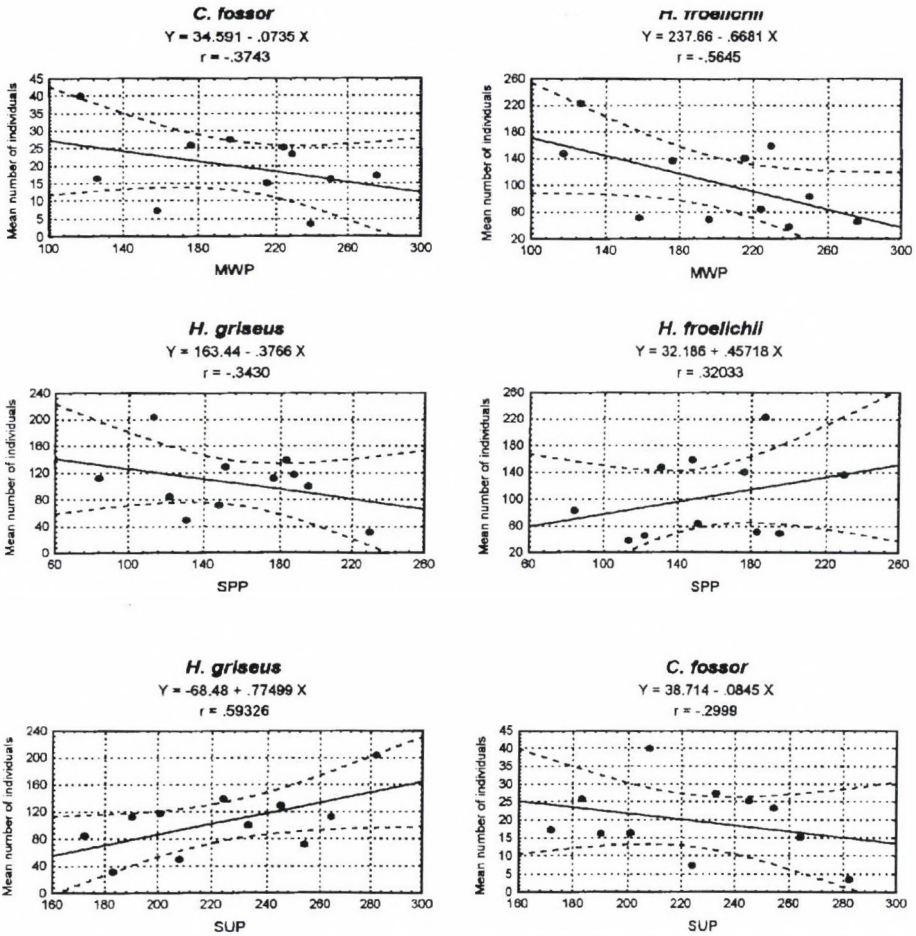

Fig. 6. Regression analysis for effects of mean amount of precipitation on population fluctuation patterns of ground beetle species. (y – axis: MWP = mean winter precipitation; SPP = mean spring precipitation; SUP = mean summer precipitation)

serious drought). *H. griseus* showed opposing tendencies, because the increase of the measure of drought (PAI, DRD, SHT) had a decreasing effect on the annual mean captures (in case of RSM, $r = 0.20$).

H. froelichii indicated no consistent tendency like the two other species. With the increase of annual drought damage (DRD) the mean capture grew (see Fig. 5) too, but in case of SHT years with higher aridity levels were associated with lower mean abundance levels ($r = 0.27$). According to regression analysis (PAI: $r = -0.05$, RSM: $r = 0.03$, NS), there was no reference correlation regarding the PAI and RSM indices, because of scat-

tered data. Comparing it with the two other carabid species it is likely, that *H. froelichii* has intermediate tendencies contrary to the effects of yearly drought, i.e. population dynamics could be dependent, on a low degree, on aridity.

The potential effects of more important climatic elements were analysed as mean summer temperature (MST), mean winter (MWP), mean spring (SPP) and mean summer (SUP) amount of precipitation. In spite of expectations, mean summer temperature had no detectable effects on the captures of any of the carabid species. Regression analysis tells us that for *C. fossor*, *H. griseus*, *H. froelichii*, values of r were 0.08, – 0.003, 0.05, respectively.

Some selected results of the regression analysis regarding the effects of different seasonal amounts of precipitation are shown in Fig. 6. The yearly captures of *C. fossor* decreased with the increase of mean autumn-winter (MWP) and summer amount of precipitation (SUP: $r = -0.3$, NS), while spring precipitation did not have considerable effect (SPP: $r = 0.16$, NS). Contrary to *C. fossor*, *H. griseus* showed inverse tendencies again with respect to precipitation: when the mean amount of precipitation increased, the mean yearly level of captures in case of MWP ($r = 0.27$, NS) and SUP increased and in case of SPP decreased. Mean total precipitation affected changes in number of individuals of *H. froelichii* in similar direction as *C. fossor*: mean captures (see Fig. 6) decreased in case of MWP and increased in case of SPP, but in case of SUP there was no considerable effect ($r = -0.14$, NS).

Captures of a succeeding year were expected to be influenced by values of some more important climatic variables from the previous year and these effects were examined by regression analysis, too. The selected variables were PAI, DRD, SHT and SUP. These were the general tendencies for each of the carabid species: as the summer amount of precipitation increased, the annual mean captures of *C. fossor* in the next year decreased (PAI: $r = -0.31$, DRD: $r = -0.22$, SHT: $r = 0.28$, SUP: $r = -0.48$, NS). For captures of *H. griseus*, only the summer precipitation of the previous year had any significant increasing effect (SUP: $r = 0.78$, P = 5%), and in case of drought indices there was not any correlation (PAI: $r = 0.03$, DRD: $r = 0.057$, SHT: $r = 0.07$). The effects of climatic variables from previous year of *H. froelichii* were similar to the effects indicated in case of mean number of individuals of *C. fossor*, except for DRD (PAI: $r = -0.24$, DRD: $r = 0.38$, SHT: $r = 0.35$, SUP: $r = -0.43$, NS).

Discussion

Seasonality of adult ground beetles

C. fossor is an eurytopic carabid species in northern area of Europe which may frequently occurs with greater abundance even in habitats under agricultural cultivation (Turin and den Boer, 1988; Turin et al., 1991). It is also known about the species that it has high flight dispersal capability. In spite of this fact, at least according to our knowledge, this is the first study that gives the detailed seasonal flight pattern of this species

based on light trapping. A bimodal distribution was found from the superponed data of wide-range light trapping of eleven years, which can be characterised by an end-of-May and a mid-July flight peak. It is likely, that the seasonality of ground surface activity of the species in Netherlands, given by Turin et al. (1977), is similar to the indicated flight pattern. The main activity period was also in May and in the early days of June, but the skewness of distribution suggests a significant July–August activity in accordance with our results of summer flight period. However, in Belgium, Desender (1983) found only the first activity peak from May to the beginning of June, using pitfall trapping. The seasonal flight pattern of *C. fossor* was considerably different from the other two *Harpalus* species not only because of the bimodality, but also because of the earlier allocation (middle of July) of summer flight maximum.

According to the analysis, the seasonality of *H. griseus* and *H. froelichii* was in significant synchrony. One reason for this is the activity period of the same length from the beginning of July to early September, the other one is the total overlap of mass flight lasting from the last decade of July to the middle of August. The results regarding the flight of these two *Harpalus* species correspond well to the seasonal dynamics of earlier light trapping in maize fields and apple orchards (Kádár and Szentkirályi, 1984; Kádár and Lövei, 1987). The distribution pattern of seasonal flight of *H. griseus* given by Honěk and Pulpán (1983) is in harmony with Hungarian data. This shows that the flight in the area of Prague, also surveyed by light trapping, started at the end of July and finished at the beginning of September, the mass flight was in August and the flight peak was in the first half of August. In Netherlands, the ground surface activity peak of *H. griseus* was in August, too (Turin et al., 1977). The seasonal distribution of ground surface activity of *H. froelichii* was also published by Turin et al. (1977) and this had two peaks: the first peak was in May and the greater one was in August. The Hungarian pitfall trapping analyses also show that this species is partially spring breeder and partially autumn breeder and this is also proved by the fact that in spring, a greater proportion of individuals had ripened eggs, while during the second activity peak from August to September this ratio was smaller (Kádár et al., unpublished).

Long-term fluctuations of carabids and the effects of climatic variations

The long-term (11 years) time-series produced by 15 light traps indicate divergent patterns for the 3 ground beetle species. There is no definite long-term increasing or decreasing trend in case of *C. fossor*. In northern areas of Europe, this eurytopic species, which is very tolerant to agricultural cultivation and lives mainly in moist and wet habitats, shows a rather increasing long-term trend in the occupied areas (Desender and Turin, 1989; Turin et al., 1991), though local decrease of *C. fossor* also occurs because of the change of habitats, as it was found on an abandoned corn field through a 12-year period (Van Dijk, 1987).

Based on the long-term patterns of light trapping in Hungary, *H. froelichii* and *H. griseus* indicated increasing and decreasing tendencies, respectively. Since 1950s in Northern Europe, the size of the occupied areas of both *Harpalus* species has declined so

much, that the otherwise rare and stenotopic species have become seriously endangered (Desender and Turin, 1989; Turin et al., 1991). Andersen (1987) proved, that the last record of *H. griseus* was found before 1900 in Norway. The decline of these two xerophilous *Harpalus* species is mainly due to the land-use change in Europe, which caused the disappearing of dry and warm habitats.

Comparing the patterns of the three carabid species, it can be say that the fluctuations of *C. fossor* and *H. griseus* were inverse and there is a connection between the maxima and minima of captures and droughty years. *C. fossor* had abundance maximum, in most cases, during strongly droughty years (1983, 1987, 1990, 1992), while in the same years *H. griseus* had minimum and vice versa. On the other hand, *H. froelichii* has an intermediate position regarding fluctuation patterns, compared to the two other species.

From the analysis of the effects of yearly climatic fluctuations, the tendencies of abundance changes can be well interpreted with the ecological requirements of each carabid species, though in most cases the values *r* of regression were not significant because the length of time series were short. According to the hypothesis, for drier and warmer seasons, a decreasing effect on population and also on captures was predicted, in case of hygrophilous *C. fossor*. Contrary to this, *C. fossor* showed unexpected reactions, e.g. as the measure of drought grew, there was an increasing tendency and with the growth of summer, autumn and winter amount of precipitation, there was a decreasing tendency of yearly mean abundance. The only explanation for this is that with the increase of measure of droughts and the decrease of soil moisture, the wet habitats of *C. fossor* dry up and the response of the population for these unfavourable changes is the increasing emigration flight activity and this gives reason for peak captures. This means that is case of *C. fossor* the increased level of captures caused by aridity indicated the increased flight activity and not the growth of population level of carabids. This idea is also supported by Honěk and Pulpán's note (1983), which states that the light trap capture of hygrophilous, riparian carabid species in dry and warm years was maximum.

Based on own light trapping results, Matalin (1994) thought that the hygrophilous species reacted by an adaptive flight to the quick changes of wet environment to colonise new, more favourable habitats even at greater distances. All this corresponds with statements of Den Boer et al. (1980), namely the *C. fossor* as wing polymorphic species having greater flight capability is able to live with higher abundance levels in unstable habitats.

Under the same investigation period the responses of *H. griseus* to the yearly climatic variations were inverse comparing with *C. fossor*, namely its mean yearly abundance level decreased with the increasing values of drought, and it increased with the growing amount of winter and summer precipitation. This species showed also population minimum (zero capture) over a 7-year light trapping period conducted near Prague (Honěk and Pulpán, 1983) in the droughty season in 1976, associated with warm and dry spring and early summer.

The inconsistent response by *H. froelichii* to the changes of aridity seems to reflect to a greater independence of its population dynamics from the climatic effects. Accord-

ing to our preliminary results it seems that *H. griseus* and *H. froelichii* are not xerophilous species but they are rather allocated within mesophilous range between the ends of xerophilous-hygrophilous scale of preference. In atlantic regions of Europe under wet climate these *Harpalus* species, known as xerophilous carabids, prefer drier and warmer habitats. However our climate in the Carpathian Basin is frequently too dry and warm for these ground beetles therefore they do not colonise the more xerotherm, unfavourable habitats. Consequently their preference can be characterised as mesophilous. This concept is confirmed by Honěk and Pulpán (1983), they found that in the droughty year the flight of xerophilous carabids were maximal but the catching rate of *H. griseus* was zero. Turin et al. (1991) also referred to this mesophilous character of both *Harpalus* species giving their humidity preference as "not dry, not wet".

By the literature the periodical warming of climate and the long-term patterns of precipitation have played an important role in climate effects on population dynamics of ground beetles. For instance, Horion (1938) detected that the greater mean temperature during in 1930s years had a favourable effect on termophilous carabids in Germany. In the Netherlands Hengeveld (1985) analysing the period between 1890–1975 found more frequent occurrence of xerophilous ground beetle species and decline of hygrophilous species because of the drier and warmer period between 1930 and 1960. He emphasised that recent decreasing trend in species richness and density of carabids were influenced by climatic factors. The large-scale regional climate change can cause long-term area expansions or contractions of ground beetles. For example, in Sweden the area expansion of many species is attributed to strong atlantization of climate during this century (Lindroth, 1972), while in northern Germany Müller–Motzfeld (1995) detected trends of area expansion of Atlantic and continental species by the climatic contrast in west and east parts over last 30 years.

In future the investigations must be conducted more intensively concerning the climatic effects on ground beetles because of the great challenge of climate change. The recent declines of the population size of carabids may be occurred frequently in a short period, within 10 years (for instance, Luff, 1982; Luff and Woiwod, 1995; Loreau, 1994; Van Dijk, 1987). However, more long-term (since last century) extended samplings confirmed the fact that high number of stenotopic, xerophilous, rare carabid species decreased and they were strongly endangered last decades (Andersen, 1987; Den Boer and Van Dijk, 1994; Desender and Turin, 1989; Desender et al., 1994; Turin and Den Boer, 1988). All of mentioned authors underlined that main cause of these drastic shifts of carabids is the land use change and human activity (i.e. agricultural intensification, habitat fragmentation and loss, soil acidification, etc.). We agree with opinion of Den Boer and Van Dijk (1994) that there is a great uncertainty in the detection of climatic effects on ground beetles under the influences made by various environmental factors which are superponed. For these reasons we intend to continue our extended light trapping investigations in future.

Acknowledgements

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Latitudinal Shifts in Spruce Budworm (Lepidoptera: Tortricidae) Outbreaks and Spruce-Fir Forest Distributions with Climate Change

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Changes in global temperatures over the next century resulting from the greenhouse effect may have profound effects on the distribution and abundance of insect populations. One general hypothesis is the poleward shift of species distributions. We investigated potential range shifts for the spruce budworm, *Choristoneura fumiferana*, in the northeastern and north central United States using maps of historical outbreak areas, climatic variables, and the distribution of spruce-fir forests in a geographic information system. We developed canonical discriminant function models of the occurrence of defoliation and the distribution of spruce-fir forests as functions of climatic variables. Using the models, we developed scenarios for defoliation and forest type changes resulting from temperature increases of 2, 4 and 6 °C. In general, predicted areas of defoliation and of the forests decreased in size with increases in temperature. As temperatures increased, distributions of defoliation and spruce-fir forests exhibited a general pattern of thinning and disappearance at their southern margins, suggesting a northward shoft of both budworm populations and spruce-fir forests.

Average annual temperatures are predicted to increase worldwide by as much as 2–4 °C by the end of the 21st century as a result of the buildup of anthropogenic greenhouse gases in the atmosphere (Houghton et al., 1996). A commonly-predicted direct effect of greenhouse warming on insects is that they will extend their ranges toward higher latitudes and higher elevations as temperatures rise (Porter et al., 1991; Sutherst, 1991; Cammell and Knight, 1992). Many insect species in North America that are currently constrained by low temperatures at the northern fringes of their distributions may begin to move northward as the climate changes. For herbivorous insects, increasing temperatures may also allow range extensions of their hosts, further facilitating their movement. Although latitudinal shifts with temperature change are a reasonable hypothesis given current knowledge of insect ecology, previous studies of distributional changes have been conducted at too limited a geographical scale to detect latitudinal movement (Williams and Liebhold, 1995) or have been in tropical regions, where such movement is less apparent (Rogers and Randolph, 1993).

In this paper, we investigate large-scale changes in the distribution of the spruce budworm, *Choristoneura fumiferana* (Clemens), the most devastating defoliator of spruce-fir forests in eastern North America. To do so, we analyze maps of historical outbreaks to develop a model of the distribution of budworm over a wide part of its range in the United States. Because potential shifts in forest types susceptible to budworm attack are critical in determining changes in defoliation, we also develop a model for the

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distribution of the spruce-fir forest type group. We use the models to extrapolate distributional changes of defoliation and spruce-fir forests under three climate change scenarios.

Materials and Methods

Map development

Maps of spruce budworm defoliation, climatic variables, and forest type were developed using the GRASS (U.S. Army Corps of Engineers, 1993) geographic information system (GIS). A GIS consists of software for the input, storage, retrieval, manipulation, and reporting of spatial data (Liebhold et al., 1993). GRASS is a raster-based GIS, in which the smallest map units (i.e., rasters) are square grid cells. All maps used a raster size of 5 × 5 km for reasons to be discussed. The geographical area of the maps covered the northeastern and north central United States, running from Maine in the east to Minnesota in the west and including the states of New Hampshire, Vermont, New York, Michigan, and Wisconsin (Fig. 1). The total area was approximately 2,300 km by 1,000 km, represented by a map grid of 464 × 202 cells.

Defoliation maps were developed from maps published in Hardy et al. (1980), which included annual maps of spruce budworm defoliation during 1938–1980 in southeastern Canada and the northeastern U.S. at a scale of 1:11,000,000. The maps were assembled from individual state and province maps that, in turn, were developed from aerial sketch maps of defoliation. Areas mapped as defoliated had defoliation visible from the air, which was generally greater than 30%. Because maps were unavailable for some states and provinces early in the series, we used maps only from 1954 to 1980. Defoliated areas on the paper maps were traced onto transparent mylar film, along with 14 georeference points located on well-defined political and natural boundaries. Transparencies then were digitized using a digital scanner at 300 dots per inch to produce binary Tagged Image Format Files, which were converted to byte-binary format files for use in the GIS. Following rubber-sheeting (discussed below), the maps on annual defoliation were added up cell-by-cell to produce a defoliation frequency map over the period 1954–1980 for use in the analyses.

Errors may have occurred in four processes of map production: aerial sketch mapping, replotting of sketch maps to produce regional coverages, tracing, and scanning. The first two processes were beyond our control, and thus, we cannot estimate errors associated with them. However, potential errors in aerial sketch mapping are well-documented (Van Sickle, 1995). The coarse grid cell size that we used (5 × 5 km) probably minimized the effects such errors. Although we exercised great care in tracing, some error was possible because the pen width represented nearly 5 km on the maps traced. Scanning error was probably minimal because each scanned dot represented less than 1 km^2 given the map scale and scanner resolution.

Maps of historical climatic variables in the conterminous U.S. were obtained from the Global Ecosystems Database (National Oceanographic and Atmospheric Ad-

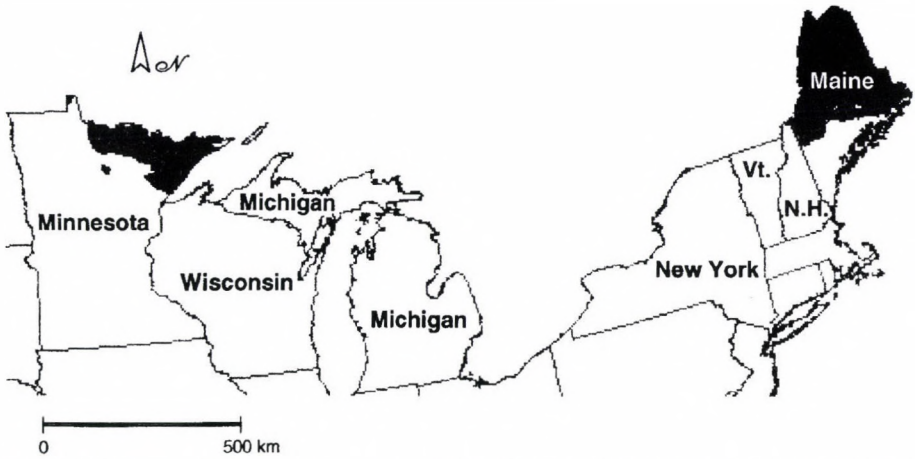

Fig. 1. Areas in the northeastern and north central United States defoliated by spruce budworm, 1954–1980. Note that defoliation distributions were available only for Minnesota and Maine

ministration, 1993). Variables included 12 monthly averages of daily temperature and 12 averages of monthly precipitation over the 40 yr period from 1948 to 1987. Temperature maps were developed using the following procedure. Monthly temperature means were obtained for 1,211 stations in the continental U.S. from the Historical Climatology Network (Quinlan et al., 1987). Given the elevation of each station, temperatures were corrected to their values at sea level using standard adiabatic lapse rates of temperature with elevation. The corrected station averages were then used to interpolate temperatures over a 10 km grid, employing a simple linear inverse distance squared algorithm (Isaaks and Srivastava, 1989). The interpolation produced a 10 km resolution raster map of sea level temperatures for the conterminous U.S. The interpolated temperatures then were corrected for elevation using a 10 km resolution Digital Elevation Model map (U.S. Geological Survey, 1990) and appropriate lapse rates to produce the final versions of the maps. Precipitation maps at 10 km resolution were developed using PRISM (Precipitation-elevation Regressions on Independent Slopes Model) (Daly and Neilson, 1992).

A map of forst types susceptible to spruce budworm was developed from a map of forest type groups produced by the U.S. Department of Agriculture (USDA) Forest Service's Forest Inventory and Analysis group (Zhu and Evans, 1992) from Advanced Very High Resolution Radiometer satellite imagery. The forest type map depicts geographical distributions of ten forest type gropus in the eastern U.S. at 1 km resolution. For use in the analysis, we extracted the distribution of spruce-fir forests (Fig. 2), which contain the primary host species of spruce budworm (Mattson et al., 1988).

The reference map to which all the map layers were standardized was a vector line map of the conterminous U.S. The map was a Lambert Azimuthal Equal Area projection, which was useful for our analyses because it equalized surface areas over the geo-

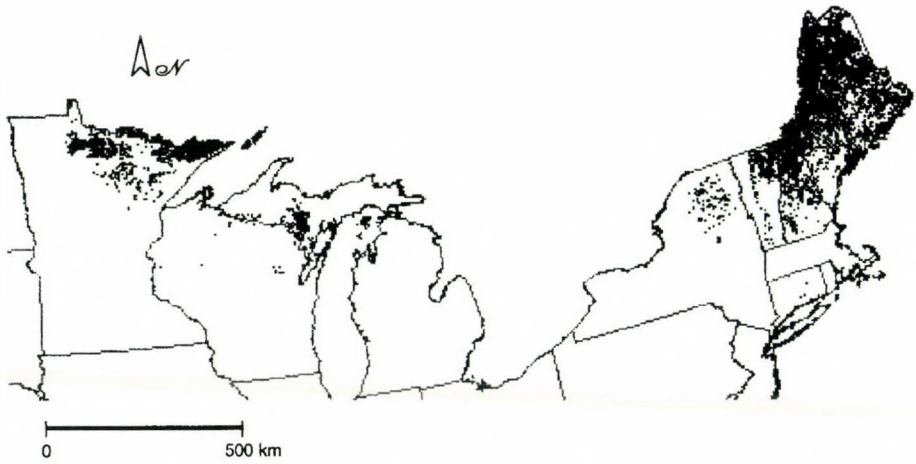

Fig. 2. Distribution of the spruce-fir forest type group in the northeastern and north central United States

graphical range of the coverage. Common georeference points were identified on the various map layers and the reference map, and the map layers were transformed to the resolution and projection of the reference map through "rubber-sheeting". Rubber-sheeting is a process by which one map is stretched mathematically to fit a reference map given a set of common georeference points whose locations are defined on both maps (Antenucci et al., 1991). The rubber-sheeting of all maps used a second-order polynomial transformation (U.S. Army Corps of Engineers, 1993). A common resolution of 5 km was used as a compromise among three conflicting considerations. First, 5 km was intermediate between the resolutions of the forest type map (1 km) and the maps of climatic variables (10 km), reducing the loss of information in the first case due to pixel aggregation and minimizing the redundancy of information in the second case due to subdivision of larger pixels. Second, 5 km represented the smallest resolution reasonable for the defoliation maps given the several sources of mapping error cited previously. Third, use of a relatively large pixel size kept the map of the wide geographical area at a manageable size (464 × 202 rasters) for statistical computations.

Predicting potential changes in the geographical distribution of spruce budworm defoliation involved two further steps: (1) fitting the occurrence of defoliation and spruce-fir forests as functions of the climatic variables and (2) extrapolating new distributions using the fitted functions under climate change scenarios. Because we were interested only in predicting the qualitative patterns of defoliation, we did not fit the defoliation frequency map described previously. Instead, we simplified the frequency map by reclassifying it into two categories of cells: those that were never defoliated and those that were defoliated at least once.

Statistical analysis

We used a canonical discriminant function as our model for predicting the occurrences of defoliation and spruce-fir forests. A canonical discriminant function predicts values of a canonical variable, Z, that has two or more discrete states as a linear function of a number of independent variables, X_i,

$$Z = a_1X_1 + a_2X_2 + \ ... + a_nX_n \ ,$$

(Manly, 1986). In our case, whether an individual cell was classified as defoliated (1) or not defoliated (0) depended on the values of the climatic variables. For modeling forest distribution, the dependent variable had the two discrete states, presence (1) or absence (0) of spruce-fir forest type. The canonical discriminant function is estimated such that it maximizes the ratio of the variation between the two groups of state values to the variation within (i. e., the F ratio) (Manly, 1986). An individual cell (i.e., observation) is classified into one group or the other depending upon the distance and relationship of its Z value to the two group means. A possible shortcoming of this approach in analyzing map data is that it does not consider the spatial structure of the data (i.e., the state of each cell is assumed to be independent of the states of its neighbors).

Rather than fitting the two functions with all 24 independent variables, we included only those that were significant in explaining the occurrence of defoliation or spruce-fir forests, using a stepwise procedure that evaluated the variables individually for their contributions to the goodness-of-fit of the discriminant function. At each successive step, the procedure chose the variable that resulted in the lowest value of Wilks's lambda among those remaining (PROC STEPDISC (SAS Institute, 1990)). The significance threshold for a variable to enter or remain in the model was 0.15.

Extrapolating climate change effects

Given the fitted canonical discriminant functions for the occurrences of defoliation and spruce-fir forests, we modified the equations to extrapolate climate change effects as follows:

$$CV = a_1(T_1 + \Delta T_1) + ... + a_{12}(T_{12} + \Delta T_{12}) + b_1P_1 + ... + b_{12}P_{12}$$

where CV is the canonical variable for defoliation or forest type, T_i are the temperature variables, ΔT_i are temperature changes for a climate change scenario, and P_i are the precipitation variables. We changed only temperature in these scenarios, although we included precipitation in the model to achieve the best fit possible. Using the relationship, we reclassified each grid cell, inserting ambient values and change values for the temperature variables. Climate change was assumed to apply uniformly across the region, and thus, the same temperature change for each variable was applied to every grid cell. We explored the range of responses in outbreak areas with three increases in temperature: + 2, + 4 and + 6 °C.

Because spruce-fir forests exist over much of the northern part of the eastern U.S. and we have data on their distribution (Fig. 2), we were interested in modeling spruce budworm defoliation over the entire range. However, we had historical defoliation data only for the states of Maine and Minnesota (Fig. 1) and were unwilling to develop scenarios from a model based directly on these data for fear of generating unrealistic results. To overcome this problem, we used a nested approach that involved three steps. First, we developed a canonical discriminant function model for the occurrence of spruce-fir forests, using data from all the states, and developed a set of projections for their range change under the climate change scenarios. Second, we fit the discriminant model for spruce budworm defoliation using only observations within the spruce-fir forest type. Third, we generated defoliation scenarios by extrapolating with the defoliation model under the climate change scenarios, but limiting the extrapolations only to grid cells projected to contain spruce-fir forests in the respective forest change scenarios. A critical assumption of this approach was that the presence of spruce-fir forests is the primary determinant of the presence of spruce budworm defoliation.

Results and Discussion

Discriminant analyses

Stepwise analysis of the spruce-fir forest distribution yielded a discriminant function of 19 of the 24 climatic variables. The squared canonical correlation, which is similar to the r^2 of a linear regression, was 0.43 for the complete model. The first three variables included, mean daily temperature in April, mean monthly precipitation in November, and mean daily temperature in July, respectively, contributed 78% of the final value of the squared canonical correlation. The relationship was highly significant, as indicated by the Wilks's lambda value of 0.569 ($F = 1166.9$; df = 19,29242; $P = 0.0001$). It provided a good fit to the spruce-fir forest data, with just 16.6% of the 29,262 observations misclassified. The model predicted a slightly greater area of spruce-fir forests under ambient climatic conditions (15.7%) than was actually observed (12.7%) (Table 1). The goodness-of-fit of the model may be assessed visually by comparing the prediction of spruce-fir presence under ambient conditions (Fig. 3a) with the actual distribution (Fig. 2). The model predicted the distribution of spruce-fir forests under ambient conditions rather well in the eastern states and less so in the western states.

The stepwise analysis of spruce budworm defoliation produced a discriminant function that included 18 of the 24 climatic variables. Comparable with the forst model, the squared canonical correlation for the defoliation model was 0.43. Seventy-five percent of the value of this correlation was contributed by the first four variables to enter the model: Mean daily temperature in May, mean daily temperature in July, mean monthly precipitation in September, and mean monthly precipitation in May, respectively. The relationship was highly significant, with a Wilk's lambda value of 0.570 ($F = 116.2$; df = 18,2768; $P = 0.0001$). Overall, the fit of the function to the data was very good, with just 13.5% of the 2,787 observations misclassified.

Table 1

Percentages of the total land area of the northeastern and north central United States (744,806 km^2) and absolute areas actually occupied and projected as occupied by the spruce-fir forest type group under ambient temperature and three climate change scenarios

Scenario	%	km^2
Actual distribution	12.7	94,482
Ambient temperature	15.7	117,186
2 °C increase	12.2	90,613
4 °C increase	10.0	74,603
6 °C increase	7.4	55,106

Climate change extrapolations

The basic pattern of forest response to increasing temperature was a decrease in the total area projected to contain spruce-fir forests (Table 1). The area decreased steadily with increasing temperature, from almost 16% under ambient conditions to less than half that amount under a 6 °C increase. The forest scenario maps (Fig. 3) exhibited a general pattern of thinning of forests in the southern end of their distribution with increasing temperature. For example, spruce-fir forests were projected to extend to the southern borders of Maine and New Hampshire under ambient temperatures. Under increasing temperature, the distribution diminished steadily from the southern ends of those states, ultimately resulting in a disappearance of spruce-fir forests from the southern half of Maine and from all but the northern tip of New Hampshire. The general pattern appeared to be a northward movement of spruce-fir forests with increasing temperature, although we cannot be certain without projections for the northern border of the forest type distribution in Canada. However, such movement is very likely. Northward distributional shifts–in some cases covering hundreds of km–have been suggested for other tree species under greenhouse warming (Davis and Zabinski, 1992).

The patterns of change in areas projected as defoliated under increasing temperatures were qualitatively similar to those for forest type, but more extreme. Whereas the area projected as defoliated under ambient conditions was almost 9% of the total, that area decreased to much less than 1% with a 6 °C increase (Table 2). The defoliation scenario maps (Fig. 4) showed similar trends to those for forest type, with a general thinning of defoliation at the southern ends of the distribution. The effect was particularly evident with a 2 °C increase (compare Figs 4a and 4b). It is important to remember that defoliation projections were constrained to areas containing spruce-fir forests under the respective temperature change scenarios. With further temperature increases, the defoliation

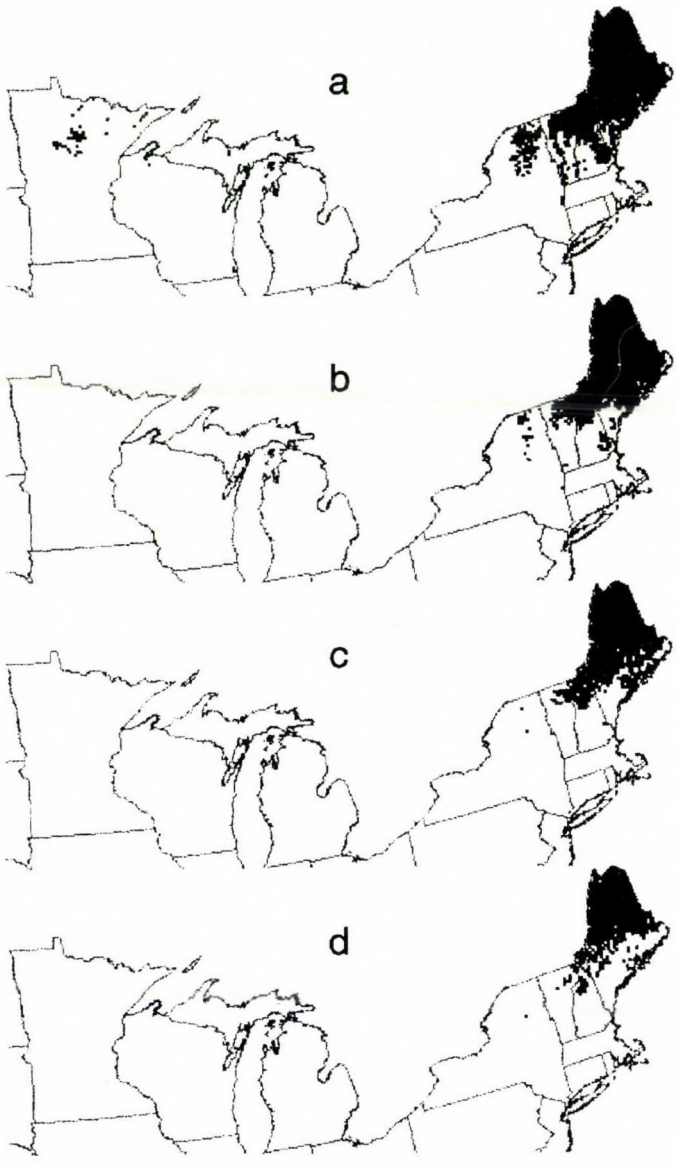

Fig. 3. Distributions of the spruce-fir forest type group predicted by the discriminant function under ambient conditions and three climate change scenarios. (a) Ambient temperature, (b) 2 °C increase, (c) 4 °C increase, (d) 6 °C increase

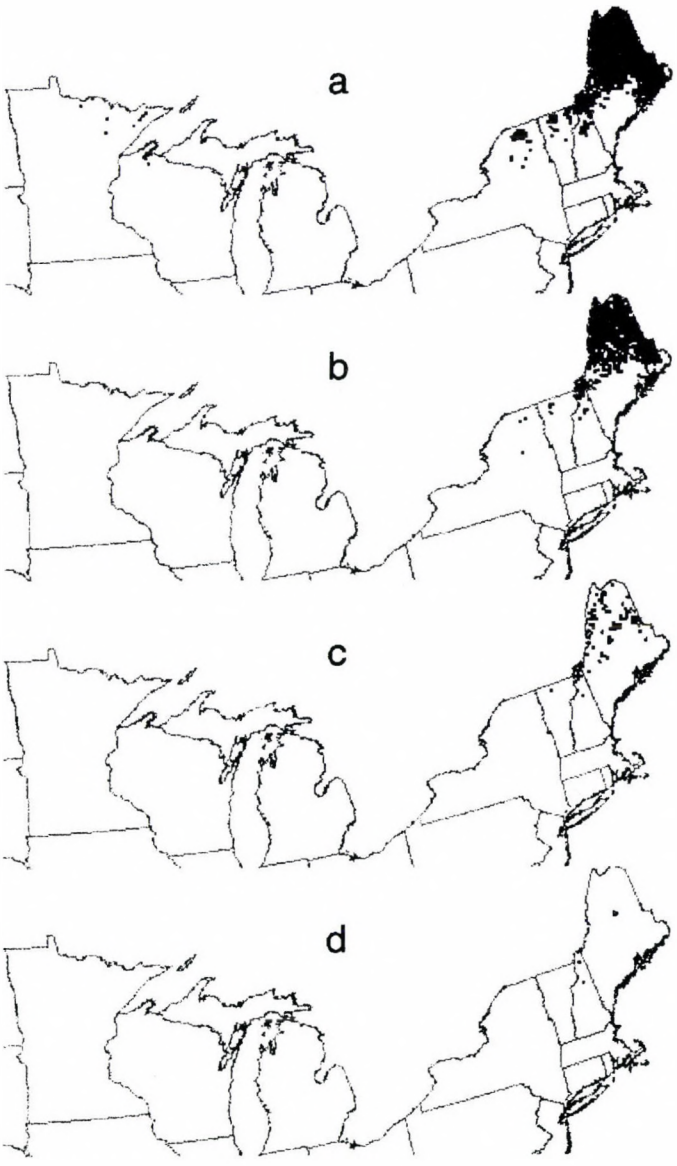

Fig. 4. Predicted areas of spruce budworm defoliation under ambient conditions and three climate change scenarios. (a) Ambient temperature, (b) 2 °C increase, (c) 4 °C increase, (d) 6 °C increase

Table 2

Percentages of the total land area of the northeastern and north central United States (744,806 km^2) and absolute areas projected as defoliated by spruce budworm under ambient temperature and three climate change scenarios

Scenario	%	km^2
Ambient temperature	8.9	66,305
2 °C increase	6.1	45,714
4 °C increase	1.2	8,858
6 °C increase	0.1	611,000

distributions generally thinned out (Figs 4c and 4d). Rather than a continued shift toward higher latitude, those distributions suggested a movement toward higher elevation because they corresponded broadly to the location of the White Mountain range. Similar distributional shifts to higher elevations under increasing temperature have been suggested for other forest defoliator species (Williams and Liebhold, 1995).

To understand the patterns of change in the distribution of defoliation, one must consider that defoliation is a process requiring both a defoliator population and a susceptible population of trees to be defoliated. Changes in the distribution of defoliation reflect simultaneous changes in both populations. Because spruce budworm populations are very mobile, they may respond quickly in tracking changes in host distribution. By contrast, trees are long-lived, and their movement is very slow. Because of the great disparity of time scales, it seems likely that the redistribution of spruce-fir forests under increasing temperature will dominate, with such changes occurring on a time scale of centuries. Shifts in spruce-fir forest distribution are reasonable from the standpoint of plant physiology. With increasing temperature in the lower latitudes and elevations, tree populations in these regions will come under increasing stress from rising metabolic costs and increased evapotranspiration (Kirschbaum and Fischlin, 1996). Without human intervention, these populations will die out–perhaps hastened in doing so by increased intensity of spruce budworm defoliation (Kurz et al., 1995). Other populations in the northern and high altitude end of the spruce-fir range will tend to diffuse northward and toward higher elevation, as those regions become favorable for growth (Davis and Zabinski, 1992; Dyer, 1995). If current climate change predictions become reality, we clearly may see considerable regional changes over the long-term in both spruce budworm populations and the forest habitats they occupy.

Acknowledgements

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Disappearance of Mass Outbreaks of *Dendrolimus pini* L. (Lepidoptera, Lasiocampidae)

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The history of mass outbreaks by the pine moth, *Dendrolimus pini* L. (Lepidoptera, Lasiocampidae) is described on basis of field studies and an extensive literature survey. The existing evidence is summarized into a diagram illustrating the history of mass outbreaks in a series of different areas from western Europe to Central Siberia. It is noted that the mass outbreaks in Central Europe started in early times and disappeared in the later half of the 20th century. In the Russian part of Europe the outbreaks started in the latter half of 19th century but disappeared about simultaneously with the disappearance in western Europe. In western Siberia the outbreaks started later and continued nearly until the end of the 20th century. It has been noted that pine moth does not occur in St. Petersburg and in a broad zone around it. The role of air pollutants for the decline of the outbreaks was tested by feeding caterpillars with pine branches from centrum of St. Petersburg and in comparison with branches from a rural area (Tichvin). The larvae which had eaten polluted branches from the city died all during their first overwintering, whereas the corresponding mortality in the rural control was only 2–4%. Obviously the gradually increasing industrial pollution has first promoted the development of outbreaks by corroding host plant resistance. In a later phase the pollutant stress has overstepped the ecophysiological tolerance limit of the herbivorous *D. pini*, too.

The periodically repeating mass outbreaks of several forest incest have disappeared at the end of the 20th century. Almost all needle and leaf devouring forest pests listed by Voronzov in 1962 (*Porthetria dispar*, *P. monacha*, *Euproctis chrysorrhoea*, *Malacosoma neustria*, *Bupalus piniarius*, *Panolis flammea* and *Neodiprion sertifer*) have lost their capacity to develop mass outbreaks or are producing only small outbreaks with short duration. Some new species have, however, entered the arena and are able to develop strong and very devastating outbreaks (e.g. *Zeiraphera diniana*, *Cephalcia abietis*, *C. falleni* and *Pristiphora abietum*) (Kalina and Skuhravy, 1985; Nuorteva, 1990).

The development of outbreaks by the new pests is in correlation to intensified acid rain, metal and nitrogen fallout as well as low altitude ozon. The reasons for the decline in the devastations by the classical pests have, on the conrary, remained unclear. In order to clear up the mystery of disappearing moth outbreaks, a case evaluation was performed on the pine moth, *Dendrolimus pini* L.

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Materials and Methods

The species *D. pini* has developed from Mediterranean ancestors in Europe during the glacial time. Its range started to expand towards east in early holocen. At he present its range has reached the lake Baikal (Rotzhkov, 1963; Amsheev et al., 1990; Epova, 1990). Great devastating outbreaks of the species have occurred in western parts of Central Europe since the 17th century (Schwertfeger, 1935) and they have continued until end of 1960s (Ziegler, 1955; Wiegand, 1956; Gevlich, 1963). In the western part of European Russia the outbreaks started 1839 and disappeared about simultaneously with the western European outbreaks (Pavlov, 1957; Grimalsky, 1971). When going towards Siberia in east the outbreaks started and disappeared gradually more and more later (Kirilov, 1958; Epova, 1990; Amsheev et al., 1990; Jnovsky, 1996).

A peculiarity in the biology of the pine moth has been that the calamities during the 19th century occurred mainly in superannuated forests (Altum, 1874; Judeich and Nitsche, 1895; Vasiljev, 1910) but during the 20th century mainly in sampling-pole age (11–33 years) (Gösswald, 1935; Ilinskij, 1965; Benedek, 1969).

The main locality for field studies was in pine stands at Middle Don river in southeastern part of the European Russia. In this area the biology of the pine moth was studied in all phases of outbreak, eruption-, reduction- and normal low phases (Malyshev, 1980, 1981, 1987, 1991). The phase of low quantity was studied during the years 1979–1996 in the region of Tikhvin (Thvina) south of the Lake Ladoga. An outbreak occurred there about 150 years ago, but not later. In addition constant observations were performed in Savala forests in the basin of River Hoper, a classical area of pine moth outbreaks (Malysheva, 1954), where an increasing population existed from middle 1970s to the beginning of 1980s (Orlovskaya et al., 1988). One observation point, where no specimens of pine moth were seen during 6 years of search, consisted of forests around the Mednoe lake 30–40 km northwest from St. Petersburg.

In addition to the above-mentioned field work, extensive literature survey was performed in order to construct a diagram illustrating the history of outbreaks in a series of areas between Western Europe and Central Siberia. It existed such an abundance of references that it was not possible to mention them all in the reference list of this paper.

In order to reveal the role of air pollutants for the pine moth, its caterpillars were fed during the years 1982–83 from third decade of August to first decade of October with pine branches collected from the park Sosnovka in St. Petesburg. Here the pines were stressed by pollutants and more than 20% of the needles had fallen away prematurely. Control branches were picked up from the forest of Tikhvin in the southern Ladoga region. It was a locality where pine moth specimens were found nearest to St. Petersburg. Its distance from the city is more than 200 km. Practically no premature fall of needles was observable there. In all 120 caterpillars were used in the experiments, 20 of them served as control.

Results

The results of the literature survey are given in Fig. 1. It is to note that the mass outbreaks in Central Europe started in early times and disappeared in the later half of the 20th century. In Siberia the outbreaks started later and continued nearly until the end of 20th century.

In the experiments where polluted urban pine needles were fed to pine moth caterpillars they died all during the first hibernation. By control caterpillars which had been fed with unpolluted rural pine needles, the mortality was only 2–4%.

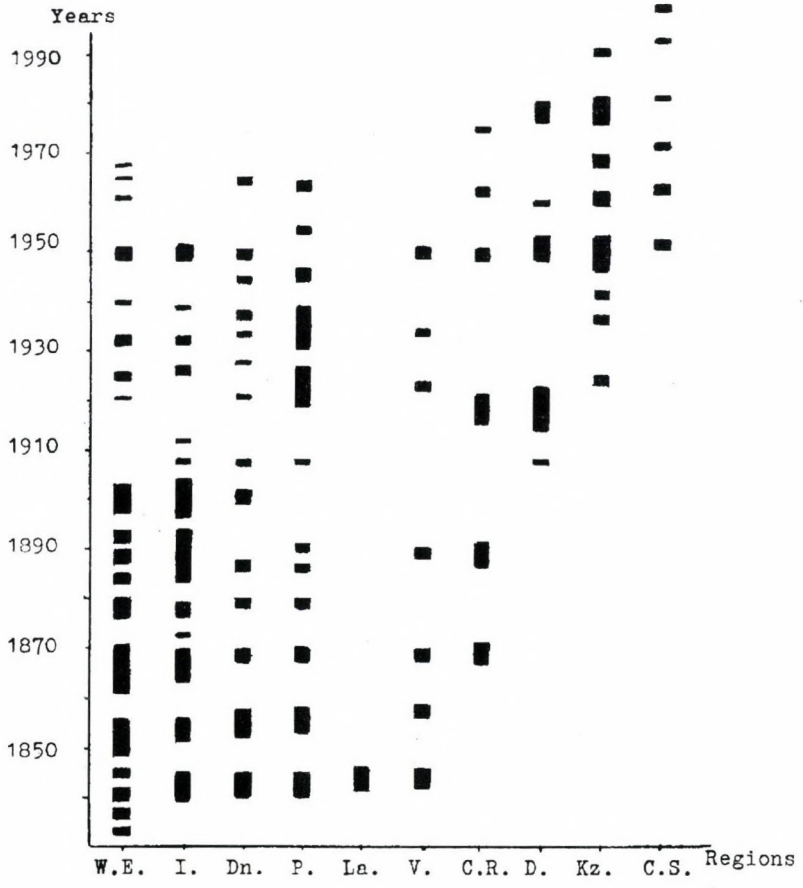

Fig. 1. Outbreaks, infestations duration of pine moth in different regions
W.E. – Western Europe, I. – Izum pine stands, Dn. – average current of Dnepr river, P. – Ukrain and Belorussia Polesj, La. – southerly of lake Ladoga, V. – average current of Volga, C.R. – Central part of European Russia, D. – river Don with Hoper and Savala, Bitjg, Medvedica, Kz. – pine stands in Kazakhstan, C.S. – south of Central Siberia

Discussion

The absence of pine moths in St. Petersburg and in its surroundings until a distance of 150–200 km gives strong indication about the harmful effects of the city pollutants for the survival of this moth. This comprehension was nicely supported by the experiment, where caterpillars died after feeding on pine needles contaminated by city pollutants.

Different kinds of pollutants may be responsible for this effect. It exists evidence only about the occurrence of metals in pine moth. Obviously it is sensitive against elevated levels of toxic metals in the food, because it is adapted for larval life on a host plant having low metal levels in the needles. This moth has also developed effective mechanisms in order to inhibit metal inflow from the food. Consequently it occurs in adult pine moths much lower levels of Al, Fe, Mn and Cd than in pine needles (Rantataro et al., 1989). Zn and Cd intake from larval food is, however, very effective, because these two metals play an important role in the physiological excretion system for other metals (Nuorteva, 1990).

Studies performed in the surroundings of metal factories in Harjavalta. Finland show that the tortricid moth *Petrova resinella* (L.) takes benefits of the physiological alterations caused by metal pollution in its host. Within 800 m of the emission source, with heaviest metal pollution, however, the moth was completely absent (Heliövaara, 1986; Heliövaara et al., 1987, compare also Nuorteva 1990 p. 52). If this model were true for *D. pini*, it would be expressed in the time scale by a gradual population increase prior to the decline, because industrial pollution has gradually increased since the beginning of the 19th century. Emissions from urban and industrial sources may well be the main regional factor determining the rise and fall of pine moth outbreaks. Other factors may bear responsibility for the cyclic fluctuations in population density, as well as for different kinds of irregularities.

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Accumulation of Metal Pollutants in Red Wood Ants and their Influence on Colony Development

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Red wood ants have achieved a dominant position in boreal forest biocoenoses through their sociality. These ants belong to the heaviest metal loaded animals in the forest ecosystem. The metal load of forests increases through industrial activity. If the metal tolerance of ants is overstepped and their populations decrease, the whole forest ecosystem may loose its stability.

Our studies revealed negative bioaccumulation of Cd and some other metals in the social food chain of red wood ant colonies. This protects larvae, pupae, sexuals and reserve ants against naturally occurring metal stress. We elevated through artificial feeding the Cd-level (4–10 ppm Cd/dwt) of Finnish and Estonian *Formica aquilonia* colonies to the level occurring in red wood ants in polluted Central Europe (40–100 ppm Cd/dwt). In Estonia this elevation damaged a single colony, but not colonies joined to supercolonies, which have a great diluting biomass and social buffer capacity. When Migula and Glowacka studied with biomarkers ants from our experiments, they found no disturbances in ants from normal colonies, but several disturbances in colonies where the Cd contents had been artifically elevated to the Central European level. It is to conclude that the few remaining red wood ant colonies in Central Europe are living at the limit of their Cd tolerance.

1. Ants as forest superorganisms

High level of sociality is the power by which the red wood ants have achieved their great importance in palearctic forest biocoenoses. They live in supercolonies, where exchange of food, information, brood, inside workers, and queens takes place between different nest mounds (Fig. 1). The number of nest mounds of *Formica aquilonia* may in some cases reach up to 15/ha. Considering that one nest contains about one million ants and that the weight of one specimen is about 10 mg, the biomass of red wood ants is about 150 kg/ha. In some red wood ant settlements in Estonia the biomass of *F. aquilonia* amounts to 300 kg/ha, i.e. 1500–3000 specimens/m^2 (Maavara and Martin, 1983). In forest communities they can have the highest biomass among other invertebrates (Hölldobler and Wilson, 1990).

In fact one can never meet such a high density of ants in the forest, because only outside workers, i. e. 10–15% of the specimens in the colony are foraging food outside the nest. About 50% of the colony members are inside workers (nurses, cleaners, and nest builders) and about 25–33% are reserve specimens, diapausing in hibernation chambers under the mound (Hölldobler and Wilson, 1990; Maarava and Martin, 1983; Zakharov, 1991). Division of red wood ants to different working groups is determined by an age

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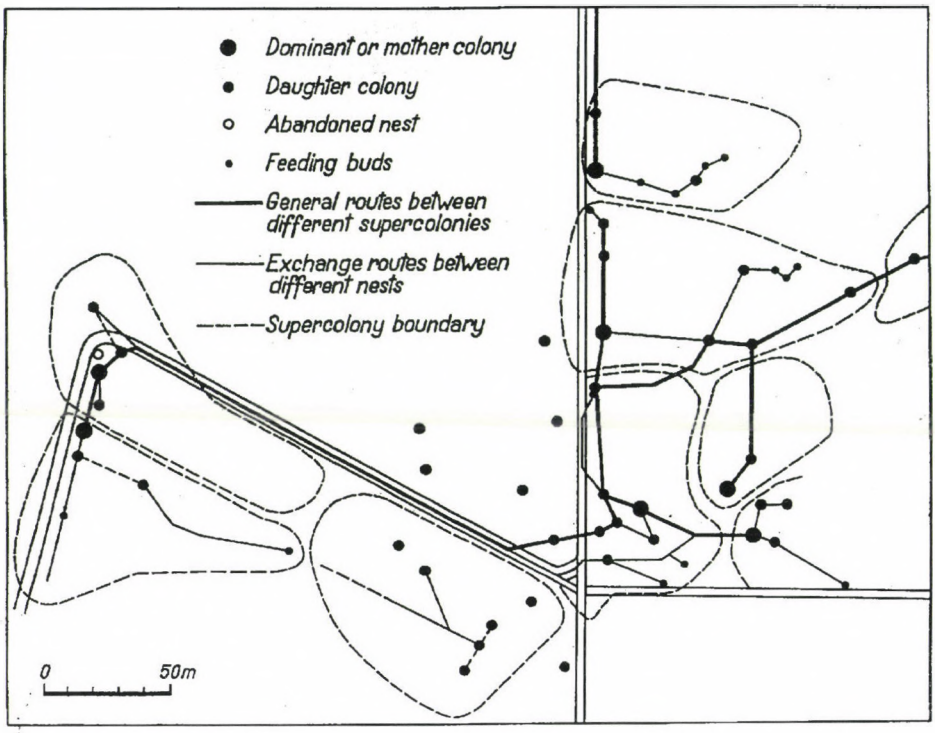

Fig. 1. Federation of supercolonies mapped in the ant protection area Akste in Polva county, Estonia (Maavara and Martin, 1983)

dependent change in behaviour. Labour division is a key to fashion the colony to a fitted, holistically functioning unit, which has an ability to adapt itself to variations in the environment (Hölldobler and Wilson, 1990).

It is a bit astonishing to note that red wood ants, wiht their exceptionally high biomass consume mainly the honeydew produced by aphids. More than 80% of their food consists of honeydew, the liquid excrements rich in sugars. In spite of the fact that honeydew is their main food, ants are also important predators of forest insects. They consume 10–20 kg insects/nest and year (Rosengren and Sundström, 1991). This consumption may in some degree prevent population increase of some pest insects, although it is known that red wood ant predation is restricted to such trees from which they collect honeydew (Otto, 1967; Laine and Niemelä, 1980; Rosengren and Sundström, 1987, 1991; Gösswald, 1989; Hölldobler and Wilson, 1990).

It is generally considered that the additional ecological power provided by sociality fashions ants to superorganisms having exceptional tolerance against environmental stresses, including poisons. The exceptionally high poison tolerance of the fire ants (*Solenopis invicta* and *S. richteri*) is well documented (Banks et al., 1978, 1985).

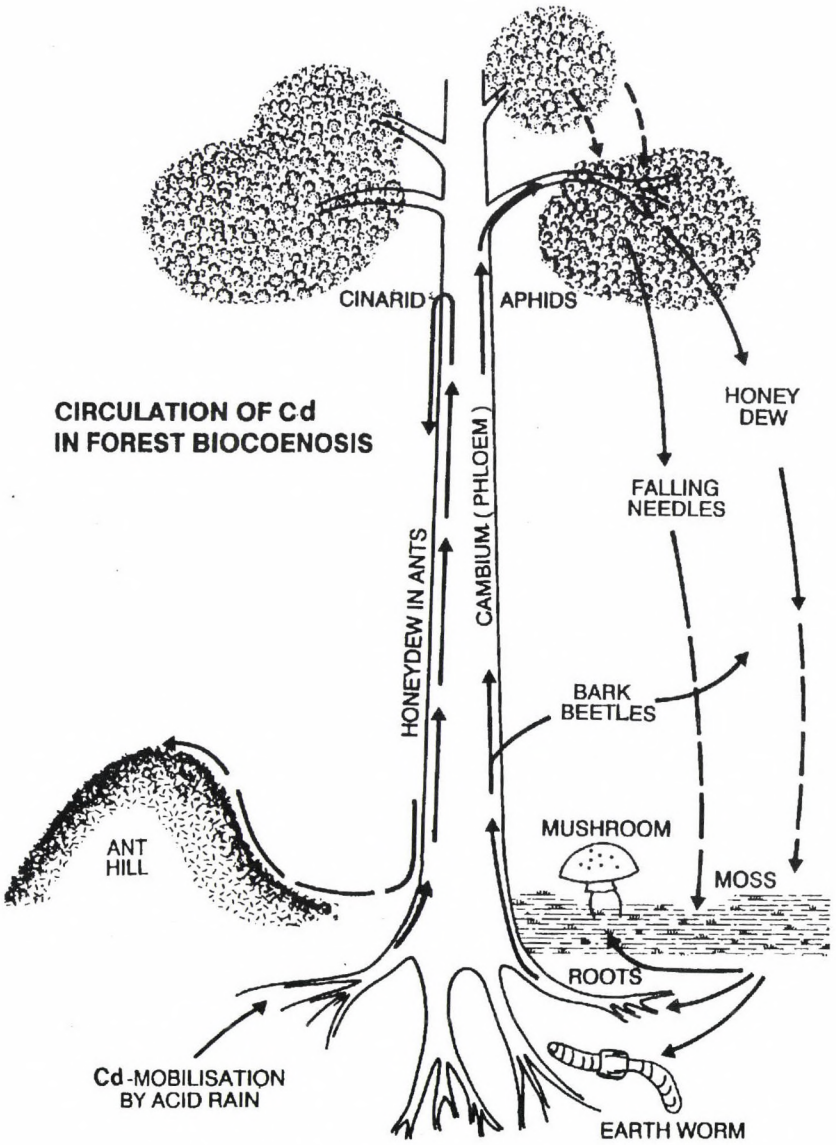

Fig. 2. Circulation of Cd in forest biocoenosis (Nuorteva, 1990)

2. Metal accumulation and distribution in ant societies

During recent years several investigators have noted in red wood ants metal contents which belong to the highest ones in forest ecosystem (Fangmeier and Steubing, 1986; Stary and Kubiznakova 1987; Ylä-Mononen et al., 1989; Nuorteva, 1990, Nuorteva et al., 1992; Oja, 1992; Migula and Glowacka, 1996). The route of acid rain solubilized cadmium and other metals from forest soil to ants is a bit complicated. First the metals go through the root system to the cambium of coniferous trees. From there the metals are taken up by phloem feeding aphids, which transfer the metals to their honeydew (Fig. 2) (Nuorteva 1991; Maavara et al., 1994). Red wood ants collect large amounts of aphid honeydew, about 500 kg/colony in a year. That is the reason why foraging red wood ants are loaded with high metal levels.

The metal contents in red wood ants mirrors the levels of solubilized metals in forest soil and in the phloem of coniferous trees. They are thus good bioindicators for the degree of metal pollution in forest ecosystems (Maavara et al., 1994). There is about ten times more cadmium and other toxic metals in red wood ants in Czech Republic and Poland – in the heart of industrialized Europe – than in the less industrialized Finland. Inside Finland the Cd levels of surface workers of *Formica aquilonia* decrease towards the north as follows:

Latitude 59–60°	Tenala	n = 24	8.5 ppm Cd/dwt
Latitude 60–61°	Perniö–Viikki–Masku	n = 81	5.3 ppm Cd/dwt
Latitude 61–62°	Tampere–Mäntyharju	n = 12	5.5 ppm Cd/dwt
Latitude 63–64°	Kokkola	n = 21	4.2 ppm Cd/dwt
Latitude 64–65°	Raahe	n = 45	3.8 ppm Cd/dwt

A collaborative study performed by Estonian, Finnish and Polish research workers showed that cadmium and some other metals do not occur at similar levels in different worker groups of red wood ants. The highest levels occur in the foragers and a negative bioaccumulation happens in the social food chain (Table 1) (Oja, 1992; Maavara et al., 1994). The poison sensitive sexuals, larvae, and storage ants at the end of this chain receive only minimal amounts of dietary metals.

3. Effectivity of the social metal tolerance systems in ants

It is important to note that the above-described negative bioaccumulation of metals in the social food chain is, in fact, a very effective metal tolerance system. Red wood ants have thus two kinds of metal tolerance systems: 1) The normal physiological one and 2) the tolerance system based on the social structure of their colonies.

In addition, social superstructures from a dilution system for poisons coming from point sources (e.g. from poison loaded carcasses, Nuorteva et al., 1978). We noted in our preliminary feeding experiments during the years 1990–91 in Tenala, Finland (see Table 3 in Maavara et al., 1994) that cadmium containing honey placed in the vicinity of two ants nests caused elevation of the cadmium level of ants, not only in the two nests but

Table 1

Mean Cd contents in different castes and worker groups of *Formica aquilonia* in an artifical feeding experiment performed in Viikki, Helsinki, Finland, April 25th – September 1st, 1991. The control nest colony was fed three times with 0.5 kg honey, Cd treated nests simultaneously three times with 0.5 kg honey containing 500 ppm $CdCl_2$. – Summarized from table 10 by Oja (1992)

Working group	No. of samples	ppm Cd (dwt) in ants	% in relation to surface workers
Control nests			
surface workers	9	7.1	100
internal workers	9	6.1	86
pupae	3	0.1	2
reserve workers	7	3.2	45
queens	1	0.5	7
Cd-treated nests			
surface workers	11	143.1	100
internal workers	11	99.6	70
larvae	1	6.1	4
pupae	2	6.5	5
reserve workers	7	36.0	25
queens	3	6.8	5

also in other nests in their neighbourhood. According to the supercolony distribution the fed cadmium diluted into a great ant biomass and its effects were buffered by social responses of different kinds. Consequently we were unable to detect some disturbances in the functions of the treated colonies. When we continued our Cd-feeding experiments, we placed a part of them to the ant protection area Akste in Estonia (Table 2). There we took in use a single completely independent ant colony. In that case we were able to observe a decrease of the nest temperature as well as insufficient brood in the next summer. In settlement-joined colonies this kind of disturbances remained unclear, although we fed more Cd and elevated the ant contents until a level occurring in the polluted Central Europe. Compensatory social mechanisms were insufficient only in cases where ant colonies were contamined with both Hg and Cd (Migula et al., 1996).

By using physiological biomarkers, prof. Pavel Migula and Dr. Elzbieta Glowacka were able to show, that the observed double tolerance system protected well the Finnish ants against the stress of naturally occurring metals. In such Finnish ants, however, whose Cd- and Hg-contents had been artificially elevated to the Central European level, the biomarker enzymes showed existence of physiological disturbances (Migula and Glowacka, 1996, Migula et al., 1993, 1996). Artificially elevated Cd- and Hg-contents inhibited, for example, the activity of enzymes participating in ATP-

Table 2

Seasonal dynamics of Cd levels (ppm/dwt) in different functional groups of *Formica aquilonia* Yarr. in the experiment in Akste, Estonia. N = normal specimens from an untreated control nest (No. 144), Cd = specimens from a nest (No. 129) fed with 500 mg $CdCl_2$ in 0.5 kg honey on 1.6.1993. Three replicates for each analysis. The elevated Cd levels in the nest 129 on 12.4.1994 resulted obviously from the fact that the healthiest ants had migrated away and the sickest had remained

Ant groups	14.5.93	8.6.93	17.6.93	20.7.93	25.8.93	12.4.94
Forager N	8.0 ± 1.5	8.4 ± 1.2	6.1 ± 0.7	7.7 ± 0.4	7.3 ± 0.8	14.7 ± 1.5
Forager Cd	5.4 ± 0.4	45.0 ± 4.6	49.3 ± 3.5	57.3 ± 7.0	51.5 ± 3.5	120.0 ± 2.7
Surface N	6.8 ± 0.4	7.9 ± 0.4	6.7 ± 0.5	6.6 ± 0.8	7.3 ± 0.9	9.5 ± 0.4
Surface Cd	9.4 ± 1.6	53.7 ± 6.4	56.3 ± 5.5	73.2 ± 3.0	61.7 ± 1.5	104.0 ± 5.0
Nurses N	4.1 ± 1.8		4.5 ± 1.9	7.3 ± 1.1	5.6 ± 0.7	6.9 ± 0.8
Nurses Cd	5.3 ± 1.2		55.3 ± 2.5	52.0 ± 11.1	34.2 ± 2.5	96.7 ± 8.4
Reserve N			3.8 ± 0.5	3.4 ± 0.4	2.4 ± 0.1	4.9 ± 0.9
Reserve Cd	2.5 ± 0.7		54.0 ± 1.4	44.5 ± 6.4		97.3 ± 2.5
Larvae N	0.05 ± 0.01			0.07 ± 0.01		
Larvae Cd	0.03 ± 0.01		0.15 ± 0.01			
Pupae N			0.07 ± 0.03	0.10 ± 0.02	0.10 ± 0.01	
Pupae Cd			0.16 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	
Queens N						0.20 ± 0.05
Queens Cd		1.2 ± 0.1				(49.0?)

synthesis. The formation of energy stores in recently emerged reserve ants is directly based on ATP synthesis. This means that their hibernation may fail. In several occasions we had difficulties to find reserve ants from Cd-treated nests, when we needed them for our metal analyses.

Our studies indicate that the Central European red wood ants are living near their tolerance limit for cadmium and some other metals. In fact the ant populations have declined there in spite of the fact that they are protected by law. Decline of red wood ant predation may be partly responsible for calamities developing by such species of forest pests, whose larvae are devouring conifer needles – e.g. sawflies and moths (Vogel et al., 1988; Nuorteva, 1990).

The results obtained through metal pollution studies have given us new elements to understand better the ways by which the social mode of life supports the success of social insects on our globe. It is also possible to say that the first work on occupational health among insects has been done (Stary and Kubiznakova, 1987; Maavara et al., 1994).

Acknowledgements

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Molecular and Physiological Biomarkers in Insects as the Tools in Environmental Hazard Assessment

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The use of biomarkers enables monitoring of stress responses, ranging from molecular to community levels. The implementation of biomarkers from insects and spiders as potential indices of biological responses in forest ecosystems under multistress from industrial pollution was proposed. The author proposes the use of detoxication enzymes, phosphoadenine nucleotides and adenylate energy charge in insects from variously polluted regions and from controlled laboratory experiments with known chemicals and dose-effect responses. A special attention is focused on use of biomarkers from red wood ants exposed in changing abiotic natural conditions to a known concentration gradient of heavy metals. Differences between responses of Central European and Nordic ants are presented and discussed. A critical aspects of the biomarker approach for ecological effects assessment are discussed.

Forest damage has long been recorded and is caused by natural phenomena or human activity, such as overgrazing, economic overexploitation and pollution (Stanners and Bourdeau, 1995). The major effects came from interaction of human activity and natural factors. Air pollution in terms of its effects on plants and animals has been regarded as the abiotic environmental stress (Heinrichs, 1988). The hypothesis of the multiple stress, indicating that air pollutants and deposits may cause irreversible changes in plant metabolism, making them more susceptible to natural stressing factors such as nutrient deficiencies, climatic factors, fires, pathogens or insect pests has been widely accepted (Nuorteva, 1990). At the final stage, with the reduced biodiversity and distrubed food chains, the forest components are often exposed directly to increased number of phytophagous insects killing the trees.

Pollution-induced stress need therefore an "early warning system". The measurements of chemical compounds in various biotic components of an ecosystem appeared insufficient and such biomonitoring is reduced only to these chemical compounds which are persistent in the environment, i.e. heavy metals. On the other hand, many compounds (pesticides) with a very short biological life may cause a serious irreversible biological effects. Classical hazard assessment has a strong limitation because the critical levels have been set only for some compounds while in reality organisms are exposed to complex and changing levels of mixtures of pollutants (Peakall and Walker, 1994).

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Concept of biomarkers

Ecotoxicology is concerned with the monitoring and prediction of the effects of anthropogenic pollutants on the biota as early as subtle changes at lower levels of biological organisation are measurable, before adverse effects may occur at higher levels (Depledge and Fossi, 1994; Peakall and Walker, 1994; Depledge et al., 1995; Van Gestel and van Brummelen, 1996). Van Gestel and van Brummelen (1996) proposed four levels of identification of effects caused by chemical compounds: (1) below individual level, as deviations of biochemical and physiological parameters and processes, using biomarkers; (2) at the individual level, as the measures of survival, growth and reproduction processes; (3) at the population level, as life-history characteristics, changes in the genetic or age structure, population dynamics; and (4) at the ecosystem level, by measurements of species composition, abundance or diversity.

The use of biomarkers in assessment of ecosystem effects enables monitoring and predicting of stress responses on various levels of biological organisation, from molecular to community level (Lagadic et al., 1994). Biochemical responses to pollutant toxicity should be related to impairments of growth or reproduction, which depend on energy utilisation and its allocation (Depledge, 1993; Migula, 1996). Also alterations in any physiological process may reduce survivorship in a population level but these relationships have not been established well (Lagadic et al., 1994; Van Gestel and van Brummelen, 1996). The "toxicological" concept in definition of biomarker given by the NRC (1987) has been modified and adapted to ecological effect assessment by Depledge (1993) as: "A biochemical, cellular, physiological or behavioural variation that can be measured in tissue or body fluid samples or at the level of whole organisms that provides evidence of exposure to and/or effects, of one or more pollutants (and/or radiations)". Recently Van Gestel and van Brummelen (1996) redefined the concept of biomarkers and restricted their application only to: "...biochemical, physiological, histological and morphological (including appearance, pigmentation, surface deformation, etc.) measurements of 'health' and exclude behavioural effects". In their concept behavioural changes, presence or absence in a given environment are characteristic for bioindicators.

Biomarkers are new, powerful tools for detecting and documenting the exposure to and effects of environmental contamination. What will be the main role of biomarkers in environmental assessment? The primary use of biomarkers in environmental monitoring is to assess the health of the species present in order to detect and identify potential problems so that unacceptable and irreversible effects, such as mass mortality, loss of commercially or ecologically important species, can be avoided (Peakall, 1992). Their use enables to determine whether or not in a specific environment organisms are physiologically normal or, in other words, allow to identify the reversible response range in order to detect the effects of stress before permanent physiological demage ensues (Forbes and Forbes, 1996). Analysis of cellular response to pollutants combine alterations in both structure and functions. If an exposure has taken place biomarker response should be related to a given degree of impairment of growth or reproductive output, or

energy utilisation (directly affects survivorship) and can be attributed to a known amount of the specific pollutant (Depledge and Fossi, 1994).

Biomarkers may be selected to one of four groups: 1) biomarkers of exposure; 2) biomarkers of effects; 3) biomarkers of effects and exposure and 4) biomarkers of latent effects (Depledge and Fossi, 1994). Both specific and non-specific are valuable. Peakall (1994) and Peakall and Walker (1994) listed various biomarkers in relation to their specificity. The list of specific cellular biomarkers is rather limited. Well known and widely used are the acetylcholinesterase inhibition assays against organophosphosphate and carbamate compounds or an inhibition of aminolevulinic acid against lead toxicity (Peakall, 1992; Lagadic et al., 1994; Peakall and Walker, 1994). The induction of various detoxication enzymes such as mixed function oxidases or glutathione transferases or esterases by various xenobiotics and endogenous compounds is less specific (Terriere, 1984). Potent general biomarkers are involved in energetic process (Migula, 1989; Migula et al., 1993; Lagadic et al., 1994). The pool size of phosphoadenine nucleotides and adenylate energy charge (AEC) may be applied as potential indicator of sublethal stress and a functional status of multicellular organism (Atkinson, 1977; Migula, 1989; Marczyk et al., 1993; Migula and Glowacka, 1996). Also free radical scavengers, such as superoxide dismutase or katalase, have been used successfully in our laboratory (Wilczek and Migula, 1996; Chrascina et al., 1996).

Biomarkers from insects and spiders

Depledge and Fossi (1994) identified three stages of investigations allowing extrapolation from a biomarkers response to the ecosystem level: (1) Identification of ecosystem at risk; (2) identification of critical species and target populations in a limited range of species occupying different trophic levels and ecological niches and (3) predicting the likely impact of chemical pollutants including measurements of a suite biochemical, physiological or behavioural biomarkers.

Sensitive and important invertebrate species of a significant importance for the functioning of the temperate zone forest ecosystems under multistress from industrial pollution have been well identified (Nuorteva, 1990; Koricheva, 1995; Migula, 1996). Small soil-dwelling microarthropods, social ants, carnivorous insects and spiders are of special importance (Maavara et al., 1994; Migula, 1996; Migula and Głowacka, 1996; Wilczek and Migula, 1996). Since insects play an essential role in natural forests and managed plantations as the dominant components of many food webs their sensitivity to pollutants may also affect other elements of an ecosystem (Koricheva, 1995). In order to allow the application of biomarkers to routine monitoring programmes some of molecular biomarkers from insects and spiders (enzyme involved in energy metabolism and detoxication processes; adenylate energy charge and pool of the phosphoadenine nucleotides) from our studies will be reported below. More attention will be also paid to experimental use of biomarkers from social insects exposed in natural conditions to a measurable gradient of a stressing factor (heavy metals).

Detoxication enzymes

Resistance of organisms to xenobiotics depends on detoxication enzymes. The important are such as microsomal oxidases, glutathione transferases and esterases which can be induced by various xenobiotics. A flavoprotein, NADPH-cytochrome reductase is one of the enzymes of the mixed function oxidase. The assays of this enzyme in phytophagous insects from a pollution gradient confirmed a positive correlation of enzyme activity with increased level of pollutants (Migula et al., 1990). Insects from different areas exposed in laboratory to excessive stressing factor (i.e. heavy metals) showed different patterns of detoxication. A stimulation characterised the Large white (*P. brassicae*) from a moderately polluted site, while insects from a heavily polluted area reduced rates of activity after cadmium and lead treatment, thus indicating a significant failure in detoxication abilities (Fig. 1; Kędziorski, unpubl.).

Spiders are particularly exposed to anthropogenic pollutants, specially, as macroconcentrators, to heavy metals (Marczyk et al., 1993; Wilczek and Migula, 1996). Detoxication enzymes depend on toxic compounds, efficiency of internal regulation processes and energy allocation. Studying these enzymes in selected species of spiders, which differ in hunting activity, type of web production and developmental processes (Wilczek and Migula, 1996), we found that detoxifying systems in spiders appeared inducible and efficient enough to maintain normal physiological responses (Figs 2–5). A web spider *L. triangularis* and a wolf spider *P. palustris* seem to be well adapted to polluted environment, but their detoxication strategy and energy distribution are different. *L. triangularis*

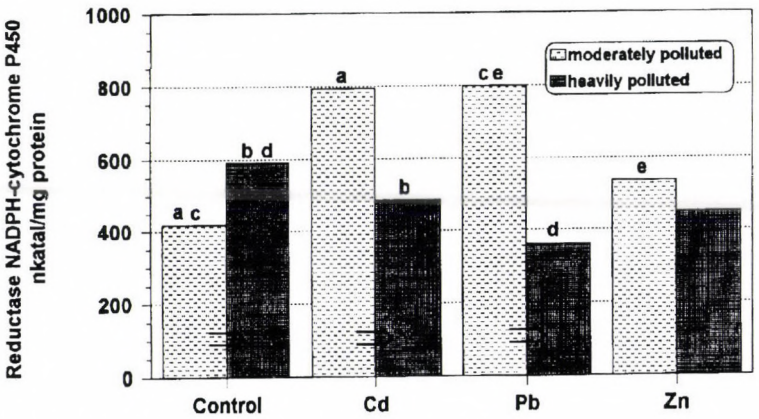

Fig. 1. Activity of reductase NADPH-cytochrome P450 in Large White L_5 caterpillars (*Pieris brassicae*) from moderately and heavily contaminated areas (Control) exposed to cadmium, lead and zinc in the laboratory. The same letter indicate significant differences of means between control and exposed caterpillars of the same age. Arrows indicate site-related significant differences of means ($p < 0.05$). (From A. Kędziorski, unpublished msc.)

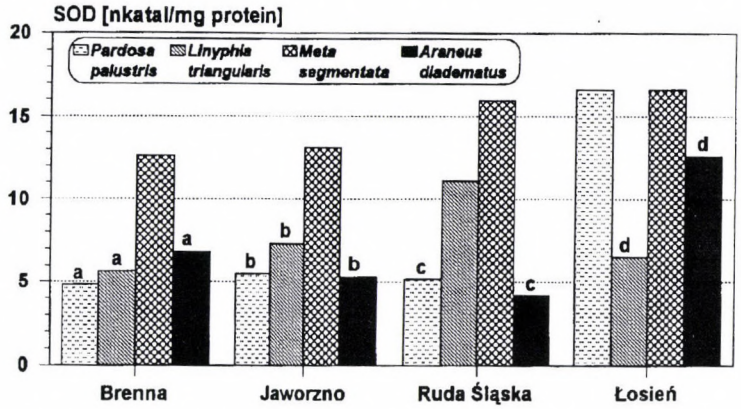

Fig. 2. Activity of superoxide dismutase (SOD) in spiders from four localities placed in increased order of industrial pollution (Poland). The same letter denotes lack of significant differences between means ($p > 0.05$)

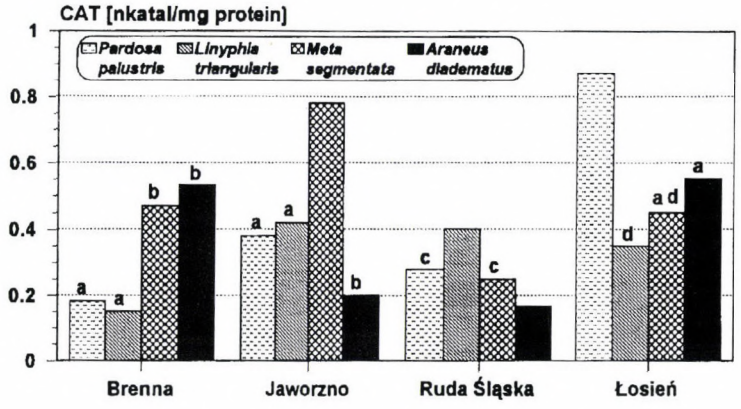

Fig. 3. Activity of catalse (CAT) is spiders from four localities placed in increased order of industrial pollution (Poland). The same letter denotes lack of significant differences between means ($p > 0.05$)

accumulate less metals but increase reactions of conjugation (GST) and elimination of free radicals (SOD). *P. palustris* accumulate more metals and activate biotransformation of xenobiotics and elimination of free radicals with higher anaerobic metabolism (Wilczek and Migula, 1996).

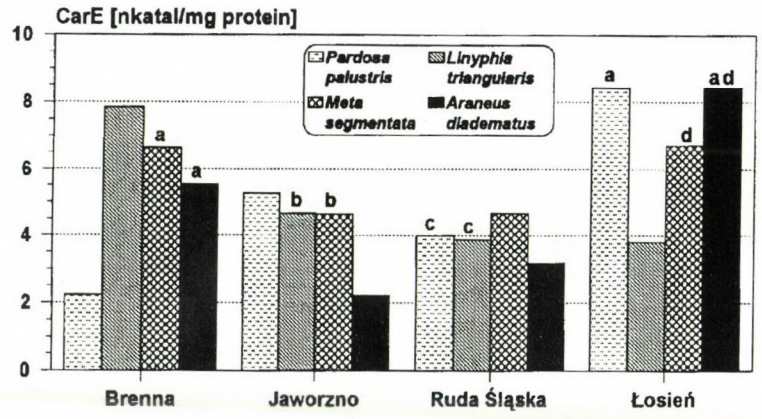

Fig. 4. Activity of carboxylesterase (CarE) is spiders from four localities placed in increased order of industrial pollution (Poland). The same letter denotes lack of significant differences between means ($p > 0.05$)

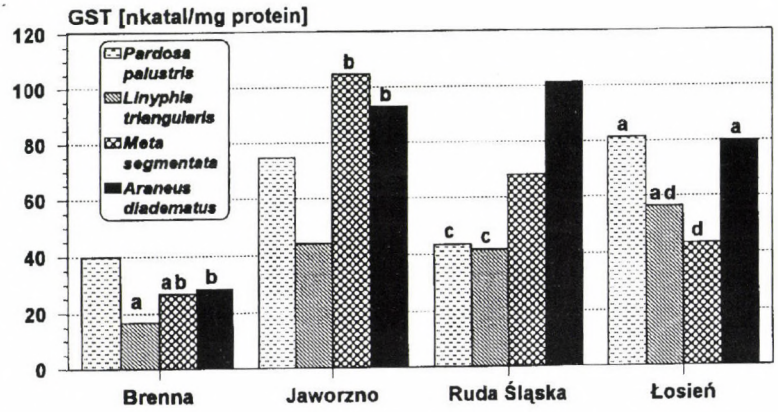

Fig. 5. Activity of glutathione S-transferase (GST) is spiders from four localities placed in increased order of industrial pollution (Poland). The same letter denotes lack of significant differences between means ($p > 0.05$)

Phosphoadenine nucleotides and adenylate energy charge (AEC)

Concentration of phosphoadenylates (ATP + ADP + AMP) and the AEC in adult insects sampled at variously polluted sites in Poland are presented in Figs 6 and 7. Insects from uncontaminated or moderately contaminated areas usually showed a higher level of both the AEC and adenylate pool size. Only in one species (*Ch. brunneus*) from heavily polluted site the AEC exceeded the values calculated for insects from more favourable

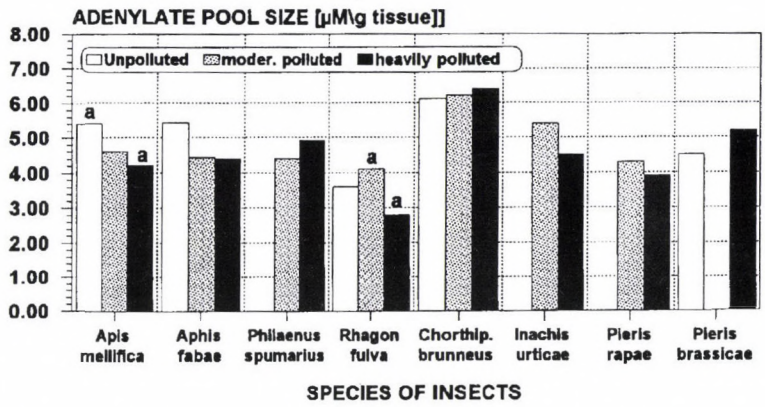

Fig. 6. Concentration of phosphoadenine nucleotides in various species of insects in relation to the level of local contamination with industrial pollutants. The same letter denote statistical signifacance of means between localities ($p < 0.05$)

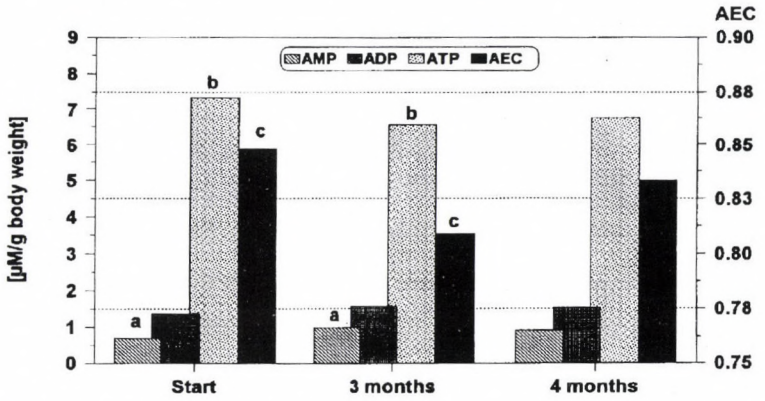

Fig. 7. The adenylate energy charge index calculated for various species of insects in relation to the level of local contamination with industrial pollutants. The same letter denote statistical significance of means between localities ($p < 0.05$)

area. In majority of insects maintaining high AEC level while withstanding anthropogenic stress the ATP level and the total phosphoadenylate pool may decrease proportionally. Our experiments with caterpillars taken from variously polluted sites exposed to excess of cadmium and lead in the natural diet have clearly shown that energetic strategy was species-dependent. The Small Tortoiseshells (*I. urticae*) maintained high level of the AEC, but reduced strongly concentration of adenylates. Reversibly, the Large white (*P. brassicae* and *P. rapae*) significantly reduced the AEC index but kept high levels of adenylates.

Spiders from the areas where heavy metals dominate as stressing factors kept high concentrations of ATP in tissues. In areas with dominating organic and gaseous pollutants the Araneids indicated high aerobic activity, but the Linyphids increased anaerobic activity and pool of adenylates while the AEC index was strongly reduced, even below 0.7 (Marczyk et al., 1993).

Social insects – biomarkers from red wood ants

Red wood ants are dominant elements in boreal forests known as potential control agents of forest pest with a high ability to cumulate heavy metals (Maavara et al., 1994; Migula and Głowacka, 1996). The source of metals for ants in mainly honeydew and their producers (Nuorteva, 1990; Whittaker, 1991; Maavara et al., 1994). Starting from the findings of Starý and Kubiznaková (1987) that red wood ants are able to cumulate high levels of metals, in a series of experiments we checked what would happen after exposure of ants to similar levels of metals in more severe Nordic conditions (coniferous forest at Tenala, Southern Finland). For this purpose selected ant colonies (*Formica aquilonia*) were fed with honey containing various concentrations of cadmium and/or methyl-mercury, provided as natural contaminant of the fish carcass. This method was applied in natural conditions during four years enabled determinations of the biochemical and physiological concentration-related effects of single metals or their joint actions and the emergence of compensatory mechanisms after repeated contamination. The biomarkers were activity patterns of metal-sensitive enzymes involved in energy metabolism or detoxication processes together with concentration of the phosphoadenine nucleotides and the AEC (used as indices of regulatory capability of metabolic processes under environmental stress). The excess of cadmium evoked metabolic disorders in workers but also in the pupal stage, but induces some detoxifying enzymes. The pool of phosphoadenylates was depleted and the AEC indices decreased (Migula et al., 1993; Maavara et al., 1994; Migula et al., 1997).

Prolonged feeding with excess of Cd and Hg caused time-dependent decrease of the phosphoadenine pool with diminished AEC index and reduced activity of ATPases, but ants were still able to maintain their energetic balance. Despite the inhibitory effects on activity of enzymes involved in energy metabolism and indirect stimulation of detoxifying enzymes, metal-dependent compensatory mechanisms were evoked in consecutive generations of ants. Ants contaminated jointly with Cd and Hg lost their abilities to cope with too high level of pollutants and even families disappeared through increased mortality or migration to other colonies (Migula et al., 1997).

Similar biomarkers were analysed in order to determine functional state and possibilities of another red wood ant (*Formica polyctena*) from five localities variously contaminated by industrial pollutants in Southern Poland (Migula and Głowacka, 1996). The analysis of various biomarkers proved that tolerance limits were not exceeded, since energetic processes were maintained in normal levels. The concentration of total phosphoadenylates was slightly lower than stated in Finnish ants poisoned with cadmium, but

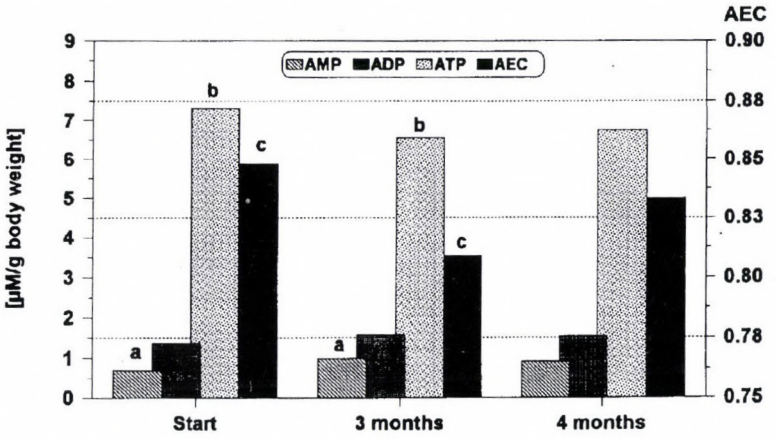

Fig. 8. Concentration of phosphoadenine nucleotides and the adenylate energy charge index calculated for *F. polyctena* workers exposed to excess of cadmium in natural conditions. Different letters denote time-related statistical significance of means within measured parameters ($p < 0.05$)

the AEC index was always above 8.8. The strategy of these ants differs from that of *F. aquilonia*, since they maintained narrow limits of the AEC due to higher variability of the phosphoadenylate pool size (Migula and Głowacka, 1996). This strategy needs more effective systems for energy restoration and needs more energy allocated for detoxication processes (Migula et al., 1989).

Comparisons between Finnish *F. aquilonia* and Polish *F. polyctena* showed a higher tolerance of metals of the latter species, proving that under more favourable climatic conditions tolerance of industrial pollutants by red wood ants would be higher. In order to check whether additional stressing factor (cadmium) may change their tolerance limits another feeding experiment with the excess of cadmium was carried out in natural conditions (mixed spruce and pine forest in Zarzecze – Beskid Mały, Carpathians). Honey with cadmium (300 µg $CdCl_2$/g honey) was offered in a similar way as it was described in earlier studies (Migula et al., 1993; Migula et al., 1997). Artifical feeding resulted in more than 9-fold increase of cadmium burden in workers (from 11.3 to 98 µg/g dry weight) and was maintained at this level for at least three months, then reduced to the level of 35 µg/g dry wight. In similar conditions the uptake of cadmium by *F. aquilonia* was higher and even exceeded 150 µg Cd/g dry weight. Since cadmium had been the only variable stressing factor for contaminated and uncontaminated ant families, all alternations in used biomarkers reflected its toxic effects. They were reflected by a significant reduction of ATP parallel with increased concentration of AMP, concomitant with decreased AEC indices (Fig. 8). The assays of enzymes involved in energy metabolism indicated only a slight decrease of isocitrate dehyrogenase ($p < 0.05$) but much stronger reduction of Na, K-ATPase, by nearly 30% (Fig. 9). Enzymes involved in various detoxifying processes showed quite a different pattern. They were stimulated: GST

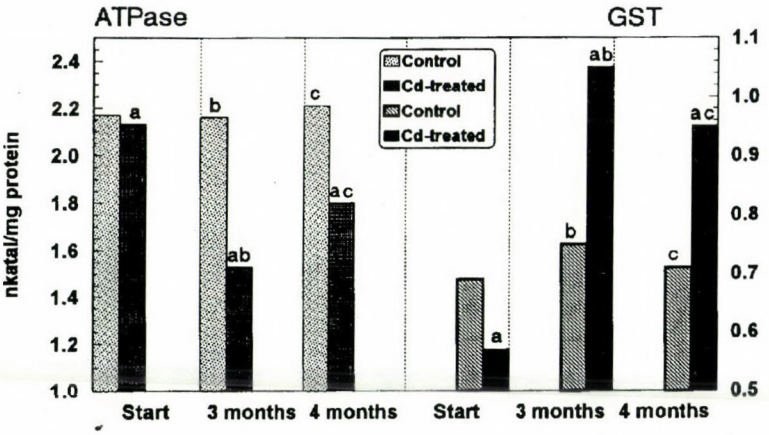

Fig. 9. Activity of Na, K-ATPase and glutathione S-transferase (GST) in *F. polyctena* workers exposed to excess of cadmium in natural conditions. The same letters denote time-related statistical significance of means ($p < 0.05$)

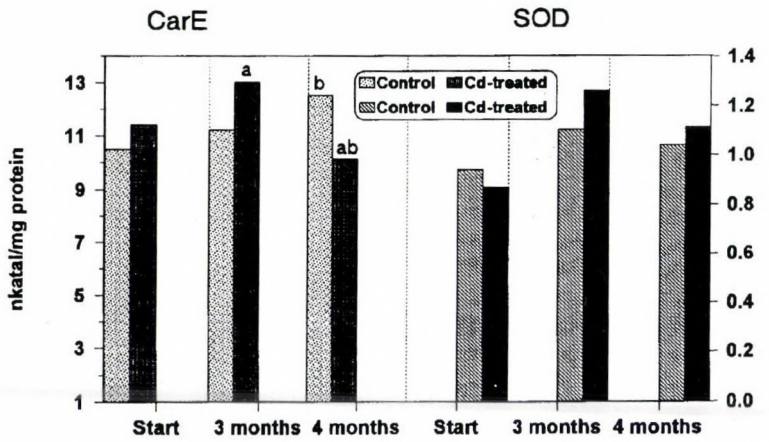

Fig. 10. Activity of carboxylesterase (CarE) and superoxide dismutase (SOD) in *F. polyctena* workers exposed to excess of cadmium in natural conditions. The same letters denote time-related statistical significance of means ($p < 0.05$)

by 45% (Fig. 9) and carobxylesterases by 17–22% (Fig. 10). Superoxide dismutase, active as free radical scavenger, remain unchanged (Fig. 10). All indicated changes were reversible, since four months after the beginning of experimental feeding the mean values of measured parameters slightly returned towards control values, parallel with the elimination of cadmium from tissues of ants (Figs 8–10). Electrophoretic analyses made in order to check whether physiological differences indicated between selected popula-

tions of *F. polyctena* may originate from their genetic variability and relatedness confirmed that only specific loci of enzymes may be influenced, but the overall variability was not affected (Migula et al., 1996)

F. polyctena appeared to be better adapted to industrial pollutants and more easily tolerated excess of a single metal than *F. aquilonia* from more severe climatic region. The last species showed much stronger correlation between cadmium load in the tissues and biochemical biomarkers mentioned above (Migula et al., 1993), but allocated more energy to detoxifying processes than *F. polyctena*. Moreover, developmental stages of Finnish ants in consecutive generations were more sensitive to pollutants (metals). Thus higher sensitivity of *F. aquilonia* might stronger reduce their ability to control the numbers of herbiovers and affects the forest condition (Whittaker, 1991; Maavara et al., 1994). Under multistress and interactive relations of various pollutants such negative effects might increase and lead to irreversible changes in biotic components causing killing of the forests.

Limitations in application of biomarkers

Biochemical measures are useful in monitoring for effects before they reach higher biological organisation level. There is no doubt that biomarkers as new tools in environmental risk assessment would help in solving the problem of environmental management. Nevertheless, there is a lot of limiting factors reducing their proper use, mainly due to lacking good links between them and a population from which an individual for measurement was taken (Weeks, 1995; Van Gestel and van Brummelem, 1996). This would be also the case for changes in enzyme activity which should be related with other responses of an organism or even with higher biological organisation level.

We should always take into account a great individual variability in a population, which is not responding homogeneously to a chemical stressor. It is yet impossible to determine what proportion of a population and which individuals must be adversely affected by pollutants before decline in the population ensues (Van Straalen, 1994).

For many biomarkers we need to know their "physiological" levels and how environmental and biotic factors will modify their responses to pollutants, relative to those seen in controlled laboratory conditions. Dose-effect relationship should be established in controlled experiments and correlations from the field studies between the level of biomarker and internal amounts of contaminants (Lagadic et al., 1994; Pekall and Walker, 1994). We do not know yet to what extent various laboratory tests will be capable of predicting likely exposure or effects of pollutants on ecosystem.

The best would be use of only non-destructive biomarkers and sampling, but more accurate measurements need to be repeated without losses in population while studying different developmental stages. Little attention has been paid to genotypically or phenotypically determined interpopulation differences in susceptibility to pollutants; biogeographical differences in pollutant toxicity, persistence of biomarker response with time. Some biomarkers are not sensitive enough to detect effects of pollutants when their

concentration is low and others might be too sensitive for use in environmental assessment (Depledge, 1993). Concluding, biomarkers will give additional information impossible to obtain from monitoring of chemical compounds in the environment or their concentration in the biota, but studies are needed to fill the gap between biomarkers and population and ecosystem characteristics.

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Dendrochronological Indication of Entomoresistance in *Pinus eldarica* Against *Dioryctria sylvestrella* Ratz (Lepidoptera, Pyralidae) in Georgia

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Eldarica pine (*Pinus eldarica*), a famous relict from the tertiary period, grows endemically on sterile hill slopes. There it has developed strong resistance not only against the environmental stresses, but also against the European stem borer (ESB) *Dioryctria sylvestrella* (Ratz.). This resistance persists when Eldarica pine is cultivated in Georgia on sterile grounds. The ESB resistance is, however, breaking down when Eldarica pines are cultivated on fertile soils, where the pines are growing faster. It is to say that silvicultural practices are responsible for the ESB damage on Eldarica pine in Georgia. In principle this kind of situation may, however, develop also in cases where the artificial fertilization or polluting fallout of nitrogen is fertilizing forest soils.

Eldarica pine is a famous endemic relict from the tertiary period. Its only known endemic population, covering 400 hectares, occurs in the area between the Black and Caspian seas. There it grows on the slopes of the mountain Eller Ouchi. The inclination of these slopes varies between 25 to 50°. The average annual precipitation is 200 mm, from which 70 mm falls during the spring–summer season (Safarov, 1962). Eldarica pines must thus have xerophytic characteristics. According to their dry resistance they have been used in reforestation of dry environments in the surroundings of Tbilisi, East Georgia. It grows there well on denuded hill slopes, on sandstone and poor unirrigated soils, from sea level elevation up to the height of 700 m. Higher up it suffers from frosts. Experience about artificial Eldarica pine cultivation exists for a time longer than hundred years. During whole this time, ESB demage has been observed in some special localities. This same pest has been observed on pine platations in Germany (Schwerdfeger, 1981), in France (Jachtel et al., 1996) and in some other countries.

The present study was performed in order to evaluate the role of soil quality, intensity of radial growth, width of year rings, number of resin ducts and the density of early and late wood for the development of ESB infestation.

Materials and Methods

The material for the dendrochronological analyses was collected in the surroundings of Tbilisi from such Eldarica pine cultures where the age of the trees was 20–25 years. There existed two categories of sampling points: 1) Those with thin humus, poor

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vegetation, and dry soil and 2) Those with thick humus, rich vegetation and moist soil. From each sampling point, 0.01 ha in size, ten samples were taken by Pressler's bore from the pine stems from a height of 1.3 m.

The width of the annual rings was measured from the samples by a light refraction eyepiece MBI-2 with a precision of 0.1 mm. The time series were processed to annual ring width indexation by a mode described by Schweingruber (1987). Radiographic densitometry was used in measurements of the early and late wood densities (Kyncl and Dobry, 1987; Schweingruber, 1987). The abundance of resin ducts in annual rings was counted from a roentgen film by using an eyepiece.

Results

The quality of the environment and Eldarica pines in relation to the degree of ESB infestation in two categories of observation points in Tbilisi in given in Table 1. Some details are described in Figs 1–3.

Table 1

The quality of the environment and Eldarica pines in relation to the degree of ESB infestation on sterile and fertile localities in Tbilisi

Observation points	Fertile (1)	Sterile (2)
Environment		
Elevation above sea level	450 m	450 m
Inclination	0–5°	5–15°
Exposure	South-East	South
Soil	Fertile	Sterile
Humus	Thick	Thin
Soil humidity	Moist	Dry
Pinus eldarica		
Structure of stand	Mixed culture	Monoculture
Age	20–25 years	20–25 years
Average height	6–7 m	4–5 m
Average stem diameter at 1.3 m	16–18 cm	20–22 cm
Indexed width of annual rings	Wide, Fig. 1	Narrow, Fig. 1
Cell size	Big	Small
Cell walls	Thin	Thick
Tissue density	Loose	Dense
X-ray radiogram + density curve	Fig. 3	Fig. 3
X-ray density maximum	0.710 kg m^{-3}	0.950 kg m^{-3}
Mode of growth	Rapid, Fig. 1	Slow, Fig. 1
Resin channels/annual ring	10, Fig. 2	4–5, Fig. 2
Dioryctria sylvestrella		
Percentage of ESB-damaged pines	80–100%	0%

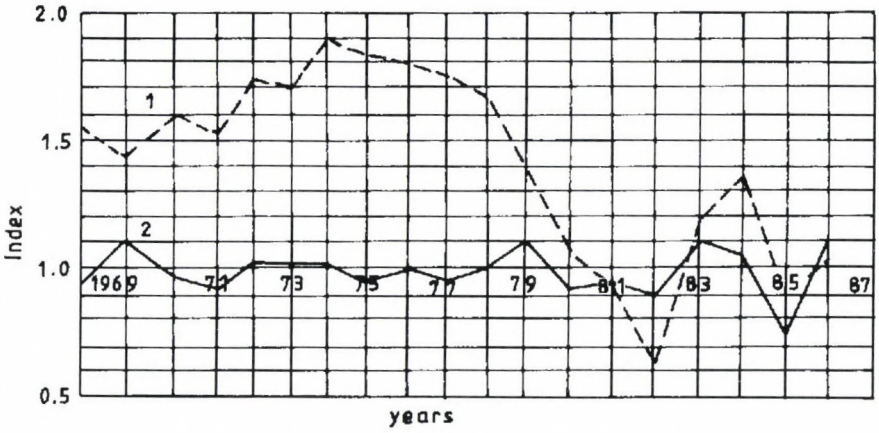

Fig. 1. Indexed values of annual rings in *Pinus eldarica* near Tbilisi in Georgia. 1) in ESB damaged trees, 2) in undamaged trees (Sukhovolsky et al., 1987)

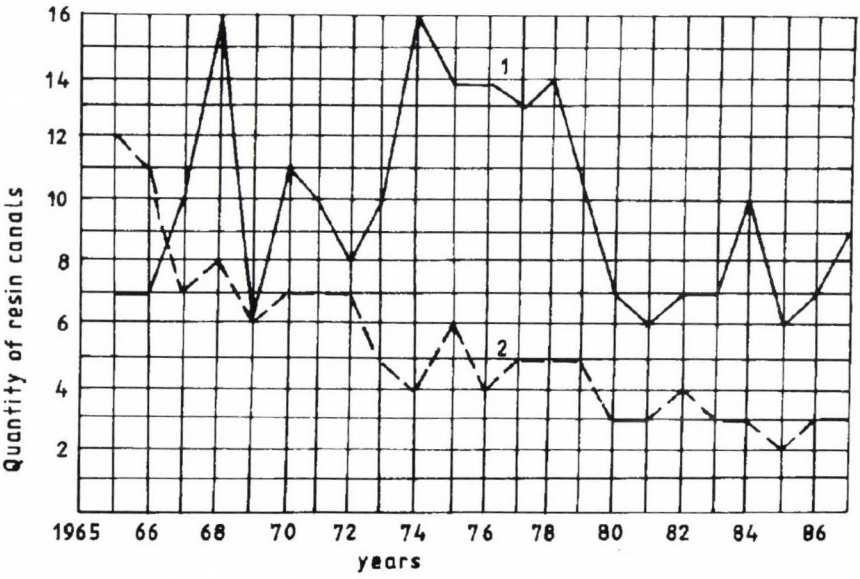

Fig. 2. Abundance of resin canals/year ring in ESB damaged (1) and healthy *Pinus eldarica* trees (2)

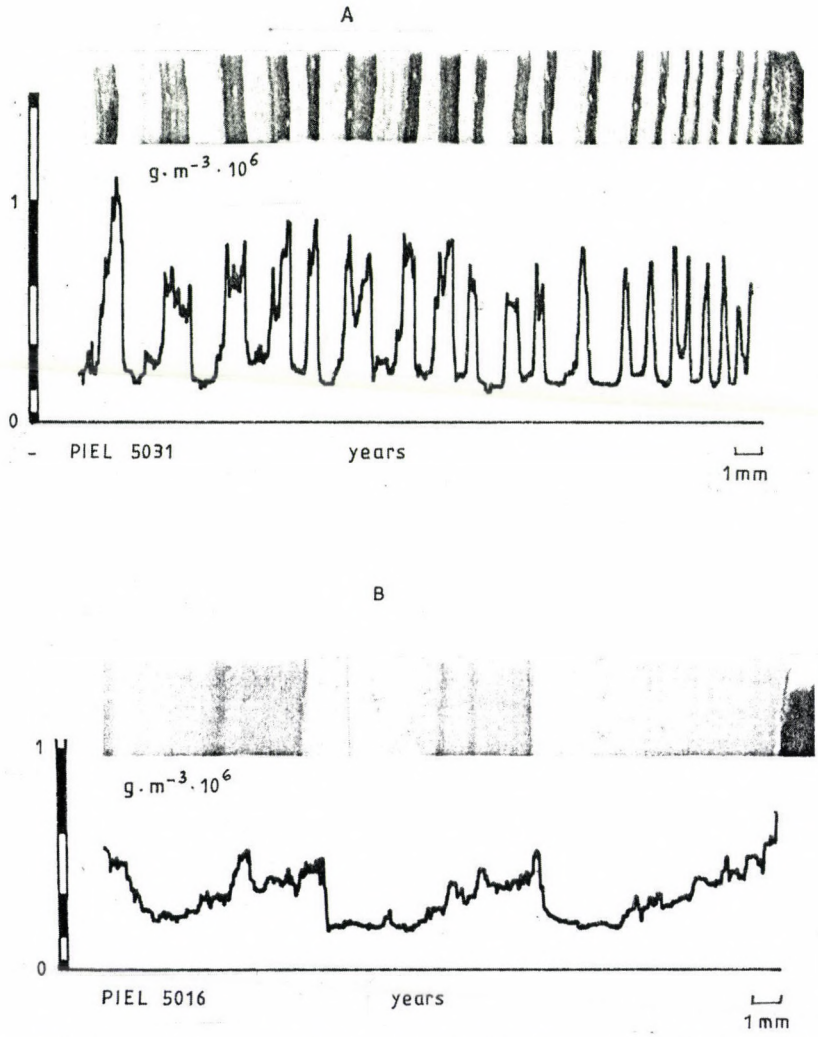

Fig. 3. X-ray radiograms and the corresponding density curves from healthy (A) and heavily ESB damaged *Pinus eldarica* trees (B) near Tbilisi in Georgia

Discussion

The results show that *Pinus eldarica* specimens growing in their sterile and stressing environment have developed strong resistance through their slow growth, not only against the environmental stresses, but also against ESB. This natural resistance is breaking down when Eldarica pines are cultivated in soils, which are more fertile than the

soils in the original biotopes of Eldarica pine. It is to say that silvicultural practices are responsible for the ESB damage on Eldarica pine in the surroundings of Tbilisi. In principle this kind of situation may, however, develop also in cases where polluting fallout of nitrogen is fertilizing forest soils.

In fact the ESB resistance was very strong in Eldarica pines growing in conditions similar to their natural environment. This was shown by transfer experiments of ESB larvae from susceptible to resistant pines. The transferred larvae died during the few first days. It was also noted that no natural infestation occurred in resistant pines growing in near vicinity to susceptible ones.

A plenty of resin was flowing out from trunks and branches infested by ESB. An anatomical study showed that the activity of ESB-larvae in the cambium induced development of resin channels. They formed one kind of defensive line against the pest. The damaged trees have in average 10 resin channels/annual ring (Fig. 2). This density is about twice as much as in the resistant trees. Harborne (1982) has shown that some compounds in the resin may in some cases act as pest attractants but in some other cases they are repellents. We analyzed the percentage of alpha-pinene in the pitch of Eldarica pines. We found 39.5% in the resistant pines, 49.7% in weakly injured, 54.0% in averagely damaged and 41.5% in strongly damaged ones.

The present paper describes one case, where silvicultural measures have promoted an outbreak of *Dioryctria sylvestrella* on Eldarica pine. Lots of other cases about anthropogenic promotion of forest insect outbreaks have recently been published. They include the important new multistress disease killing forests in Europe and Northern America. Anthropogenically initiated pest outbreaks will obviously take a central position in the working field of forest entomology. It existed by the 20th International Congress of Entomology in Firenze two symposia, which can be considered as messengers for this: "The role of air and soil pollution in development of forest insect outbreaks" chaired by P. Nuorteva and "Effect of the possible global warming on the insect diversity and distribution" chaired by F. Kozar.

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Population Dynamics of a Herbivore in an Industrially Modified Landscape: Case Study with *Melasoma lapponica* (Coleoptera: Chrysomelidae)

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Factors affecting population dynamics of willow feeding leaf beetle, *Melasoma lapponica*, were studied on Kola peninsula, NW Russia. This leaf beetle was common within the impact zone of the Severonikel copper-nickel smelter, contary to unpolluted habitats. In 1991–1996, peak beetle densities were associated with birch- and willow-dominated communities in moderately polluted zone. Mass appearance of *M. lapponica* was presumably promoted by increased abundance of willows in secondary communities replacing coniferous forests and decrease in densities of predators due to habitat deterioration and/or toxicity problems. Population levels of *M. lapponica* within the impact zone seem to be related to host-plant quality. Ambient SO_2 in concentrations occurring within the deforestated zone improved foliar quality of willows for *M. lapponica*, but close to the smelter high SO_2 concentrations may be directly toxic for larvae. Although metal pollutants may be toxic as well, no detrimental effects were revealed in experiments with *Salix borealis*, the species most preferred by *M. lapponica*. Combination of negative direct and positive indirect effects explained density pattern along pollution gradient, with high density in moderately polluted sites and low sensity in heavily polluted sites. Outbreak of *M. lapponica* in moderately polluted sites resulted in severe damage of *S. borealis* already in 1993. Defoliation appeared to be stressful for willows, as demonstrated by an increase in leaf fluctuating asymmetry. Feeding on stressed plants decreased beetle performance and may have contributed to decline of beetle populations in the outbreak sites in 1995–1996. We conclude that pollution seemed to create prerequisites for beetle outbreak, but did not suppress plant responses to previous-year(s) damage.

Industrial emissions are known to affect population densities of insects (Riemer and Whittaker, 1989; Kozlov, 1990; Heliövaara and Väisänen, 1993), sometimes provoking outbreaks of forest pests (Führer, 1985). Density changes of herbivorous insects are usually explained by the combination of (i) changes in host plant quality, (ii) altered densities of natural enemies, (iii) direct toxicity of pollutants, and (iv) changes in plant community structure, e.g. in abundance of the main host plants (Hughes, 1988). However, the relative role of mechanisms acting as density regulators in industrially modified landscapes is poorly understood and existing data are sometimes contradictory (Riemer and Whittaker, 1989; Kozlov, 1990; Whittaker and Warrington, 1990). Furthermore, damage induced alterations on plant quality (Haukioja, 1980, 1990; Karban and Myers, 1989) have seldom been considered in studies on pollution impact on herbivores (Neuvonen et al., 1990; Suomela and Neuvonen, 1997). Furthermore, long-term (more than for three successive years) observations on insect densities along pollution gradients are scarce (Selikhovkin, 1992). In this paper, we summarize our knowledge on the relative importance of factors affecting population densities of the leaf beetle *Melasoma lapponica* in sites with different pollution loads.

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Materials and Methods

Study area

The area surrounding the city of Monchegorsk on the Kola Peninsula, NW Russia (68° N, 33° E), is one of the most extreme examples of terrestrial pollution in the boreal zone. Sulphur and heavy metals, emitted by the Severonikel smelter since 1939, have caused widespread destruction of soils and vegetation. Coniferous forests which were dominant in the impact zone during the preindustrial time have completely vanished up to 10 km south and 6 km north of the smelter, and have been replaced by willow- and birch-dominated communities and industrial barrens. The total area influenced by aerial pollution exceeds 10 000 km^2 (Kozlov and Haukioja, 1995). In 1994, mean ambient SO_2 concentration near the smelter during the growth seasons was ≈ 250 $\mu g/m^3$, and foliar nickel in *Salix borealis*, the favourite host of *M. lapponica*, reached 250 μg/g (Zvereva et al., 1995a, b, and unpublished data).

Ten study sites were established in 1993 along two transects, 1 to 47 km from the smelter. The most distant site (47 km S) has nearly background pollution level and zero density of *M. lapponica*.

Insect–plant system

M. lapponica is a medium-sized (5–8 mm length) black-and-red patterned leaf beetle. In our study area, it feeds on willows only, although aspen, birch and alder have been also reported as its host plants in central Europe (Koch, 1992). Beetles hibernate in soil and appear on willows at the time of leaf flush; copulation occurs during the early summer. Females produce on the tree batches of 30–40 eggs, and place them on leaf underside. Young larvae feed gregariously whereas the last instars disperse over host individual and pupate in the beginning of August on the upper surface of uppermost leaves on the shoot. Beetles emerge in mid-August, feed for some days and then move to the overwintering places.

Eight willow species (*S. borealis*, *S. caprea*, *S. phylicifolia*, *S. hastata*, *S. lapponum*, *S. glauca*, *S. myrsinites*, *S. lanata*) have been recorded in our study plots (Zvereva et al., 1995a, b). Nearly 80% of *M. lapponica* fed on *S. borealis* and *borealis*-dominated hybrids; the remaining 20% fed mostly on *S. caprea* and *S. phylicifolia* (Zvereva et al., 1995a, b).

Field surveys

Densities of overwintered beetles were estimated by 10-min counts on bushes of all willow species in 1993–1996 (Zvereva et al., 1995a). Larval densities and pattern of host-plant use were evaluated in plots 2 × 25 m size (four plots in each study site) by recording species, sex and size of all willow bushes and counting *M. lapponica* larvae on each bush (Zvereva et al., 1995b). To account for parasitism rates, all larvae and prepu-

pae were sampled from 10 bushes of *S. borealis* at each study site at the beginning of pupation and reared in laboratory.

Measurements of leaf size, shoot length, and leaf fluctuating asymmetry were conducted in five clones of *S. borealis* in each study site (Zvereva et al., unpublished). Foliar metals were determined in leaves collected from these bushes in mid-July by either X-ray fluorescence (Kozlov et al., 1995) or ICP-spectrophotometry. Ambient concentrations of SO_2 were estimated by passive lead-dioxide absorbers (the method describes by Barkan, 1993) exposed in our plots during the growth season in 1994.

Field experiments

Effects of SO_2 on larval performance were studied in 1994 by fumigation of naturally growing bushes of *S. borealis* enclosed in plastic chambers (Kozlov et al., 1996). Direct and indirect effects of SO_2 were separated by comparing larvae fumigated together with their hosts and larvae reared outside chambers but fed fumigated foliage.

Suitabiliy of unpolluted habitats for *M. lapponica* was evaluated in 1994–1995 by introducing egg batches to the site where this leaf beetle had not been discovered. Larval development has been followed, and hibernated beetles were searched next year after introduction.

Laboratory experiments

Host-plant quality was assessed by rearing larvae in laboratory (Zvereva et al., 1995a, b). We recorded duration of larval development, relative growth rate of larvae, pupation rate and weight of emerged beetles. The preference of host plants originating from sites which differ in pollution load and previous-year defoliation level was assessed by trials with leaf disks (Zvereva et al., 1995b; Zvereva and Kozlov, 1996).

Results and Discussion

Beetle densities

As in Fennoscandia, the distribution of *M. lapponica* is patchy on Kola peninsula. We have found only two populations of this leaf beetle outside polluted zone: in Khibiny Mts. and 20 km SW Dalnie Zelentsy, close to the Barents sea shore. Both these populations were recorded in open habitats at the altitudinal/latitudinal tree line.

In 1989 we observed high density of *M. lapponica* 1 km N of the Severonikel smelter. Although no counts were conducted in 1989, we noted that beetle densities decreased with an increase in distance from the smelter. However, already in 1991 the highest densities were observed in moderately polluted sites. Beetle densities in these sites increased since 1991, reaching the peak in 1994, and then started to decline, whereas in both heavily and slightly polluted plots densities were at about the same level during 1991–1996 (Zvereva et al., 1996a, and unpublished data).

Host plant vigour and foliar quality

In our studies, neither leaf fluctuating asymmetry (FA) which is a non-specific stress indicator (Clarke, 1992) nor vigour parameters, such as leaf size or shoot growth, or leaf water content, or the proportion of generative shoots, indicated pollution-induced stress on *S. borealis* (Zvereva et al., unpublished data). Still foliar quality of willows varied with pollution: plants originated from moderately polluted site (data of 1993: Zvereva et al., 1995a) and plants fumigated with SO_2 in the field experiment (Kozlov et al., 1996) were better quality hosts for *M. lapponica* in comparison with controls. Even the highest ambient concentrations of SO_2 recorded near the smelter (ca 1000 $\mu g/m^2$) increased suitability of *S. borealis* foliage for *M. lapponica* (Kozlov et al., 1996).

Bushes of *S. borealis* were severely damaged, and some of them were almost completely defoliated by *M. lapponica* by August of 1993–1996 in two moderately polluted sites (Zvereva and Kozlov, 1996, and unpublished data). FA of *S. borealis* showed no among-site variation in 1992, but significantly increased in high density sites in 1994–1995, indicating that defoliation was stressful for *S. borealis* (Zvereva et al., 1997).

Before the first severe defoliation, in July 1993, indices of larval performance of *M. lapponica* in high density sites were either better or the same as in low density sites. However, in 1994 and 1995, the years following severe defoliation of willows in high density sites, performance of *M. lapponica* (in terms of survival, developmental time and beetle weight) decreased with increasing beetle density. Retarded larval growth in high density sites was related to decreased consumption rate, whereas efficiency of conversion of ingested food was similar in high and low density plots. Consistently with performance experiments, preference trials demonstrated that beetles both avoided (low proportion of injured disks) and rejected after a short feeding (low consumption per injured disk) *S. borealis* from high density sites, choosing plants from low density sites (Zvereva and Kozlov, 1996). These results indicated defoliation-related decrease in quality of *S. borealis* foliage (Zvereva et al., 1997).

Leaf FA of *S. borealis* increased in high density plots in 1994, the year after large scale defoliations. Since this coincided with decrease in beetle performance, and the plot-specific indices of beetle performance and FA were negatively correlated both in 1994 and 1995, FA may indicate induced resistance in this willow species (Zvereva et al., 1997).

Natural enemies

Although more than 1000 field-collected egg batches of *M. lapponica* hatched in the laboratory, no egg parasitism has been observed. In the field, egg batches were sometimes damaged by larvae of unidentified syrphid flies; nearly all batches introduced to clean sites were destroyed by forest ants (*Formica* spp.). Thus, low abundance of *Formica polyctena* and other ants near the smelter (Kozlov, 1997) may substantially contribute to density increase of *M. lapponica* in the impact zone.

Two species of parasitic flies infested larvae of *M. lapponica*: *Cleonice nitidus-*

cula Zett. (Tachinidae) and *Megaselia rubricornis* Schmitz (Phoridae). Parasitism rate depended on neither beetle densities nor the level of pollution (Zvereva et al., 1996a).

Both nymphae and adult bugs (*Lygocoris rugicollis* Fallén, *Psallus aethiops* Zett., *Anthocoris nemorum* L., *Orthotylus boreellus* Zett.) were frequently observed feeding with larvae and prepupae of *M. lapponica*. Occasionally, larvae of Coccinellidae and Syrphidae as well as some spiders fed with young beetle larvae. However, densities of predators have not been monitored in our sites.

Densities of redstarts, Siberian tits and pied flycatchers were 2 to 23% of the reference level in severely polluted habitats, and 16 to 61% of reference level in moderately and slightly polluted habitats (Gilyazov, 1993), suggesting decrease in predation pressure on *M. lapponica* with an inrease in pollution. Importance of predation was indirectly demonstrated by our introduction experiment: although dozens of *M. lapponica* beetles emerged from pupae by August 1994, only two beetles were found next June.

Toxicity of pollutants

Neither preference tests nor performance trials with *S. borealis* revealed detrimental effects of heavy metals (Zvereva et al., 1995a; Zvereva and Kozlov, 1996). Sulphur dioxide, a major gaseous pollutant emitted by the Severonikel smelter, was toxic for *M. lapponica* larvae in concentrations exceeding 1000 $\mu g/m^3$, as revealed by fumigation experiments (Kozlov et al., 1996).

Habitat changes

Human-induced environmental deterioration around the smelter resulted in expansion of willow-dominated communities and thus created beneficial conditions for *M. lapponica*. Densities of *S. borealis* in the impact zone were 3 to 10 times higher than in reference sites; however, within the impact zone willow densities did not vary with pollution, and the density variation of *M. lapponica* was not related to abundance of the most preferred host plant (Zvereva et al., 1996a). Thus, environmental deterioration just increased the proportion of habitats suitable for *M. lapponica*.

Since forest decline in the impact zone resulted in increased illumination of willow bushes, we tested the hypothesis that illumination itself may improve host-plant quality. Five bushes of *S. borealis* growing in small clearings in an unpolluted site (36 km S of the smelter) were compared with five bushes growing under dense forest canopies and receiving only 0.3–0.4 of visible radiation reaching well-illuminated bushes. In preference trials (conducted in 1996) beetles did not distinguish between these two groups of bushes whereas larvae survived better and pupated earlier when fed with leaves from shaded bushes. Thus, light did not improve foliar quality of *S. borealis*, which means that factors promoting beetle outbreak in open habitats are not related to changes in plant quality due to higher illumination. However, we cannot exclude the importance of direct effects of illumination on leaf beetles, neither the effects of higher daily temperatures in open habitats.

Effects of bush size on beetle performance were studied in 1996 by comparing small, medium-sized and large bushes growing in similar light conditions in an unpolluted site. We revealed no size-related variation in survival or in developmental the whereas variation in beetle weight was significant. Beetles fed on foliage of large- and medium-sized bushes attained only 90% of weight of beetles fed on leaves from small bushes. Since the proportion of small (= young) willow bushes in transitional communities is generally higher than in both slightly and in heavily polluted habitats, better quality of young plants may somewhat contribute to density increase of *M. lapponica* in moderately polluted sites.

Conclusion

Mass appearance of *M. lapponica* in the impact zone of the Severonikel smelter was presumably promoted by forest decline, caused by aerial pollution, fellings and fire. We suggest that increased abundance of willows in secondary communities replacing coniferous forests, and decrease in densities of forest ants, birds and possibly other predators due to habitat deterioration and/or toxicity problems, allowed wide distribution of leaf beetle over the impact zone.

Population dynamics of *M. lapponica* within the impact zone seem to be related to host-plant quality. Although willows demonstrated no signs of pollution-induced stress even in the most polluted areas, their suitability for herbivores varied with pollution. Sulphur dioxide in concentrations occurring within the deforestated zone (70 to 1000 $\mu g/m^3$) improved foliar quality of willows for *M. lapponica* and thus positively affected leaf beetle performance. However, close to the smelter, both SO_2 (in concentrations 200 to 1000 $\mu g/m^3$) and foliar metals may cause toxicity problems for leaf beetles. Thus, moderate SO_2 concentrations (50–150 $\mu g/m^3$) are most profitable for *M. lapponica*: they improve foliar quality but are not yet toxic for larvae. Consistently, we recorded density increase in moderately polluted sites between 1989 and 1994.

Beetle outbreak in moderately polluted habitats resulted in severe damage of *S. borealis*. Some bushes were nearly completely defoliated by August of 1993–1996. This damage appeared to be stressful for willows: leaves produced next year(s) after defoliation demonstrated higher fluctuating asymmetry. Plants stressed by herbivory were poorer quality hosts for the next generations of herbivores, i.e. defoliation triggered delayed induced resistance in *S. borealis*. Feeding on stressed plants decreased beetle performance and thus may have contributed to the density decrease at moderately polluted sites in 1995–1996.

We concluded that pollution created prerequisites for beetle outbreak, but did not suppress induced plant responses to defoliation. Our findings demonstrate that factors independent of pollution level, primarily plant responses to previous-year(s) damage, should not be ignored when studying pollution effects on herbivore population dynamics.

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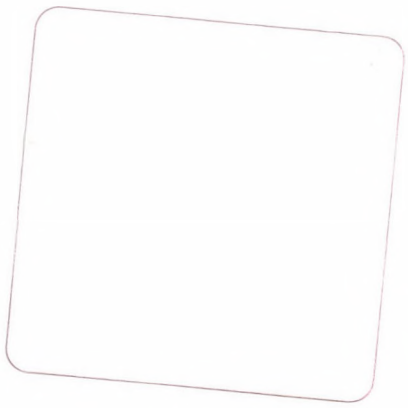

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Contents

Acta Phytopathologica et Entomologica Hungarica 32 (3–4), pp. 259–264 (1997)

Effects of Environmental Stress Caused by Elevated CO_2 Levels on the Activity of Glutathione S-Transferase in Plants

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Effects of environmental stress caused by elevated levels of carbon dioxide on the level of glutathione S-transferase (GST) activity were investigated in leaf tissues of various plant species growing in the proximity of two natural CO_2 springs in central Italy. Leaf tissues of *Phragmites australis* grown in elevated CO_2 concentrations at the CO_2 spring of Bossoleto contained significantly reduced GST levels as compared to control plants. Similar, but statistically not significant changes in GST levels of *Chenopodium album, Quercus ilex, Quercus pubescens* and *Quercus robur* were detected.

Levels of atmospheric CO_2 are expected to double by 2100 (Watson et al., 1990). Investigations on the long-term effects of such changes on plant species are complicated by lack of adequate facilities (Guehl et al., 1994). CO_2 springs in central Italy provide a unique research tool for such studies, since these sites have a long history of elevated CO_2 in a natural environment, with the benefit of plants acclimated and adapted to elevated CO_2 and subjected to natural selection (Miglietta et al., 1993; Amthor, 1995). Previous studies on woody plants at the CO_2 springs indicated that rising CO_2 concentrations may have an effect on stomatal structure and size (Miglietta and Raschi, 1993), leaf and whole-plant transpiration (Tognetti et al., 1996, 1997), carbon physiology (Körner and Miglietta, 1994; Johnson et al., 1997) and foliage metabolism (Chaves et al., 1995; Jones et al., 1995; Barták et al., 1997).

Among the wide variety of plant processes influenced by environmental stress, significant alterations have been reported in the thiol status and activities of several thiol-related enzymes in various plants (Polle and Rennenberg, 1994; Rennenberg and Brunold, 1994; Knörzer et al., 1996; Kőmíves et al., 1997). Glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine) and structurally similar related tripeptides were shown to be the most abundant thiol components in plants (Polle and Rennenberg, 1994; Rennenberg and Brunold, 1994). Significant alterations in the activities of the enzyme glutathione S-transferase (GST, EC 2.5.1.18) were found in different plants exposed to a wide range of stress effects, including exposure to environmental pollutants (Kőmíves et al., 1997). Apparently, there are no reports on the effects of elevated CO_2 levels on the

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GST activity in plants. In the present study the effects of elevated atmospheric CO_2 on the level of GST activity were investigated in various plant species growing at the natural CO_2 spring of Bossoleto in central Italy.

Materials and Methods

Plant material and study site

The research was carried out at the natural CO_2 spring of Bossoleto (43° 17′ N, 11° 35′ E and 350 m.a.s.l.) located near Rapolano Terme (Siena, central Italy) and at the natural CO_2 spring of Orciatico (43° 26′ N, 0° 40′ E and 270 m.a.s.l.) located near Laiatico (Pisa, central Italy). The climate at both sites is characteristic of the Mediterranean region. At the study site of Bossoleto plants grow on the flanks of a circular doline, while at the study site of Orciatico plants grow on a NW-facing moderate slope; detailed information on the sites is given elsewhere (Miglietta et al., 1993; Körner and Miglietta, 1994; van Gardingen et al., 1995). Concentration gradients are enhanced under stable (windless) atmospheric conditions. The H_2S and SO_2 concentrations in the springs is very low and cannot be considered harmful to plants (H. Rennenberg, Univ. of Freiburg, pers. comm). A large part of both sites is forested with a coppice stand in which *Q. ilex* and *Q. pubescens* are the main species. The control sites were chosen as being characterized by similar morphology, light exposure, soil nutrient availability, plant age and association. Leaves of *Chenopodium album, Quercus ilex, Q. pubescens, Q. robur* and *Phragmites australis* were sampled from plants growing both at the control sites and at different levels of atmospheric CO_2 at the spring sites following the vertical CO_2 concentration gradient along the flanks of the doline (Bossoleto, at the bottom of which the CO_2 concentration is at the local maximum), or on the slope (Orciatico). Sampled materials were kept on dry ice, then transferred to the freezer at –80 °C until enzyme assays.

GST enzyme activity

For GST enzyme assays cell-free homogenates were prepared at 0–4 °C. Leaf material (0.5 g) was frozen at –80 °C, pulverized with a mortar and pestle, and suspended in 3 ml cold 0.2 M TRIS/HCl buffer (pH 7.8) containing 3% soluble polyvinylpyrrolidone and 0.1 mM EDTA-Na_2. The homogenate was strained through muslin and centrifuged at 8000 g for 20 min. The supernatants were used as enzyme source. GST activities were determined spectrophotometrically at 340 nm (using 1-chloro-2,4-dinitrobenzene as substrate) as described previously by Uotila et al. (1995). All the reagents are from Sigma (St. Louis, MO, U.S.A.).

Statistical analysis

Enzyme activity data presented are averages calculated from three independent samplings involving three replicates each and standard deviations. Significance of differences was evaluated by Student's *t*-test.

Results

Effects of elevated CO_2 on leaf GST levels

Measurable levels of GST were found in all plant species studied, regardless of their site of growth. A substantial reduction was observed in GST activity in the leaf tissues of *P. australis* grown under elevated CO_2 concentrations at the springs as compared with the control plants (Fig. 1). Similar, but statistically not significant changes in the GST levels of *C. album, Q. ilex, Q. pubescens* and *Q. robur* were detected (Fig. 1).

Discussion

Exposure of plants to elevated CO_2 levels results in the expression of different stress symptoms (Schwanz et al., 1996). In leaf tissues of *Quercus robur* and *Pinus pinaster* trees, elevated CO_2 caused significant alterations in the activities of the antioxidative enzymes superoxide dismutase (EC 1.15.1.1), ascorbate peroxidase (EC 1.11.1.11) and catalase (EC 1.11.1.6), leading to the conclusion that growth under elevated CO_2 might reduce oxidative stress to which leaf tissues are normally exposed (Schwanz et al., 1996).

A reduction in glutathione pool, although not statistically significant, was evidenced on wheat plants growing under elevated CO_2 in the open (Badiani et al., 1997); however, in these plants GSH level was reduced, while GSSG underwent a concomitant increase. Similar results were obtained by the same authors on soybean (Badiani et al., 1994). In an experiment on alfalfa response to elevated CO_2 performed in open top chambers (OTC), no changes in GSH pools and in GR were evidenced, while GR was reduced in plants growing in OTC at ambient CO_2, in comparison to plants growing in the open, probably in consequence of higher temperatures (Sgherri et al., 1996). Glutathione content has been reported to be enhanced in pine and to be reduced in oak leaves (A. Polle, pers. comm.) under elevated CO_2; in conclusion, no clear relationship between glutathione content and CO_2 enrichment seems to exist.

Previous studies showed that GST enzyme levels are sensitive indicators of environmental chemical stress in plant tissues, usually responding through strongly induced activities (Kőmíves et al., 1997). Thus, massive and rapid elevation of GST was detected in maize (*Zea mays*, Sari-Gorla et al., 1995), tobacco (*Nicotiana tabacum*, Gullner et al.,

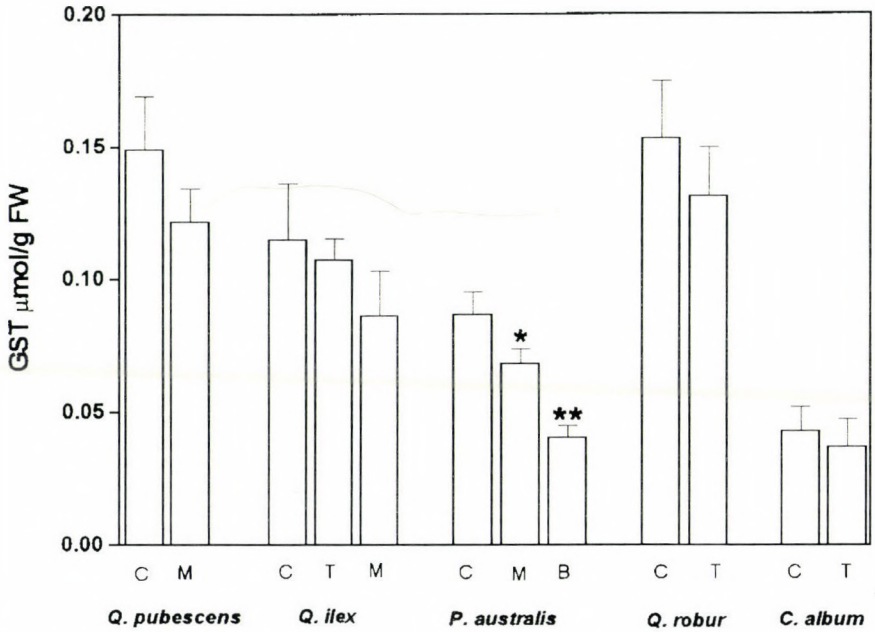

Fig. 1. Glutathione S-transferase (GST) enzyme activities in leaf tissues of plants growing at the control sites (C) and on the top (T), at the middle depth level (M) and on the bottom (B) of the Bossoleto CO_2 spring. *Significantly different from the control at $P \leq 0.05$. ** Significantly different from the control at $P \leq 0.01$.

1991), and in wheat (*Triticum aestivum*, Uotila et al., 1995) exposed to environmental stress caused by herbicides and heavy metals. Herbicide safeners stimulate *de novo* GST synthesis by activating the transcription of genes (Jepson et al., 1994). The observed reductions in the GST activities in leaf tissues of plants growing at elevated CO_2 levels (Fig. 1) may denote a lack of stress conditions. It is worth noting that differences were only found in *Phragmites australis* that grows at the lower depth in the Bossoleto. In fact in this site CO_2 concentrations are very high for a large part of the day, and a localized greenhouse effect takes place in the morning (van Gardingen et al., 1995). The only indication of a similar drop in GST activities was observed following virus infections of sorghum (*Sorghum bicolor*) plants (Gullner et al., 1995). Although a clear explanation has not been given for this unusual response to viral challenge, it is highly probable, that elevated CO_2 and viral infections influence GST enzyme gene expression differently, even if the result (a reduced enzyme level) is the same. Possibly, the observed reductions in GST activities represent a late section of a time-course curve of an induction-type response. In this case the stress-induced GST activities remain at high levels only for a few days, then start to decline at different rates, depending on the nature and the intensity of stress. However, the role of elevated CO_2 in reducing the sensitivity to stress in plants (at the leaf level) cannot be ruled out.

Our results suggest that altered GSH metabolism, among other plant processes influenced by CO_2 enrichment, may have a role in the plant response to biotic and abiotic stress under global climatic change conditions. Further studies are necessary to elucidate the interactions between elevated CO_2 levels and the thiol metabolism in plants.

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Submicroscopic Evidence of Bacterially Induced Resistance in Tobacco Leaves

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A prototrophic HR (hypersensitive reaction)-negative mutant of *Pseudomonas syringae* pv. *phaseolicola* was used to induce the early induced resistance (EIR) and the late induced resistance (LIR) in tobacco. This mutant also served as an indicator of submicroscopic host responses in non-treated, heat shocked, and dark-treated leaves. Heat shock (50 °C, 20 s) and dark are known to suppress EIR and LIR, respectively. Intercellular spaces of the non-treated and heat-shocked leaves contained dividing bacteria at 6–12 hpi. At 24–48 hpi the ratio of dividing bacteria decreased with a concomitant increase of an intensely electron-dense, distorted bacterial form frequently embedded in an amorphous, electron-dense matrix. Around 12 hpi paramural papillae appeared as site-specific plant responses to the neighbouring bacterial cells. Occurrence of papillae correlated closely with the distorted, possibly damaged bacterial form. The highest number of dividing bacteria and the largest microcolonies indicated the partial lack of EIR in heat-shocked leaves, but neither the distorted bacteria nor papillae could be connected with EIR. In the dark, compared to the other two treatments, less distorted bacteria constituted looser microcolonies after 24 hpi, while papilla formation was suppressed. Thus a relationship was found between papilla formation, the distorted bacterial cells and LIR.

Plant cells recognize the presence of bacteria and rapidly develop localized types of responses. Ultrastructural studies have shown that different types of bacteria, such as saprophytes, incompatible pathogens, or pathogenic mutants, induce similar responses (Al-Mousawi et al., 1983; O'Connell et al., 1990; Brown and Mansfield, 1988; Brown et al., 1995). These bacteria multiply in the extracted plant intercellular fluid, but can not within a responding plant tissue (Somlyai et al., 1986; Willis et al., 1990). Not only pathogens but also saprophytes trigger expression of plant defense genes (Jakobek and Lindgren, 1993; Meier et al., 1993). These facts imply an operative, non-specific, locally induced resistance mechanism apart from the incompatible-specific hypersensitive reaction (HR), that results in the decline of bacterial population (Hevesi et al., 1981).

Two locally induced forms of resistance have been described in tobacco: the early induced resistance (EIR) and the late induced resistance (LIR). The EIR exists from 3–6 hpi till about 20 hpi (Burgyán and Klement, 1979), and is inhibited by a short heat-shock (Visnyovszky et al., 1983) or a protein synthesis inhibitor, cycloheximide (Bozsó et al., 1997). The EIR is able to prevent induction of HR (Burgyán and Klement, 1979; Klement et al., 1997), while the LIR, that corresponds to the "induced resistance" described by Sequeira (1975) requires more time (about 24 h) and intense illumination to develop, but it is more effective for a longer time (about six days) than EIR since it suppresses

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both the incompatible (HR) and compatible (disease) symptoms (Lovrekovich and Farkas, 1965).

A number of electron microscopic studies on plant-bacterium interactions involve saprophytes (Smith and Mansfield, 1982; Brown and Mansfield, 1988; O'Connell et al., 1990; Brown et al., 1995) or *hrp* (hypersensitive reaction and pathogenicity) mutants (Bestwick et al., 1995; Brown et al., 1995; Fett and Jones, 1995) as a bacterial partner. While similar ultrastructural phenomena (e.g. appearance of a pellicle and granular material around bacterial cells) are described, the interpretation of the results varies considerably due to the individuality and complexity of the model systems. For example, very different roles and importance are attributed to the envelopment of bacteria at the plant cell wall (cf. e.g. Bestwick et al., 1995; Hildebrand et al., 1980; Bonatti et al., 1979; Mazucchi and Bazzi, 1982) in the plant defense reaction. Results are more congruent if reference is made to localized alterations within plant cells affected by bacteria: the changes usually begin with a localized convolution of the plasma membrane, increased synthetic and transport activities and sometimes end up with a deposition of differently shaped and structured cell wall appositions, papillae. Papillae are regarded as protective structures involved in creating an antibacterial environment (Peng and Kuč, 1992; Bestwick et al., 1995; Brown et al., 1995).

Our aim was to examine the ultrastructural nature of EIR and LIR. To separate EIR and LIR, tobacco leaf samples from the following three treatments were compared: heat-shock (to inhibit EIR); dark (to inhibit LIR) and control (normal growth condition where both EIR and LIR are active). We used a single inoculum prepared from a HR-negative mutant of *Pseudomonas syringae* pv. *phaseolicola* to induce both of these resistance forms and to indicate their effect.

Materials and Methods

Bacteria

We used a HR-negative mutant (No. 1250), isolated by Somlyai et al. (1986), of *Pseudomonas syringae* pv. *phaseolicola* for the inoculations in order to avoid the hypersensitive collapse of the infected leaves. Mutant No. 1250 causes neither the HR in the non-host tobacco, nor any disease symptom in a susceptible bean host. However, it is capable of inducing the EIR and LIR (unpublished results). Although this mutant was not characterized genetically, it is probably a *hrp* mutant. To prepare the inoculum, late exponential-phase bacterial cells grown at 25 °C in King's B broth (King et al., 1954) were collected in a nicrofuge and resuspended in sterile distilled water at a density of 1–2 × 10^9 CFU/ml.

Plants

Tobacco *Nicotiana tabacum* cv. "Samsun" plants were kept in a greenhouse until 2–3 months of age. They were transferred to growth chambers (set to 20 °C, 16 h photoperiod; no light for the dark treatment) one day before inoculation. We inoculated intervenial areas from the middle part of fully expanded, young leaves with a hypodermic syringe fitted with a 26 gauge needle (Klement, 1963). The experiments were carried out at 20 °C. The treatments of plants were as follows:

1. control (only inoculated with bacteria, both types of induced resistance are expressed): continuous light after inoculation;

2. heat-shock treatment (to inhibit EIR for at least 10 h): attached leaves were submerged into a 51 °C water bath for 15 s, inoculated and then the plants were put under continuous light;

3. dark treatment (to inhibit LIR): plants were kept in complete darkness throughout the 24 h preinoculation and the experiment, and in minimal light for inoculations and sampling.

Samples were taken 2, 4, 6, 12, 24 and 48 h after inoculation, using a 9 mm (diameter) cork borer.

Sample preparation and electron microscopy

Leaf discs were fixed in 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4) for 12 h in sealed vials at 4 °C, with a change of buffer after 2 h. Small (ca. 1 mm^2) pieces were cut and post-fixed in 1% osmium tetroxide. Tissue was stained in 5% uranyl acetate for 1 h and dehydrated in a series of ethanol concentrations, and finally in propylene oxide. It was impregnated in propylene oxide: SPI Pon Araldite resin (1:1) for 1 h and then in the undiluted resin overnight. Blocks directed in BEEM® capsules were polymerized at 60 °C for 48 h. With an MT 7000 ultramicrotome, ultra thin (50 nm) sections were made for examination under a Zeiss EM 910 transmission electron microscope. For quantitative assessments we examined at least 30 bacterial attachment sites per sample. Every sample derived from a combination of a treatment and a sampling time. Fifteen attachment sites were selected from two separate blocks of leaf tissue prepared from the same inoculation site.

Results

The *P. syringae* pv. *phaseolicola* HR-negative mutant did not cause macroscopic symptoms in tobacco even at a density of 10^9 CFU/ml, however, there were distinctive changes seen in the bacterial cells and their environment, the plant's inter- and intracellular spaces. The changes were followed in heat-shocked leaves (inhibition of EIR), in leaves kept in the dark (LIR is inhibited) and in control leaves (neither EIR nor LIR are inhibited) from 2 to 48 hpi.

Changes in bacterial number and morphology in intercellular spaces

Two main bacterial types could be distinguished: 1. one type was only slightly electron-dense, had a normal, elongated and rounded shape with discernible inner structure (thereafter: normal cells, e.g. Fig. 1A). The bacteria showing signs of division (thereafter: dividing cells, e.g. Figs 2A, 2B and 3A) or signs of blebbing (i.e. 20–30 nm dia. vesicles were extruded from their surface, e.g. Figs 2A and 2C) also belonged to this type. 2. The other type was usually strongly electron-opaque and had an irregular, concave shape with sometimes lobed contour (hereafter: distorted cells, e.g. Figs 1D and 2D). Between these two types there were transitional stages (thereafter: transient forms, e.g. Figs 1B, 1C and 3C) as well.

In the early period (2–6 hpi) in all treatments the bacterial cells were normal, most of them stayed alone but later (12–48 hpi) the majority established microcolonies (e.g. Figs 1B and 3B) that were largest in the heat-shocked (9–10 cells/colony/section) and smallest in the dark (5–6 cells/colony/section) treatment. Most dividing cells (at 6 and 12 hpi) were seen in the heat-shocked cells (Fig. 4). In the control and heat-shocked tissue, from about 12 hpi the percentage of dividing cells began to decrease steadily, while the percentage of distorted bacterial cells increased drastically to above 40% (Fig. 5) till 48 hpi. This trend did not apply to the dark treatment, where from 24 hpi the ratio of distorted cells remained around 12% with a concomitant increase in the percentage of dividing cells (Fig. 4). However, the decrease of the ratio of dividing cells was also seen in the dark between 12 and 24 hpi, when many transient forms appeared. In all treatments, some bacteria were blebbing, at a maximal ratio (20–25%) between 12 and 24 hpi.

We assessed bacterial density of the microcolonies, i.e. the average distance between bacterial cells in a colony. These distances decreased during the experiment in all treatments: from 1.7 to 0.3 μm in the control; from 2.7 to 0.4 μm in the heat shocked and from 3 to 1.1 μm in the dark. It was obvious that the distorted bacterial cells were three times more densely packed within colonies (e.g. Figs 1D and 2D) than the normal cells (e.g. Fig. 3D).

Envelopment of bacteria

We recorded two basic kinds of envelopment located around and among bacterial cells: 1. a membrane-forming material (hereafter pellicle) around bacterial cells or microcolonies and connected to plant cell walls (e.g. Figs 1A and 2D); 2. differentially electron-dense granular or fibrillar matrix appearing among bacteria or on the outer side of the plant cell wall (e.g. Figs 1A, 1B and 2C). The amount and frequency of both forms increased with time in all treatments (Table 1). Most pellicles and matrix were seen in the control and least in the dark treatment. The extremely electron-opaque granular matrix was especially common at 24 and 48 hpi in the control and heat-shock treatment (Figs 1C and 2D, Table 1), in frequent association with the distorted bacterial types. On the contrary, this form of envelopment was rare and the ratio of uncovered cells was twice as high in the dark, as compared to the control or heat-shocked treatments.

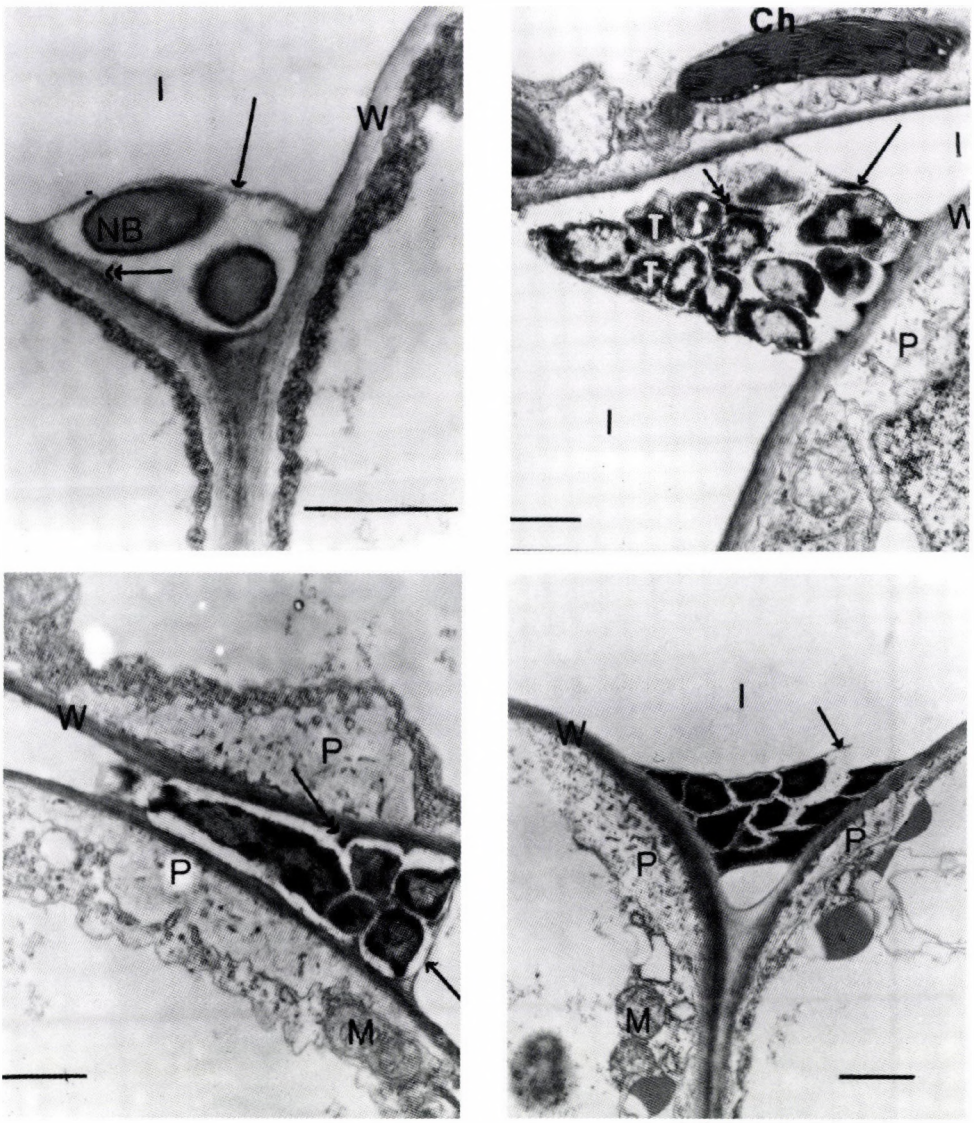

Fig. 1. Interaction between tobacco leaf cells and an HR-negative mutant of *P. syringae* pv. *phaseolicola*. Control (light) treatment. A, 6 hpi. B, 12 hpi. C, 24 hpi. Most bacteria are of the transient or distorted type. D, 48 hpi. Colony of distorted, intensely electron-dense bacterial cells in association with a papilla. Ch, chloroplast, I, intercellular space, M, mitochondrion, NB, normal bacterial cell, P, papilla, T, transient bacterial cell from between normal and distorted, W, plant cell wall. Single-headed arrows, pellicle, double-headed arrows, granular-fibrillar matrix. Horizontal bars, 1 μm

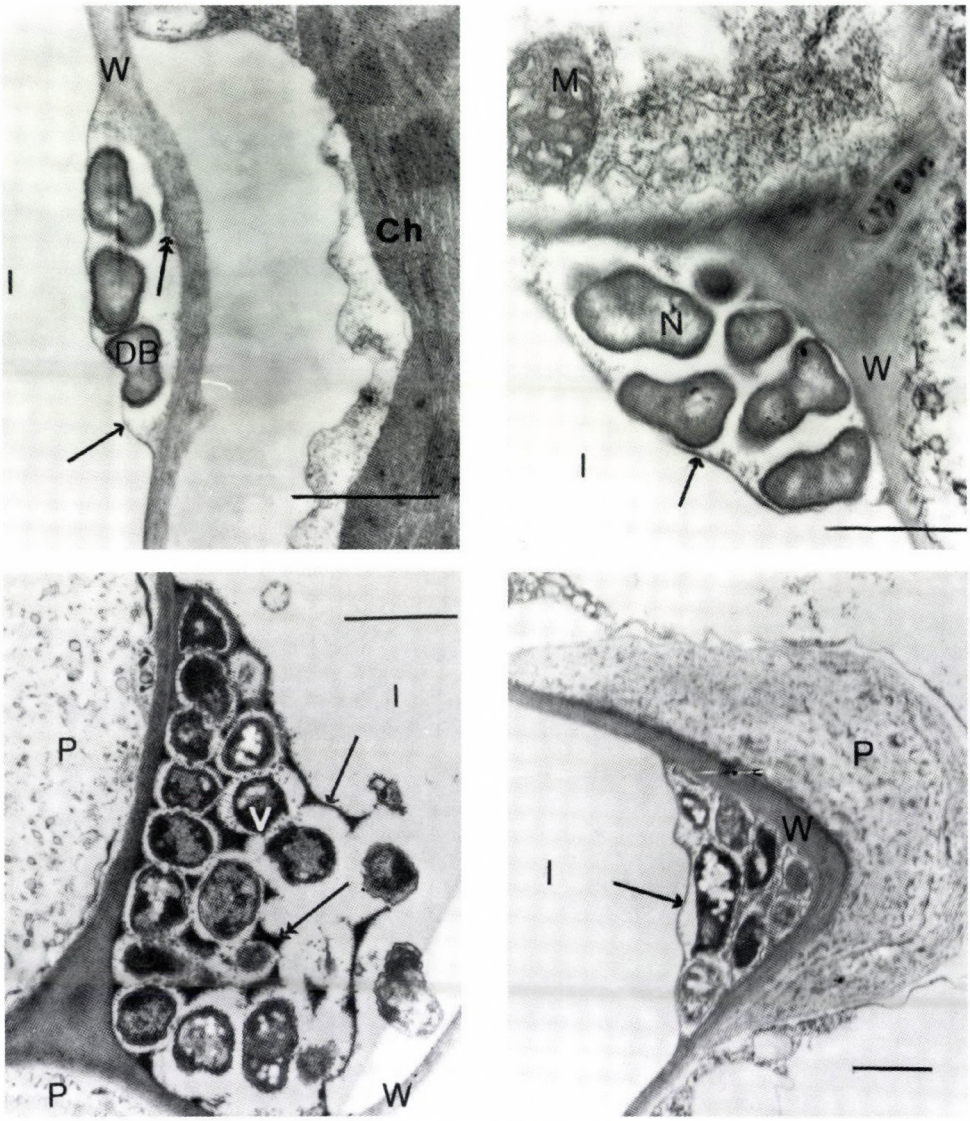

Fig. 2. Interaction between tobacco leaf cells and an HR-negative mutant of *P. syringae* pv. *phaseolicola*. Heat shock treatment. A, 6 hpi. B, 12 hpi. C, 24 hpi. D, 58 hpi. Ch, chloroplast, DB, dividing bacterium, I, intercellular space, P, papilla, V, blebbing bacterial cell, W, plant cell wall. Single-headed arrows, pellicle double-headed arrows, granular-fibrillar matrix. Horizontal bars, 1 μm

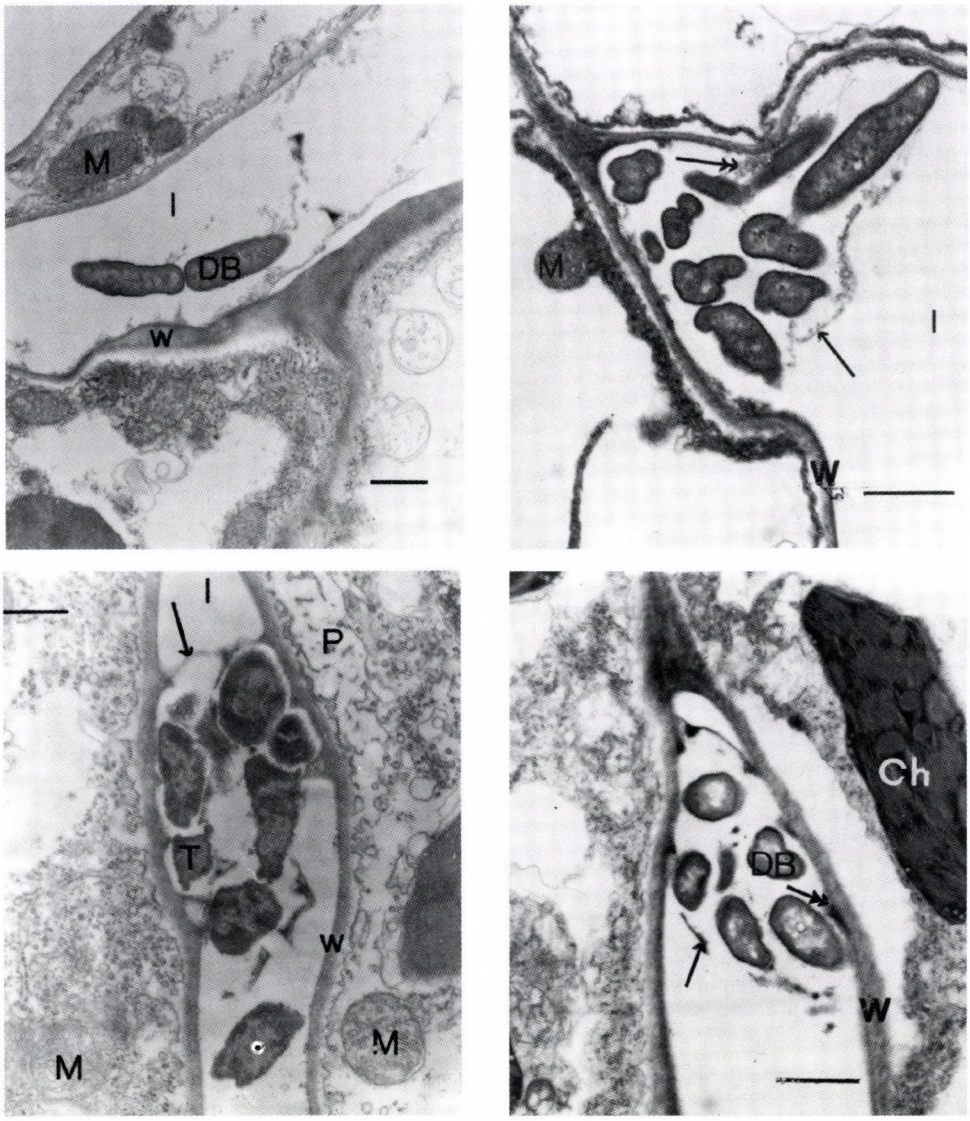

Fig. 3. Interaction between tobacco leaf cells and an HR-negative mutant of *P. syringae* pv. *phaseolicola*. Dark treatment. A, 2 hpi. The water from the inoculum has not yet evaporated, there is much of a loose granular-fibrillar material in the intercellular space. B, 12 hpi. The pellicle looks similar to the matrix. C, 24 hpi. Many transient bacteria and a poorly developed papilla. D, 48 hpi. Bacteria look normal, there is no papilla. DB, dividing bacterium, I, intercellular space, M, mitochondrion, N, normal bacterial cell form, P, papilla, T, transient bacterial cell from between normal and distorted, W, plant cell wall. Single-headed ar rows, pellicle, double-headed arrows, granular-fibrillar matrix. Horizontal bars, 1 μm

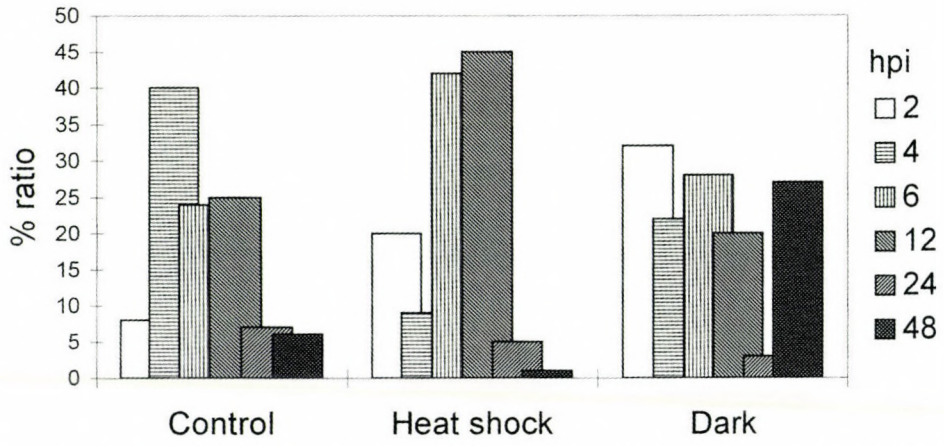

Fig. 4. Percentage of dividing cells of *P. syringae* pv. *phaseolicola* HR-negative mutant in the intercellular space of tobacco leaves

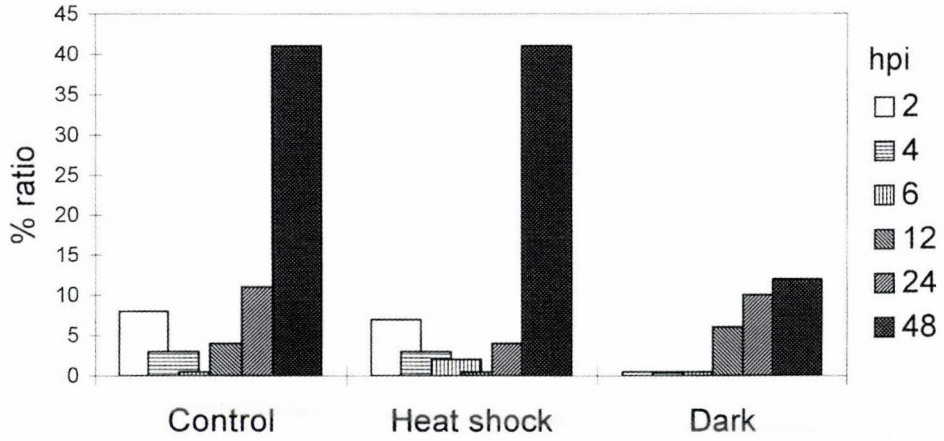

Fig. 5. Percentage of distorted cells of *P. syringae* pv. *phaseolicola* HR-negative mutant in the intercellular space of tobacco leaves

Changes in the infected plant cells

The most evident feature of the responding plant cells to this HR-negative mutant was the highly localized formation of papillae, i.e. layered deposits inside the cell wall opposite the bacterial attachment site. In this study we concentrated primarily on the papillae, although some other alterations, such as damage of plant cells, were also observed. The papillae were seen at or after 12 hpi in the control leaves (Figs 1B, 1C, 1D

Table 1

Overview of ultrastructural changes in tobacco leaves inoculated with a HR-negative mutant of *P. syringae* pv. *phaseolicola*

hpi	Dividing bact.[a]	Distorted bact.[b]	Very distorted bact.[c]	Papilla development[d]	Opaque covering matrix[e]	Pellicle[f]
			Control			
2	–	–	–	–	–	+
4	+++	–	–	–	–	++
6	++	–	–	–	–	++
12	++	+	–	+	–	++
24	–	+++	+	+++	++	++
48	–	+++	+++	++	+++	+++
			Heat-shock			
2	+	–	–	–	–	–
4	–	–	–	–	+	++
6	+++	–	–	–	–	++
12	+++	–	–	–	–	++
24	–	+++	–	+++	+++	++
48	–	+++	+++	+++	++	++
			Dark			
2	+++	–	–	–	–	++
4	++	–	–	–	–	+
6	++	–	–	–	–	+
12	+	++	–	–	+	++
24	–	+++	+	+	+	++
48	++	+	+	–	–	++

[a] Data according to Fig. 2: –, 0–10%; +, 10–21%; ++, 22–30%; +++, above 30%
[b] Data according to Fig. 3a: –, 0–15%; +, 16–30%; ++, 31–45%; +++, above 45%
[c] Data according to Fig. 3b: –, 0–8%; +, 9–16%; +++, above 33%
[d] Data according to Fig. 4: –, 0–1; +, 1–2; ++, 2–3; +++, above 3
[e] Ratio of attachment sites: –, 0–10%; +, 11–20%; ++, 21–30%; +++, above 30%
[f] Quantity in abitrary units: –, 0 or very low; +, low; ++, medium; +++, high

and Fig. 6). The heat shock seemed to delay this process, however after 24 hpi we saw more complex papillae in the heat shocked (Figs 2C and 2D) than in the control tissue (cf. Fig. 6). In the dark the development of papillae was remarkably suppressed (cf. Figs 1D, 2D and 3D, see also Fig. 6). The thickness of papillae for one attachment site (sum of thickness/sum of all attachment sites per treatment) was 270, 420 and 50 nm in the control, heat-shocked and dark-treated leaves, respectively. This was in part attributable to the lower frequency of papillae in the dark (it was 8% compared to 26% in the control

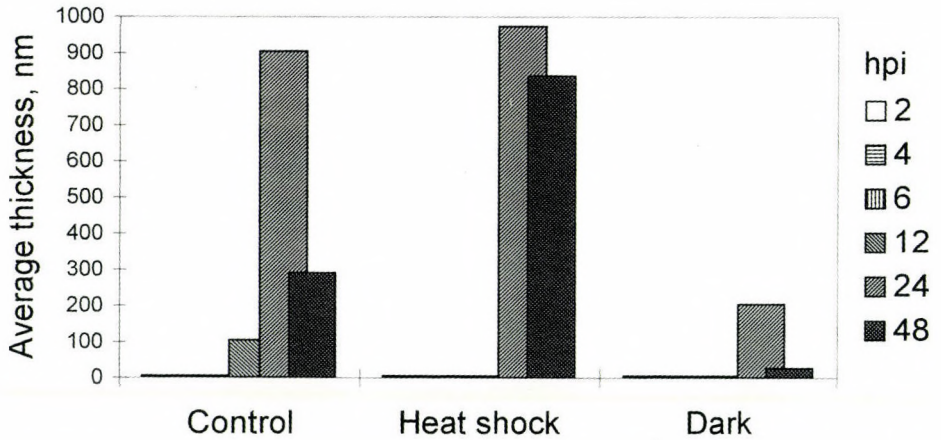

Fig. 6. Development of papillae as a localized response of tobacco cells to a *P. syringae* pv. *phaseolicola* HR-negative mutant

and 42% in the heat-shock treatment), but existing papillae were also 50% thinner (500 nm in average) than in the other two treatments (both around 1 μm).

During the development of a plant response the appearance of papillae changed. Early (about 12 hpi), complicated vesicular structures between the cell wall and cytoplasmic membrane appeared (Fig. 1B); later ornate, usually thick appositions emerged having alternate layers of electron-opaque and lucent materials (e.g. Figs 1D and 2D). Only the amount (average thickness) of the early papilla forms found in the dark was comparable to the values of the other two treatments: all late forms in the dark were either severely depressed or even lacking.

Discussion

When a tobacco leaf is infiltrated with bacteria other than compatible pathogens, EIR and later LIR are induced. Both host responses are macroscopically symptomless. EIR is known to suppress growth and HR-inducing activity of a challenge incompatible bacterium (Burgyán and Klement, 1979; Hevesi et al., 1981), LIR was shown to be effective against both incompatible and compatible challenge bacteria (Lovrekovich and Farkas, 1965; Sequeira, 1975). It is probable that induced plant responses like EIR or LIR are responsible for the failure of pathogenic mutants or saprophytes to establish themselves within leaves. One can conceive that the inhibitory effects of EIR and/or LIR may be evidenced by changes in the ultrastructure of bacterial cells. In the present study we used an HR-negative prototrophic strain of *P. syringae* pv. *phaseolicola* not only to induce EIR and LIR but also as an indicator of their effect. Besides bacterial

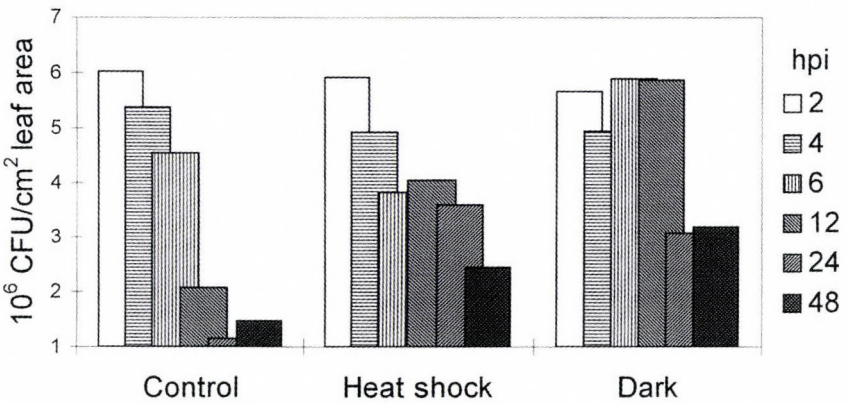

Fig. 7. Culturable cell numbers of *P. syringae* pv. *phaseolicola* HR-negative mutant obtained from tobacco leaf tissue

morphology we examined other submicroscopical events like envelopment of bacteria in the plant intercellular spaces and localized formation of wall appositions within plant cells.

Changes in bacterial morphology reflect a resistance mechanism

In this study, many signs implied a resistance in effect (Table 1). The bacteria had not lost their capacity to multiply in the plant, which means that the intercellular space was suitable for their multiplication up to 12 hpi. The ratio of dividing bacterial cells to the total cell number was found to be highest when EIR was inhibited by heat-shock. Between 12 and 48 h, presumably due to activated plant defense mechanisms, the distorted bacterial form appeared and there seemed to be a transition toward this form in the control and heat-shocked leaves (heat shock is able to delay the EIR only for about 6–8 hpi). The intercellular environment contrarily affected the ratio of the dividing and the distorted bacterial cells. The distorted, electron-opaque cells became predominant between 24 and 48 hpi in the control and heat-shocked leaves. Moreover, at 24 and 48 hpi about half of those cells that were not distorted also lost their dividing activity (data not shown). Especially in the control leaves, by this time the majority of the bacterial population comprised non-culturable or non-viable cells (Fig. 7). Therefore we regard the distorted bacteria as damaged or dead.

The ratio of the homogeneously electron-dense (no inner structure discernible) distorted cells to the total cell number did not increase when the LIR was suppressed (dark treatment), in contrast to the control or heat-shock treatment, after 24 hpi (Table 1). However, there was a high ratio of transient forms in the dark at 24 hpi that suddenly dropped until 48 hpi. Parallelly the normal, dividing cells reappeared. This suggests that at least the transient bacteria are not necessarily dead, they may be static, with a chance

of recovery. And this chance seems to be given only in the dark, although we saw only quantitative (not morphological) differences in distorted cells between the control and the dark treatment.

Possible role of papillae and LIR in generating the distorted bacterial form

There were several other changes in correlation with the ratio of the distorted cells and the presence (or absence) of EIR or LIR. The frequency of the distorted bacterial cells and the amount of papillae was high in the heat-shocked and control leaves and low in the dark treatment. In the heat-shocked and control leaves, where both EIR and LIR are present at 24 and 48 hpi, most transient and distorted bacterial cells (78% and 71%, respectively) were associated with papillae. From our results it is not possible to think far beyond correlation, but given the fact that many types of bacteria (heat-killed, saprophytic, *hrp* mutant) can induce papilla formation it is tempting to imply a role of papillae in the generation of the distorted bacterial form, i.e. in the change toward irreversible damage. This would also be consistent with suggestions made by other investigators, i.e. paramural deposits may restrict diffusion of either microbial or plant-derived (e.g. nutrients) metabolites through the plant cell wall (O'Connell et al., 1990), thus influencing bacterial metabolism. Moreover, constituents (hydroxyproline-rich glycoproteins) of papillae have also been found on the outer side of the plant cell wall, within the matrix covering the bacteria (Bestwick et al., 1994; Brown et al., 1995). The fact that in the absence of LIR (dark treatment) 67% of transient cells were observed without any papilla response at 24 hpi and that the ratio of transient cells dropped in favour of normal cells (Figs 4, 6 and Table 1) at 48 hpi suggests that papillae are not responsible for the initial damage of bacteria (reflected by the transient form), but their presence is needed for a more effective antibacterial activity seen in the control and the heat-shock treatment at 48 hpi.

In the heat-shocked leaves there was a delayed but stronger papilla response compared to the control. This may have been triggered by the bigger microcolonies resulting from the inhibition of EIR, since we found that more attaching bacteria were more "successful" in inducing a papilla than less bacteria. Papillae developed later than the EIR. Therefore we conclude that papillae are not directly connected to the EIR. Our results suggests that the papilla formation may be connected with the development of the LIR and the bacterial cell damage is a consequence of this process. This conclusion is further confirmed by the following observations:

1. Different (saprophytic, heat-killed pathogenic or pathogenic mutant) bacteria that trigger papilla formation can also induce LIR in tobacco leaves, indicating an aspecific interaction.
2. The time sequence of the development of LIR coincides with the appearance of papillae (after 12 hpi).
3. Compatible bacteria suppress both LIR (otherwise they were unable to cause disease) and papilla development (Brown et al., 1995).

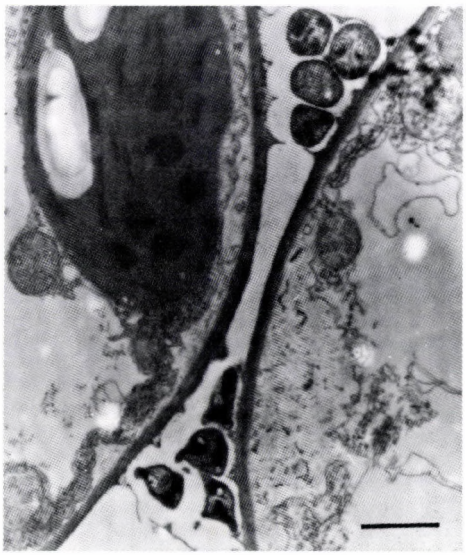

Fig. 8. A papilla response and its putative effect is highly localized. The group of distorted bacteria are associated with a large papilla, the group of normal cells above are not. Control treatment, 24 hpi. Bar, 1 μm

4. Electron micrographs show that the plant cell response is highly localized to the attachment site of distorted bacterial cells. There are irregular distorted bacteria close to a papilla and to normal cell types only two micrometers away (Fig. 8). Therefore, not all bacterial cells are damaged by this local plant cell response in the inoculated tissue. This was established when the live bacterial number in inoculated tobacco leaves was measured by plate count method. These investigations also proved that the live bacterial number was the highest when the LIR was suppressed in the dark (Fig. 7).

Possible role of envelopment

Earlier studies regarded the envelopment of bacteria (the pellicle and the amorphous, electron-dense matrix among bacterial cells) as signs or means of active immobilization (Goodman et al., 1976; Sequeira et al., 1977; Benhamou, 1991; Bestwick et al., 1995), an insignificant phenomenon in terms of defense (Cason et al., 1978; Bonatti et al., 1979; Al-Issa and Sigee, 1982; Ebrahim-Nesbat and Slusarenko, 1983; Jones and Fett, 1985), an advantageous environment for the bacteria enclosed (Mazzuchi et al., 1982) or even an artifact of inoculation (Hildebrand et al., 1980). In our experiments the pellicle appeared early (at 2–4 hpi). It was more frequent and more manifest with time (Table 1). Later, around the bacteria enclosed in the pellicle, an electron-opaque, granular matrix emerged, embedding the bacterial cells. This matrix was seen frequently around the distorted cells in the control and heat-shocked treatment and not so often in

the dark. Though the pellicle frequently appeared together with the dense amorphous matrix among bacterial cells, it seems that they are not results of the same or correlated process. Thus, the pellicle may have been shaped by physical forces working on air-water-interfaces, which supports earlier studies (Hildebrand et al., 1980; Jones and Fett, 1985). The growing quantity of covering material and the dense granular matrix at later stages (24 and 48 hpi) may not be explained with only condensation of a water-soluble material of plant cell wall origin. It possibly involves accumulation of substances originating from biological activity. Because pellicles are common in both control and the dark treatments, pellicles may not correlate with the concurrently developing resistance, like LIR or EIR. The amorphous matrix in which both normal and distorted bacterial cells were embedded contains hydroxyproline-rich glycoproteins that are also present in papillae (Bestwick et al., 1995; Brown et al., 1995). Because the number of distorted bacterial cells embedded in this matrix increases in time (and they are closer to each other than the normal cells, data not shown), it is possible that the amorphous matrix may play a role i) in the agglutination of invading bacteria (Leach et al., 1982; Swords and Staehelin 1993) or ii) in formation of an antibacterial condition within the encapsulated colony (Peng and Kuč, 1992) or iii) in forming a physical barrier that makes plant's inhibitory substances more and bacterial attempts to obtain nutrients less effective (Bestwick et al., 1995).

Since the EIR and LIR overlap in time, we had to inhibit either of them for distinction. Our treatments are not completely inhibitory to EIR or LIR and might affect bacteria not only through the inhibition of EIR and LIR. We could see weak papilla formation as well as distorted bacterial cells in the dark treatment. Rather than inhibiting resistance, the heat-shock may stimulate bacterial growth via e.g. leakage of nutrients into the intercellular space. However, we only could measure low and transient (from 1–4 hpi) leakage of electrolytes (data not shown) from the heat-shocked tissue, which alone probably does not support sustained bacterial growth. But quantitative assessments of the micrographs showed clearly that when the EIR was inhibited the ratio of dividing and the number of culturable bacterial cells was highest. Working with *hrp* mutants of *P. syringae* pv. *syringae* we also observed attenuation of an otherwise normal bacterial growth suppression and superinduction of *hrp* genes in leaves after the leaves were heat-shocked (Bozsó et al., 1997). The micrographs also showed that when the LIR was inhibited the frequency of papillae and the percentage of distorted bacteria was the lowest.

We conclude that the submicroscopic changes (papilla formation – bacterial cell damage) are independent from the HR and can be outward signs of the LIR.

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Hungarian Plum Pox Virus Isolates Represent Different Serotypes

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Fifteen representative isolates were selected from more than hundred samples of plum pox virus (PPV) according to their serological reactions in agar-gel diffusion tests. Isolates were inseparable on the basis of symptoms on test plants. Using monoclonal antibodies raised against different (external, intermediate and internal) epitopes of PPV in indirect ELISA (IDAS) the presence of both M and D serotypes has been demonstrated, indicating that both types were common in Hungarian orchards. Some isolates showed intermediate relationships between the two main serotypes.

Plum pox potyvirus (PPV) is the most destructive viral pathogen of stone fruits in Europe (Németh, 1968; Lecocq et al., 1988) causing serious yield loss in Hungarian peach, apricot and plum plantations. First occurrence has been reported by Atanasoff (1932) in Bulgaria. By the import of the propagating materials it was rapidly spread in Europe. PPV infection in Hungary was first reported from plum by Szirmai (1948), but later became a common pathogen of apricot (Husz and Klement, 1950) and peach (Németh, 1963). Recently we isolated PPV from almond trees, too. The virus distributed by more than ten different aphid species in a non-persistent manner (Leclant, 1973; Jenser et al., 1980; Basky et al., 1996; etc.). The pollen and seed transmission has been proved by Szirmai (1961), Németh and Kölber (1983). Because of the variability and wide distribution of the pathogen various "necrotic", "yellow" and "intermediate" strains have been described according to the pathogenicity and symptom severity on test plants (Sutič et al., 1971). In Hungary only the presence of yellow strain was recorded (Roy and Smith, 1994).

More useful classification has been mady by Kerlan and Dunez (1979) on the basis of serological cross-reaction in agar-gel diffusion tests. Two basic serotypes have been described: the severe M (Marcus) strain originated from apricot trees from northern Greece, and a milder D (Dideron) apricot strain, isolated in southeastern France. In Hungary only one (Sk38 of Dr. M. Németh) isolate was characterized as a typical M type (López-Moya et al., 1994a, b) and sequenced by Palkovics et al. (1993).

Our previous studies on test plants demonstrated that PPV isolates were different, indicating their heterogeneity. In this paper three monoclonal antibodies (MAbs) were compared to verify their specificity to fifteen Hungarian PPV isolates for strain determination.

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Materials and Methods

More than hundred samples were collected from apricot, peach almond and plum orchards and from natural hosts of PPV, like *Lycium halimifolium*. Isolates were maintained by mechanical inoculation on GF 305 peach seedlings and on *Nicotiana benthamiana*, under greenhouse conditions. Origin of selected fifteen isolates are listed in Table 1.

Plant virus assay and diagnosis were predominantly based on serological techniques, especially the Outchterlony double diffusion test (Kerlan and Dunez, 1979) and the ELISA (Clark and Adams, 1977). Polyclonal antiserum was prepared previously in our laboratory (Tóbiás and Papp, 1990) against the Rankovic isolate, a typical member of D serotype. Monoclonal antibodies were raised against the internal (3C6 1,5 mg/ml), intermediate (4DG11 1,3 mg/ml) and external (1EB 1.3 mg/ml) sequences of D type Spanish 1.15 isolate (López-Moya et al., 1994a, b). Antisera were diluted 1 to 1000 in phosphate buffered saline (PBS) or in coating buffer (CB). Indirect ELISA (I-DAS ELISA) was preformed according to Converse and Martin (1993) using *Staphylococcus aureus* Protein-A conjugate and horseradish peroxidase enzyme. The evaluation of tests were made by a Lab System photometer at 492 nm. Positive reactions were arranged according to the values of extinction.

Selected test plants were mechanically inoculated in normal greenhouse conditions. Efficiency of inoculation was tested on the basis of symptoms and/or on ELISA. Plant samples were ground (1:5, w:v) in a PBS-Tween buffer, supplemented with 20 nM sodium diethyldithiocarbamate and 2% polyvinylpyrrolidone. After grinding, the extracts were rapidly clarified by centrifugation in Eppendorf centrifuge.

Results

In DAS-ELISA all the used PPV isolates reacted positively with D type polyclonal antiserum (Table 2). As strong, as well as week reactions have been detected. In this study the *N. benthamiana* plants were inoculated at the same time, meaning that differences in absorbancies were due to the serological heterogeneity of isolates, not from the host plants.

Monoclonal antiserum raised against the internal epitope of PPV coat protein (BC6) was able to distinguish only one isolate (Pd20) which gave negative reaction (Table 3), indicating the unique nature of this isolate. The MAb of intermediate sequences (4DG11) were able to recognize neither Pd20 nor Lh1 isolates (Table 4). It was known, that this MAb reacted only with isolates of D serotype. From the negative reactions with MAb 4DG11 we could conclude that the M serotype were represented by Pd20 and Lh1 isolates in our collection. Some isolates (Pd7, Pd17, Pc13, Pd16) gave very week reactions with this MAb. In these cases we were not able clearly separate the isolates. However, the strong positive reactions (Pd3, Pd9, Pc12, Pa1 and Pd15) with Mab

Table 1

Source of the used plum pox virus isolates

Abbreviations	Place	Host plant
Pd3	Fehérgyarmat	Prunus domestica L.
Pd4	Fehérgyarmat	Prunus domestica L.
Pd7	Fehérgyarmat	Prunus domestica L.
Pd9	Újfehértó	Prunus domestica L.
Pd11	Budapest	Prunus domestica L.
Pc12	Keszthely	Prunus cerasifera Ehr.
Pd14	Becehegy	Prunus domestica L.
Pd17	Kisar	Prunus domestica L.
Pa1	Becehegy	Prunus amygdalus Batch.
Ra*	Cacac	Prunus domestica L.
Pc13	Budapest	Prunus cerasifera Ehr.
Pd15	Borsod	Prunus domestica L.
Pd16	Borsod	Prunus domestica L.
Pd20	Karcag	Prunus domestica L.
Lh1	Nagymaros	Lycium halimifolium Mill.

* Rankovic isolate (D serotype homologous antibody)

Table 2

Serological reactions of some Hungarian PPV isolates to polyclonal antiserum in IDAS ELISA system

Polyclonal (PPV-D)antiserum				
Pd3	Pd4	Pd7	Pd9	Pd11
++	+	++	+++	++
Pc12	Pd14	Pd17	Pa1	Ra
++	++	+	++	+
Pc13	Pd15	Pd16	Pd20	Lh1
+	+++	++	+	+

Extinction values

– = 0.000 – 0.024 (3 × of negative control)
\+ = 0.025 – 0.100
++ = 0.101 – 0.150
+++ = 0.151 –

Table 3

Serological reactions of some Hungarian PPV isolates
to a monoclonal antiserum in IDAS ELISA system

Monoclonal antiserum (PPV 5.15) of CP internal sequences (BC6)				
Pd3	Pd4	Pd7	Pd9	Pd11
+	++	+++	+++	+
Pe12	Pd14	Pd17	Pa1	Ra
++	++	+++	+++	+
Pe13	Pd15	Pd16	Pd20	Lh1
+++	+++	++	–	+++

Extinction values
– = 0.000 – 0.025 (3 × of negative control)
\+ = 0.026 – 0.040
++ = 0.041 – 0.050
+++ = 0.051 –

Table 4

Serological reactions of some Hungarian PPV isolates
to a monoclonal antiserum in IDAS ELISA system

Monoclonal antiserum (PPV 5.15) of CP intermediate sequences (4DG11)				
Pd3	Pd4	Pd7	Pd9	Pd11
+++	++	+	+++	+++
Pe12	Pd14	Pd17	Pa1	Ra
++	++	+	+++	++
Pe13	Pd15	Pd16	Pd20	Lh1
+	+++	+	–	–

Extinction values
– = 0.000 – 0.025 (3 × of negative control)
\+ = 0.026 – 0.040
++ = 0.041 – 0.050
+++ = 0.051 –

Table 5

Serological reactions of some Hungarian PPV isolates to a monoclonal antiserum in IDAS ELISA system

Monoclonal antiserum (PPV 5.15) of CP external sequences (1EB)				
Pd3	Pd4	Pd7	Pd9	Pd11
+++	+	++	++	+++
Pc12	Pd14	Pd17	Pa1	Ra
+++	+	–	++	+++
Pc13	Pd15	Pd16	Pd20	Lh1
–	+	+	+	–

Extinction values
– = 0.000 – 0.024 (3 × of negative control)
+ = 0.025 – 0.100
++ = 0.101 – 0.150
+++ = 0.151 –

Table 6

Symptoms of PPV isolates on selected test plants

Isolates	Test plants*					
	AMIMA	CHEQ	CHEFO	NICPH	NIOBE	NIOCL
Pd3	LCh	LCh	LL	LL	ChDf	S+
Pd4	LCh	LCh	LL	LL	Vb	–
Pd7	+	S	LL	LL	ChDf	S+
Pd9	LCh	S	LL	LL	Vb	S
Pd11	–	S	LCh	LL?	Vb	S+
Pc12	S	S	LL	LL	ChDf	S+
Pd14	–	LCh	LL	LL	ChDf	–
Pd17	LCh	LCh, S	LL, S	LL	VbDf	S+
Pa1	LCh, S	Ch1, LL	LL	Ch	S	
Ra	S	S?	Ch1, LL	LL	Vb	S
Pc13	–	S	–	LL	Vb	S
Pd15	–	LCh	LL	LL	?	S
Pd16	–	LCh	LL	LL	?	S
Pd20	–	LCh	LL	LL	Vb	S
Lh1	–	LCh	–	PPLL	Df	S, Dw

Key: – = symptomless, serologically negative, + = symptomless, serologically positive (latent), SM = systemic mosaic, LL = local lesions, Ch = chlorotic, chlorosis, Vb = vein banding, Df = deformation, VN = venial necrosis, PP = pin point, Dw = dwarfing

*Abbreviations: Horváth, J. (1993a, b). Acta Phytopath. 28, 21–58 and 257–354.

4DG11 demonstrated the presence of D type isolates. Similar consequences could be drawn from the reactions with the MAb of the external sequences (Table 5).

Symptoms on indicator plants listed in Table 6 were different among the isolates. No possibility of separation was given on the basis of the symptoms and serological properties of the two mayor serogroups. The symptoms on *Chenopodium foetidum* were also variable, both the yellow and the necrotic type of symptoms occurred and some isolate were not able to infect this plant. *C. murale* was immune against all the isolates. *C. quinoa* reacted with local or systemic symptoms to the infection, but the type of symptoms was not related to the serological classification, similarly to the *C. foetidum*. *Nicotiana benthamiana* and *N. clevelandii* proved to be systemic host to all isolates, but the symptom severity was not dependent on serotypes. *Nicandra physaloides* got generally local reactions, or nothing, in one case only litle, pin-point lesions appeared on the inoculated leaves. According to our results the used indicator plants were not suitable to separate the different serotypes.

Discussion

Plum pox virus is a typical member of Potyvirus genus (Barnett, 1992). Its flexuous helical rod-shaped particles are about 700 nm long and 11 nm in diameter, wherein the genom is a single-stranded RNA consisting about 9.7 kilobasis pairs (Lain et al., 1989; Maiss et al., 1989; Palkovics et al., 1993). The RNA 5′ end is linked to the small protein (VPg) and the 3′ end is polyadenylated (Hari, 1981). The RNA helix is covered by about 2000 copies of single coat protein of about 32–36 kD (Ravelonandro et al., 1988).

PPV strains were first separated on the basis of symptom severity on indicator plants like *Chenopodium foetidum*: yellow, necrotic and the yellow-necrotic (intermediate) types were separated (Sutič et al., 1971). In our experiments all the three type of reaction occurred, but the type of symptoms did not correlate with the serological classification. Similarly any other test plants provide a reliable basis for differential diagnosis of PPV strains.

Potyviruses could be separated on the basis of their serological properties. The N-terminal end of the coat protein (CP) located on the surface of the capsid are virus specific, whereas the core regions are similar to other potyviruses (Shukla et al., 1988; Shukla and Ward, 1989). Similarly to other potyviruses, PPV isolates were separated first according to the double immunodiffusion tests: a severe M and a milder D serotypes. These groups have been found to be related to the recent classification of PPV isolates created on the basis of monoclonal antibodies raised against the different regions of the PPV-CP (López-Moya et al., 1994a, b) and by molecular diagnostic methods (Wetzel et al., 1991; Bousalem et al., 1994). By specific MAb-s strains could be differentiated and classified more precisely (Adamolle et al., 1994; Cambra et al., 1994; Candresse et al., 1994; Deborré et al., 1994; López-Moya et al., 1994a, b; Asensio et al., 1995). The only investigated Hungarian PPV isolate (SK68) proved to be as a typical M type (Deborré et al., 1994).

Hungarian PPV isolates we tested previously in test plants as well as in agar-gel diffusion tests showed a large variability. Serological tests of some representative isolates from Hungary with monoclonal antibodies detected the presence of D serotypes too (López-Moya et al., 1997). In this study we have demonstrated the heterogeneity of PPV isolates. It has been proved that both strains, M and D, were common in Hungarian orchards. Some isolates were clearly separable as M or D types, however few of them seemed to be intermediate between the two main serotypes. The heterogeneity of these isolates needs more detailed more studies based on molecular analysis.

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Susceptibility of Host Plants to Belladonna Mottle and Turnip Yellow Mosaic *Tymoviruses*: Multiplication and Distribution

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The susceptibility of host plant to the multiplication of belladonna mottle *tymovirus* (BeMV, isolate BeMV-M from Croatia) and turnip yellow mosaic *tymovirus* (TYMV, isolate TYMV-Y65 from Slovenia) was assessed from virus concentration (mg/ml) in systemically invaded leaves of plants infected under glasshouse conditions. The order of the decreasing susceptibility with BeMV was: *Datura stramonium* (3.9), *Nicotiana megalosiphon* (3.8), *N. clevelandii* (3.3), *Petunia hybrida* (3), *N. glutinosa* (2.2), *Physalis alkekengi* (1.4), *Capsicum annuum* (1.3); with TYMV it was: *Brassica chinensis* 'Michihli' (3.4), *B. rapa* var. rapa (turnip) 'Kranjska' (2.8), *Sinapis arvensis* (1.4) and *S. alba* (1.2). In *D. stramonium* and turnip plants concentration of BeMV and TYMV, respectively, fell from leaf lamina via stem and leaf petiole to root. In the leaves of glasshouse infected and field infected turnip plants the TYMV concentrations were comparable. A relatively high virus concentration in the fleshy root of the latter plants was higher in the central than in the peripheral part. The concentration was determined in low speed centrifuged plant sap by the single radial immunodiffusion method.

Data on concentration of viruses and of their free nucleic acid and protein constituent in plants have been used in experiments to find out the sites of constituent synthesis and assembly in the cell, to study inhibitory effect of some substances on constituent (virus) synthesis, to explain virus movement within plants and some further points of virus-host relationship (Francki and Matthews, 1962; Matthews, 1991). They are also useful in choosing suitable hosts for virus purification and for serological experiments, the latter especially with crude or clarified infectious plant sap. This choice can be of interest even in viruses appearing in host plants in a comparatively high concentration if they are a common object of investigation. Turnip yellow mosaic *tymovirus* (TYMV) particularly, is such a virus. Detailed studies on virus concentration, that is its distribution within the plant, can be of interest in the domain of plant breeding for virus resistance (Wiesner and Krause, 1990) and in some other practical aspects of virus infections (Stobbs et al., 1991).

In this paper the term virus multiplication is used to mean the increase of virus content i.e. virus concentration in an infected plant to approximately maximal value. Virus multiplication largely depends on the properties of a particular virus, its strain or isolate and also on host (plant species, cultivar, genetic composition) and environmental factors (Gibbs and Harrison, 1976; Matthews, 1991). Following this, it was assumed that the concentration of a particular virus in its host plants – in their leaves, the main sites of

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the synthesis of the viruses investigated – reflects in a directly proportional way the susceptibility of the plants to virus multiplication. This is in the case that different host species are grown and infected under equal conditions (the points mentioned above, too, simultaneous inoculation of the plants in comparable physiological states) and investigated simultaneously for virus concentration as in all of them an intensive process of virus multiplication is already established.

In the present work several plant species were investigated, mainly under glasshouse conditions, for their susceptibility to the multiplication of belladonna mottle *tymovirus* (BeMV) and TYMV and also for virus distribution in the main vegetative parts of plant.

Materials and Methods

Included in the experiments were BeMV (isolate BeMV-M) from Croatia (Štefanac, 1974) and TYMV (isolate Y65) from Slovenia (Mamula et al., 1966). Additionally, a TYMV turnip isolate from Croatia (Juretić, unpublished; see Results, 2.2.) was included in the experiments as well. Virus concentration was determined by the single radial immunodiffusion (SRID) method. The procedure utilized by Juretić and Mamula (1978) was followed in all details. These included the use of: 3 ml of gel medium (0.9% 'Difco' bacto agar mixed with undiluted antiserum at a ratio 20:1, v/v) per microscope slide, the same polyclonal antisera homologous to virus isolates BeMV-M and TYMV-Y65 (preserved with glycerol they had titres of 1/128), sample (virus) in the form of a supernatant obtained after low speed centrifugation of infectious crude plant sap, incubating serological reactions for 48 h at temperatures of 20–25 °C, and the same calibration curves. Higher or lower titre values of the BeMV-M and TYMV-Y65 antisera reported by Juretić and Mamula (1978, 1980) were due to use, in titre determination, of the microprecipitin test in liquid and/or inadequate virus concentration.

Each BeMV-M and TYMV-Y65 was inoculated mechanically onto a group of plants belonging to different species by using crude sap of systemically infected *N. megalosiphon* and turnip leaves, respectively, as inoculum, 500 mesh carborundum and glass spatula. The plants were inoculated (two leaves per plant) as they had, depending on the species, three or four expanded true leaves suitable for inoculation. Throughout the experiments they were grown in a glasshouse in natural light and a temperature ranging from 20 to 35 °C. TYMV field infected turnip ('Kranjska') plants were taken at random. Samples investigated separately were: leaf (two to three successive, expanded and completely systemically invaded younger leaves, with a part of their petioles, per plant), stem, root, fleshy root (the main root was somewhat fleshy also with glasshouse infected turnip plants) and, additionally, parts of some of these i.e. leaf lamina (blade) without midrib, and leaf petiole (stalk) together with leaf midrib. Leaves always showed more or less marked symptoms. Samples were taken from seven or eight plants of each species. During the experiments the investigated turnip plants were in the developmental stage of rosette. Preliminary SRID experiments with a few plant specimens from the mentioned groups of inoculated plants were performed.

Fig. 1. Systemic symptoms of belladonna mottle *tymovirus* (BeMV) in *Datura stramonium* leaf (glasshouse infection).

Results

1. Virus concentration in glasshouse infected host plants

1.1. BELLADONNA MOTTLE *TYMOVIRUS* (BeMV)

Measurements were taken at the end of March from the leaves of seven plant species of the family Solanaceae (Fig. 1). Generally, BeMV concentration was comparatively high in all the investigated hosts; differences of up to three times were recorded. With prolonged infection in *D. stramonium* and *N. megalosiphon* the virus concentration fell off (Figs 2 and 3, Table 1).

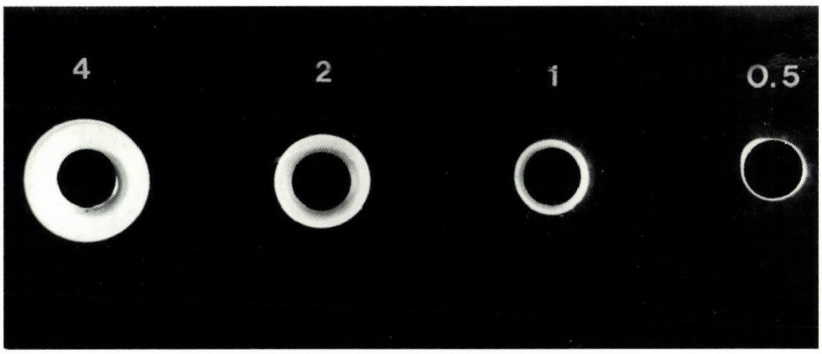

Fig. 2. Single radial immunodiffusion reactions of purified belladonna mottle *tymovirus* (BeMV, isolate BeMV-M) in concentrations of 4, 2, 1 and 0.5 mg/ml

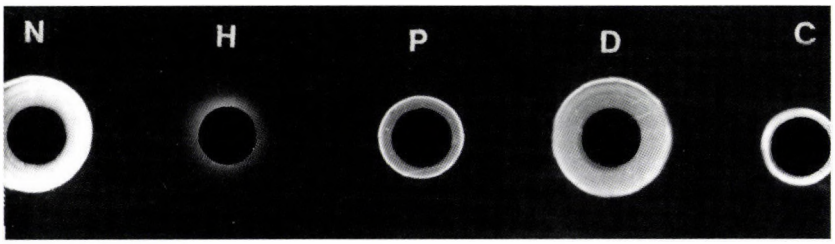

Fig. 3. Single radial immunodiffusion reactions of belladonna mottle *tymovirus* (BeMV, isolate BeMV-M). Reactions of clarified plant sap from leaves in infected plants. *Nicotiana megalosiphon* (N), *Physalis alkekengi* (P), *Datura stramonium* (D), *Capsicum annuum* (C). H, healthy plant sap (preliminary experiment)

1.2. TURNIP YELLOW MOSAIC *TYMOVIRUS* (TYMV)

Virus concentration was investigated at the end of April in each of two *Brassica* and *Sinapis* species (Fig. 4). As with BeMV, a relatively high concentration was found in the plants investigated. Distinct concentration differences were recorded between two genera (see Table 1).

Table 1

Concentration of belladonna mottle *tymovirus* (BeMV) and turnip yellow mosaic *tymovirus* (TYMV) in systemically infected leaves of host plants

Hosts/Viruses	Virus concentration (mg/ml) on days after inoculation			
	20	30	45	60
Capsicum annuum L. local cultivar (medium-sized)/BeMV	1.3			
Datura stramonium L./BeMV	3.9		2.4	1.3
Nicotiana clevelandii Gray/BeMV	3.3			
N. glutinosa L./BeMV	2.2			
N. megalosiphon Heurck et Muell./BeMV	3.8		1.8	0.8
Petunia hybrida hort. ex Vilm./BeMV	3.0			
Physalis alkekengi L./BeMV	1.4			
Brassica chinensis L. 'Michihli'/TYMV		3.4		
B. rapa L. var. rapa 'Kranjska'/TYMV		2.8		
Sinapis alba L./TYMV		1.2		
S. arvensis L./TYMV		1.4		

Fig. 4. Systemic symptoms of turnip yellow mosaic *tymovirus* (TYMV) in turnip (*Brassica rapa* var. rapa) leaf (glasshouse infection)

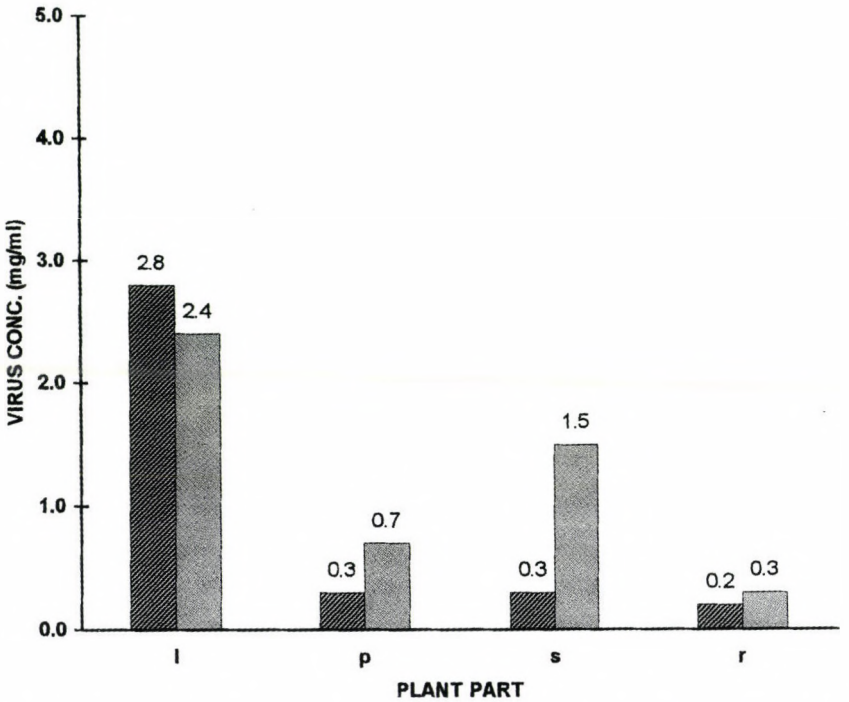

Fig. 5. Virus concentration in experimentally infected plants. Dark columns, belladonna mottle *tymovirus* (BeMV, isolate BeMV-M) in *D. stramonium*. Light columns, turnip yellow mosaic *tymovirus* (TYMV, isolate TYMV-Y65) in turnip. l, leaf lamina; p, leaf petiole; s, stem; r, root

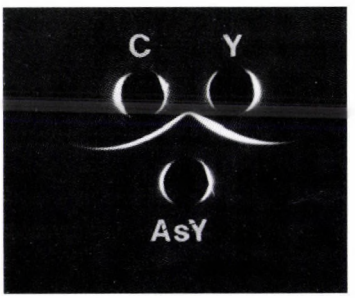

Fig. 6. Serological identification (double radial immunodiffusion method, DRID) of a turnip isolate of turnip yellow mosaic *tymovirus* (TYMV, isolate C) from Croatia. Y, isolate TYMV-Y65; AsY, antiserum to isolate TYMV-Y65

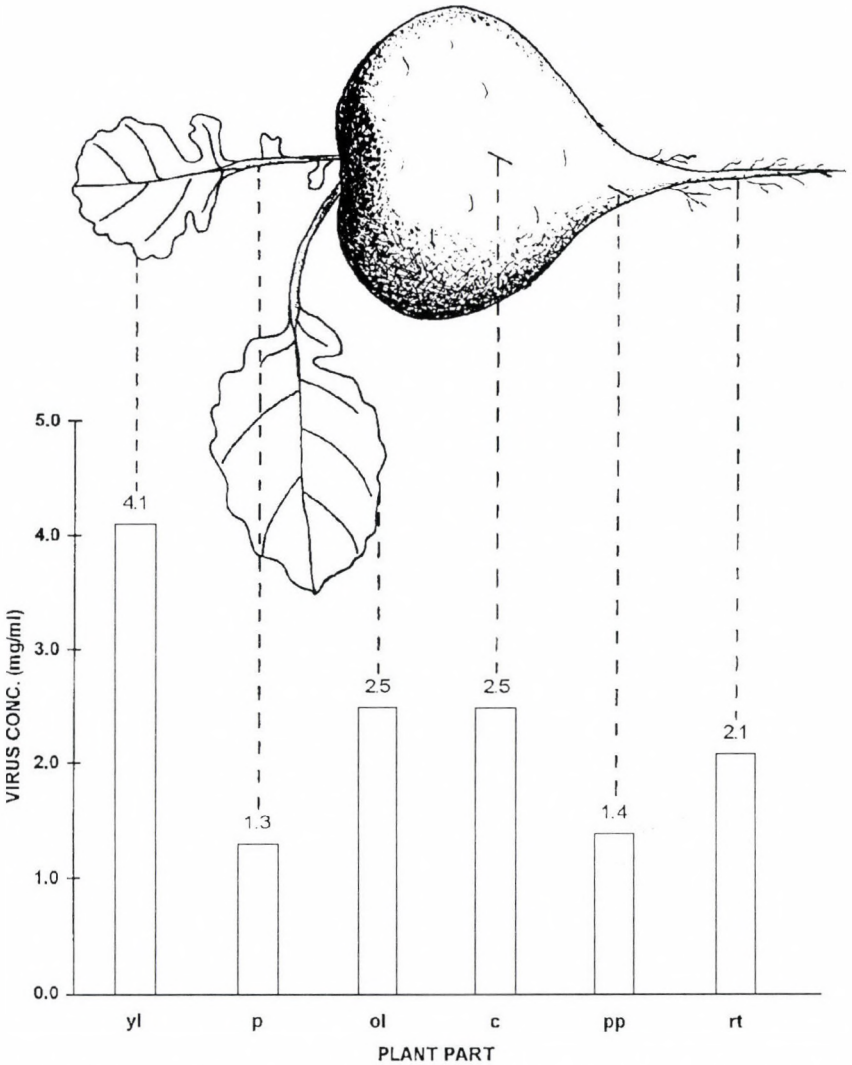

Fig. 7. Turnip yellow mosaic *tymovirus* (TYMV) concentration in naturally infected field turnip plant. yl, young leaf; ol, older leaf; p, leaf petiole; c, fleshy root central part; pp, fleshy root peripheral part; rt, fleshy root tip

2. Virus distribution within a plant

2.1. GLASSHOUSE INFECTION

2.1.1. BELLADONNA MOTTLE *TYMOVIRUS* (BeMV)

Figure 5 shows BeMV distribution in *D. stramonium* 35 days after the inoculation of the plants. A conspicuous difference in virus concentration between the main parts of the plant is evident, showing the highest concentration in leaf lamina and a considerably lower one in the other plant parts.

2.1.2. TURNIP YELLOW MOSAIC *TYMOVIRUS* (TYMV)

The general schema of virus distribution in 'Kranjska' turnip plants 40 days after inoculation was similar to that with BeMV in *D. stramonium*. However, a remarkably high TYMV concentration was detected in the stem (see Fig. 5).

2.2. FIELD INFECTION

The experiments were done with TYMV only. Infected turnip plants were collected at the end of September. The plants displayed a full scale of systemic symptoms, so they certainly had been infected for at least one month. Virus isolate from those plants was serologically indistinguishable from the TYMV-Y65 isolate (Fig. 6). The results showed the highest TYMV concentration in younger leaves. A fairly high concentration was recorded in the fleshy root and also in the root tip (Fig. 7).

Discussion

Comparatively very high concentrations of *tymoviruses* in their hosts (systemically infected leaves) have commonly been recorded, based on purified virus yields ranging from 0.5 mg/g (e.g. Shukla and Gough, 1975; cf. Mamula, 1985; Ribeiro et al., 1996) to 2 mg/g (Mamula, 1969; Lee et al., 1979). There have also been reports of those of up to c. 4 mg/g for a strain of eggplant mosaic *tymovirus*, EMV (Waterworth et al., 1975) and 6 mg/g (measured directly in plant extracts) for TYMV Cambridge type strain ('Edinburgh' isolate) in *Brassica pekinensis* cv. Wong Bok (Chinese cabbage), a plant closely related to *B. chinensis* (Matthews, 1958; Francki and Matthews, 1962). However, an approximately real concentration of a virus in a plant is always more or less higher than shown by the quantity of purified virus yield because of virus loss which occurs during purification process (Matthews, 1991); the use of *n*-butanol, for instance, can sometimes cause consederable yield losses (Horváth et al., 1976; Lee et al., 1979). In the mentioned as well as in some further examples in this text section purified virus yield or virus content in plants was expressed on the basis of virus weight (mg) per unit (g) of fresh leaf tissue weight. Depending on water content in plant tissue it should be increased by some percentage if extrapolated to plant sap volume (mg/ml). With *B. chinensis*, for instance, a value of about 25% could be appropriate with thoroughly homogenized leaf tissue (Mamula, 1985).

This paper gives evidence of differences among the susceptibilities of BeMV and TYMV hosts to virus multiplication, considering the prerequisites quoted at the end of this paragraph, except, possibly to some extent, plant genetic composition. With BeMV the highest susceptibility was in *D. stramonium* and *N. megalosiphon* and the lowest one in *C. annuum* and *P. alkekengi*, while with TYMV it was higher in *Brassica* than in *Sinapis* spp. Moline and Fries (1974) isolated from *D. stramonium* (also *N. glutinosa*) c. 1.25 mg/g of BeMV-PM isolate = BeMV-I strain, while Lee et al. (1979) isolated from pepper (*C. frutescens*) over 2 mg/g of BeMV-K strain. The present conclusion concerning the susceptibility of *Brassica* spp. is supported, to some extent, by our previous findings on virus concentration (mg/g) in leaves of the following plants: field infected (a TYMV isolate serologically indistinguishable from TYMV-Y65) hybrid *Brassica* 'Perko' PVH (1.1) – concentration determined in the same way as in the present work (Juretić and Mamula, 1990) and glasshouse infected (isolate TYMV-Y65) plants *Lunaria annua* (0.8), *Raphanus sativus* var. niger (1.0), *B. rapa* var. rapa 'Kranjska' (1.3) and *B. chinensis* 'Michihli' (1.4) – concentration determined spectrophotometrically in purified virus preparations. Only in the case of the last four plants was the length of (TYMV) infection conspicuously greater i.e. 70–80 days (Mamula, 1985); in all the other examples of BeMV and TYMV concentration quoted herein it was comparable to those in our present experiments. However, concentration (i.e. multiplication) of a virus in a single host species can differ, sometimes considerably, depending on the factors mentioned in the introduction, and also on the quantity of infective virus in inoculum, length of infection and some other conditions (Gibbs and Harrison, 1976; Matthews, 1991). Accordingly, concentration of isolates BeMV-M in *N. megalosiphon* and TYMV-Y65 in *B. chinensis* have been reported that differed from the present ones (Juretić and Mamula, 1978, 1980).

Regarding the fall of BeMV and TYMV concentration in prolonged infections our results agree with many analogous findings in various viruses. A conspicuously higher virus concentration in leaf (leaf lamina) than in other plant parts, which we found with both viruses (less with TYMV), is also a common phenomenon in virus infections of the mosaic type (and possibly also of the mottle type) (Matthews, 1991). However, TYMV concentration was unusually high in the stem and also leaf petiole. This paper gives evidence that also in field turnip plants TYMV can accumulate in a comparatively high concentration in leaf petiole, but especially in the fleshy root and even in its tip. Even higher TYMV concentration (extrapolated to 1 ml of plant sap) were found in the fleshy root (1.8 mg) of field infected 'Perko' PVH plants than in the leaf (1.4 mg) (Juretić and Mamula, 1990), which resembles the distribution of two beet viruses in some plants (Wiesner and Krause, 1990). Contrarily, in a related rutabaga plant, turnip mosaic *potyvirus* (TuMV) reached a much lower concentration in the fleshy root than in the leaves (Stobbs et al., 1991). As known, in virus movement through the plant, resulting in virus distribution within the plant, the plant (host) and virus genes as well as environmental factors play a significant role (Matthews, 1991).

There are some limits on comparisons between the BeMV and TYMV concentrations reported here (e.g. different hosts, not simultaneously performed experiments, etc.). A possible procedure error, arising from serological reactions not being completed (Juretić and Mamula, 1978), could not substantially influence our results.

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Evaluation of *Cucumis sativus* L. Germplasm for Field Resistance to the Powdery Mildew

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Thirteen cucumber (*Cucumis sativus* L.) accessions were evaluated for resistance to the powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*) under conditions of natural infection. Plants were maintained in a glass isolation cage under standard cultivation (garden soil covered with the black plastic mulch) but without chemical protection against powdery mildew. During vegetative growth, the infection degree (ID) was assessed five times by evaluating leaf surfaces (ID-L) and stems and petioles (ID-S) covered by mycelia of powdery mildew. Resistance was expressed as percentage of maximum infection degree (%maxID and/or Σ%maxID, respectively) and as "Area below curve" of the disease infection progress (ABC-L) and (ABC-S). Data were analyzed by analysis of variance and means were separated by multiple range tests. The cultivar Corona F1 was found to be the most susceptible (ABC-L = 1806 and ABC-S = 1004). Three accessions were not infected on the stems and petioles during the whole growing period. Their ABC-L values were comparably lower than the other accessions tested (PI 435946 = 543, PI 426170 = 277, PI 390258 = 241). Data indicate that significant differences exist among the accessions tested in field resistance to powdery mildew.

Cucumber (*Cucumis sativus* L.) is among the most popular vegetable crops of the family Cucurbitaceae in central Europe (Esquinas-Alcazar and Gulick, 1983). In the Czech Republic, cucumbers are traditionally used for fresh market consumption or as gherkins for the processing industry.

Powdery mildew of cucurbits is one of the most destructive foliar diseases in both temperate and subtropical climates conditions (Sitterly, 1978). In temperate growing areas, powdery mildew is caused by two fungi from the class Ascomycetes, order Erysiphales; *Erysiphe cichoracearum* DC. ex Mérat and *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Pollaci (Braun, 1987). Their occurrence on cucumber within the territory of the former Czechoslovakia was verified during 1979 and 1980 (Lebeda, 1983). This species spectrum continued to persist in the Czech Republic during 1990s. *Erysiphe cichoracearum* is recently the predominant fungus in the Czech Republic (Křístková and Lebeda, 1996).

In spite of the enormeous reduction in cucumber yield caused by downy mildew (*Pseudoperonospora cubensis*) during the last decade in the field (Lebeda and Schwinn, 1994), the destructive effect of powdery mildew is more common in glasshouses and plastic tunnels. Attention is now being paid to resistance research because the heterothallism of both pathogens (McGrath, 1994; Yarwood, 1978) will undoubtly be a continual source of genetic variability of the fungus complicating host-pathogen interactions. Be-

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cause different levels of field resistance depends also on the local spectrum of physiological races of the pathogens (Corbaz and Taillens, 1994) it is necessary to evaluate host-pathogen relationships directly in the region of cucumber cultivation. The production of powdery mildew resistant cultivars could substantially reduce the use of fungicides and have a positive effect on biological control (Aalbersberg and Stolk, 1995). Several wild *Cucumis* species show a relatively high level of resistance to the powdery mildew (Lebeda, 1984) but these are cross-incompatible with cultivated cucumber (Lebeda and Křístková, 1993). Thus searching for sources of resistance within *Cucumis sativus* is most advisable. During the last decades, breeding for resistance to *E. cichoracearum* and *S. fuliginea* in Europe used the NPI line as source of resistance (Kooistra, 1968). Resistance in this genotype and related cultivars is linked with expression of leaf chlorosis during autumn and winter cultivation. This chlorosis causes yield loss, and limits the practical use of these cultivars (Zijlstra and Groot, 1992). Sources of resistance which do not exhibit negative chlorotic effects would be extremely valuable for cucumber improvement (Aalbersberg and Stolk, 1995; Zijlstra and Groot, 1992).

Thus, a study was designed to evaluate the level of resistance among 13 different cultivars and accessions of *Cucumis sativus* to the natural infection of powdery mildew. Identification of such resistant germplasm would provide genotypes for the cucumber breeding.

Materials and Methods

In 1993, 13 cucumber (*Cucumis sativus* L.) accessions were evaluated for the resistance to powdery mildew of cucurbits (*Erysiphe cichoracearum, Sphaerotheca fuliginea*) under conditions of natural infection at the SEMO Ltd. Breeding Station (Smržice, Czech Republic). Seeds of elite hybrids were provided by the breeding company L. Daehnfeldt A/S (Denmark), and the North Central Regional Plant Introduction Station (Ames, Iowa, USA) supplied plant introductions (PI) of diverse origin. This set included four commercial cultivars of salad cucumbers (Corona F1, Flamingo F1, Profito F1 and Futura F1), and genetic resources of salad cucumbers (PI 390258/Japan/, PI 418964 /China/, PI 432870/China/, PI 478365/China/ and PI 483342/China/), pickling cucumbers (PI 426170/Philippines), PI 435946/Soviet Union/ and PI 506461/Soviet Union/) and the androecious accession PI 163222/India/. Plants were sown on 21 May in a glasshouse, and then transplanted to a glass isolation cage containing local garden soil covered with the black plastic mulch. Each accession was represented by four plants, spaced within rows at approximately 30 cm and between rows at 50 cm. Standard cultivation procedures were employed such that plants were trained vertically on strings, watered to avoid leaf wetting, and treated with Omite 57E (0.05%) four times (10 and 23 June, 16 July and 4 August) to protect against spider-mite. In the isolation cage, infection of powdery mildew was caused by a mixture of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*. Their determination was based on microscopic analysis of their imperfect stage (conidia) (Lebeda, 1983).

Reaction to powdery mildew was evaluated five times starting with the first appearance of symptoms and continuing until the end of vegetation (80 days after transplanting). The infection degree (ID) was assessed visually on leaves (ID-L) and on stems and petioles (ID-S). A rating 0–9 scale was used (0 = without any disease symptoms, 5 = 40–50% of the host surface covered by mycelia, 9 = over 80% of the host surface covered by mycelia). For each accession, the total infection degree (ΣID-L, ΣID-S), was calculated as a sum of all values obtained. The infection degree (ID) and the total infection degree (ΣID) were also expressed as percentage of maximum infection degree (%maxID) and as percentage of the total infection degree (Σ%maxID). For each accession, the values "Area below curve" (ABC) of the disease infection progress for leaves (ABC-L) and stems and petioles (ABC-S) were calculated as well. Data were subjected to one-way analyses of variance and mean separation was performed using the Scheffe multiple range tests (Koschin et al., 1992).

Results and Discussion

During vegetative development, a mixture of both pathogens (*Erysiphe cichoracearum, Sphaerotheca fuliginea*) occurred on host plants. *E. cichoracearum* was the predominant pathogen.

On the 38th day after transplanting (1st evaluation), pustules of sporulating mycelium were recognized on leaves of all plants of cvs. Corona F1 and Flamingo F1, and accession PI 163222. Although these accessions were infected on the stems and petioles 54 days after planting (2nd evaluation), no visual symptoms were observed on the other accessions evaluated.

During the third evaluation (62 days after transplanting), the leaves of accessions PI 390258 and PI 506461 showed only very weak symptoms of powdery mildew infection (approximately 10 pustules per plant). A similar level of infection was observed on the stems and petioles of accession PI 483342 at the last evaluation (80 days after transplanting).

Although infection was initially observed on the oldest leaves in a majority of the entries examined, the accessions PI 418964, PI 435946 and Profito F1 expressed initial symptoms on leaves of the middle part of the plant.

The powdery mildew infection progress is shown in Figs 1 and 2. The values ABC and Σ%maxID for each genotype are expressed in Tables 1, 2 and 3. Cultivar Corona F1 was the most susceptible to powdery mildew where leaves, stems and petioles were considered. This result is in good agreement with data from Zijlstra and Groot (1992) who considered cv. Corona as highly susceptible to *S. fuliginea*. Cv. Flamingo F1 showed relatively high stem susceptibility what is not in agreement with Aalbersberg and Stolk (1995) who observed expression of stem resistance in this genotype. Conversely, the most resistant accessions were PI 426170 and PI 390258, which displayed mycelia sporadically on leaves, while their stems and petioles did not develop symptoms of infection during this study. The cv. Profito F1 also showed this level of resistance and had

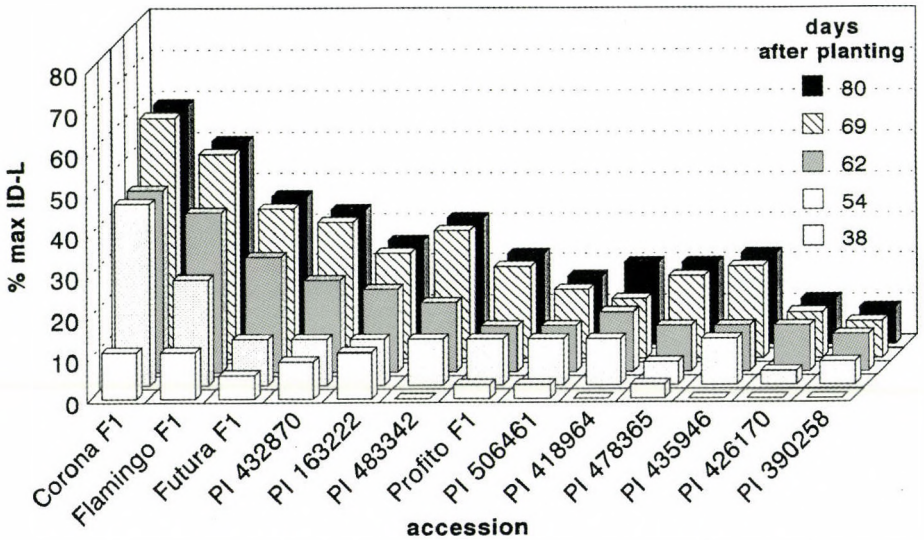

Fig. 1. The powdery mildew infection degree on the leaf surface (%maxID-L) of *Cucumis sativus* accessions during vegetation

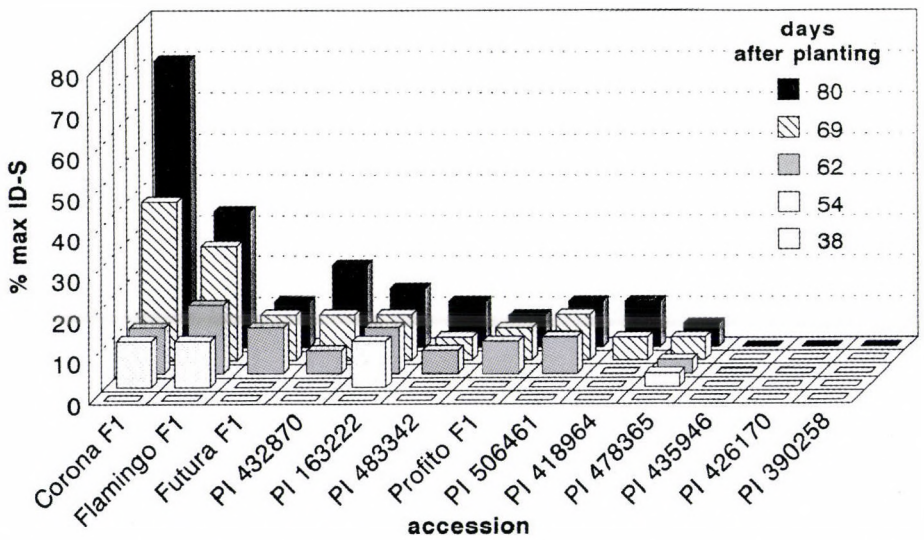

Fig. 2. The powdery mildew infection degree on the stems and petioles (%maxID-S) of *Cucumis sativus* accessions during vegetation

Table 1

Field resistance of *Cucumis sativus* accessions to powdery mildew as expressed in ABC and Σ%maxID for the leaves area (L)

Cultivar, accession	ABC-L	Σ%maxID-L
Corona F1	1806.525	48.4 a
Flamingo F1	1409.700	39.0 ab
Futura F1	912.975	26.2 bc
PI 432870	854.700	24.2 bc
PI 163222	732.600	20.8 bc
PI 483342	707.625	20.0 bc
Profito F1	572.700	15.5 c
PI 435946	543.900	14.8 c
PI 418964	521.700	12.6 c
PI 506461	482.850	13.1 c
PI 478365	457.875	13.3 c
PI 426170	277.500	8.1 c
PI 390258	249.750	7.2 c

Lower case letters distinguish among homogeneous groups as defined by Scheffe's multiple range analysis, P = 0.05 (same also for Tables 2 and 3)

Table 2

Field resistance of *Cucumis sativus* accessions to powdery mildew as expressed in ABC and as Σ%maxID for the stems and petioles (S)

Cultivar, accession	ABC-S	Σ%maxID-S
Corona F1	1004.550	29.1 a
Flamingo F1	610.500	19.8 b
PI 163222	305.250	10.6 bc
PI 432870	255.300	8.2 bc
PI 506461	249.775	6.9 bc
Futura F1	244.200	7.4 bc
Profito F1	190.250	5.2 c
PI 483342	155.400	5.0 c
PI 478365	130.425	4.0 c
PI 418964	116.550	3.7 c
PI 435946	0.000	0.0 c
PI 426170	0.000	0.0 c
PI 390258	0.000	0.0 c

Table 3

Field resistance of *Cucumis sativus* accessions to powdery mildew as expressed in ABC and as Σ%maxID for the sum (ABC) of leaves, stems and petioles (L+S)

Cultivar, accession	ABC (L+S)	Σ%maxID (L+S)
Corona F1	2811.075	77.5 a
Flamingo F1	2020.200	58.8 ab
Futura F1	1157.175	33.6 bc
PI 432870	1110.000	32.4 bc
PI 163222	1037.850	31.4 bc
PI 483342	863.025	25.0 c
Profito F1	762.950	20.7 c
PI 506461	732.625	20.0 c
PI 418964	638.250	16.3 c
PI 478365	588.300	17.3 c
PI 435946	543.900	14.8 c
PI 426170	277.500	8.1 c
PI 390258	249.750	7.2 c

been identified as a genotype with high partial resistance by Zijlstra et al. (1995). Profito F1 suffers from leaf chlorosis when cultivated in autumn, winter and early spring (Groot et al., 1992). During our investigation, we observed no expression of chlorosis.

For each accession, the infection progress on leaves was very similar to the infection progress on stems and petioles (Figs 1 and 2). Also the total infection degree of leaves (Σ/maxID-L) corresponded with the total infection degree of stems and petioles (Σ%maxID-S) (Tables 1 and 2).

When comparing two expressions of disease severity on host plants (ABC and Σ%maxID), it is evident that both expressions are positively correlated, and that cultivar differences exist. The comparable phenomenon was observed in *Cucurbita pepo* (Lebeda and Křístková, 1996).

In spite of the relatively limited genetic variability of the species *Cucumis sativus* L. (Dijkhuizen et al., 1996; Lebeda and Křístková, 1993), a significant and potentially valuable level of resistance for field infestation to powdery mildew exists. Moreover, in accessions with high levels of field resistance, we did not observe the typical expression of leaf chlorosis. These observations confirm the value of evaluating diverse germplasm for sources of resistance to causal agent for powdery mildew in cucurbits.

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Authors are obliged to Dr. M. Kršková for help with the statistical treatment of results. Prof. Dr. J. E. Staub and Dr. M. Widrlechner are acknowledged for valuable comments on the manuscript.

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Abutilon theophrasti Medik. – a new host for *Sclerotinia sclerotiorum* (Lib.) de Bary in Croatia

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Velvetleaf (*Abutilon theophrasti* Medik.), newly introduced and very aggressive weed in arable crops in Croatia, is found to be the host of *Sclerotinia sclerotiorum*. Symptoms on infected plants occurred on basal stem parts as well as on upper plant parts, including fruits and seeds. That *S. sclerotiorum* is cause of velvetleaf plants decay was proved by artificial infections. *S. sclerotiorum* is very important parasite of many crops, especially sunflower. In preliminary trials *S. sclerotiorum* isolate from velvetleaf was highly pathogenic for sunflower. Occurrence of white rot in velvetleaf can increase inoculum density of *S. sclerotiorum* in soil.

Velvetleaf plants infected with *Sclerotinia sclerotiorum* were recorded on several locations in eastern Croatia during summer and autumn 1996.

Velvetleaf is one of the most aggressive weeds in row crop, such as sunflower, soybean, maize and sugar beet. Control of this weed is often unsuccessful since available herbicides are not sufficiently effective.

Occurrence of *S. sclerotiorum* on velvetleaf was described for the first time by Dillard et al. (1991) in the USA. Boland and Hall (1994) included velvetleaf in the index of plant hosts of this fungus. This is the first record of *S. sclerotiorum* on velvetleaf in Croatia.

Many species of weed plants can be alternative hosts for fungal parasites of arable crops (Helbig and Caroll, 1984; Mc Keen and Thorpe, 1973; Anikster, 1982; Heiny and Weidemann, 1990). Because of rapid spreading and increasing importance of velvetleaf in arable crops in Croatia, occurrence of *S. sclerotiorum* on this weed may be important for maintenance of inoculum density in soil.

The results of our preliminary research are presented in this paper.

Materials and Methods

Diseased *Abutilon theophrasti* plants from soybean, sugar beet (location Petrijevci) and sunflower (locations Bizovac and Čepin) fields, were first inspected in the field, and later the whole plants or their parts were taken for laboratory tests.

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Infected plant tissues and sclerotia were washed under running tap water for 30 minutes, surface sterilized in 70% ethanol for 30 seconds, rinsed twice in distilled water and blotted dry. *S. sclerotiorum* was isolated on potato-dextrose agar (PDA, pH 6.2) in Petri dishes, from sclerotia and from infected tissues of stem, fruit and seed. Cultures were incubated at 20 °C in thermostat.

To prove that *S. sclerotiorum* is cause of velvetleaf plants decay and to evaluate virulence of *S. sclerotiorum* isolate from *Abutilon theophrasti* on sunflower, artificial infections of velvetleaf and sunflower plants (hybrids Fakir and Sunce) were done. Ten velvetleaf and 20 sunflower (10 hybrid Sunce and 10 hybrid Fakir) plants were inoculated with isolate A (isolated from diseased *A. theophrasti* plants) and the same number of plants with isolate S (from sunflower). Control group had 30 plants (10 velvetleaf, 10 Sunce, 10 Fakir). Inoculum used for inoculations was four day old PDA culture produced form sclerotia collected from naturally diseased velvetleaf and sunflower plants (location Bizovac, 1996). Small agar plugs (5 × 5 mm) with mycelium were used to infect healthy plants – velvetleaf plants 5 weeks and sunflower 3 weeks old. Stems were rinsed with distilled water and agar plugs pressed on them (side with mycelium on the stem). Inoculation site was covered with cotton wool moistened with distilled water and with aluminium foil, to prevent drying out. Control plants were wrapped with cotton wool and foil, but without agar plugs. Plants were kept in growth-cabinet at 23 °C, 90% relative humidity and 12 hours of light per day. Four days after inoculation, inoculum and the cover were removed. Plants were examined daily to record development of lesions on the stems, beginning of wilting and lodging.

After the experiment was finished, *S. sclerotiorum* was re-isolated from diseased plants.

Results and Discussion

First symptoms of disease in velvetleaf in field were recorded during midsummer, when preliminary research of mycopopulation of weed species in arable crops was started.

Certain number of plants showed symptoms of basal stem rot (Fig. 1), but this type of disease was less frequent in velvetleaf compared to its occurrence in sunflower or soybean. More frequently, symptoms appeared on upper stem parts and petioles (but not on blades). In some plants these symptoms were combined with basal stem rot. Fruits and seeds were infected in several cases. Appearance of infected velvetleaf plants was typical for *S. sclerotiorum* – diseased stem parts were bleached, leaves lost their turgor and were hanging on the stems. Dense mycelium was developed on some plant parts. The pith of infected plants was more or less destroyed and numerous sclerotia developed in it, as well as on stem surface. Top parts of sprouts were brown, softened and hung down. Infected fruits were light brown and seeds in them shriveled and bleached.

The *S. sclerotiorum* isolate from velvetleaf grew very well on PDA developing white compact cottony colonies. At the edge of Petri dishes typical sclerotia, white in the

Fig. 1. Basal stem rot (*S. sclerotiorum*) on naturally infected *A. theophrasti* plant

beginning and black later, were formed. Sclerotia formed on naturally diseased plants were irregular in size and shape, while culture-produced sclerotia had rather regular hemispherical shape and uniform size. Culture growth characteristics and production of sclerotia in *S. sclerotiorum* isolate form velvetleaf did not differ significantly from characteristics of the same parasite isolated from sunflower.

Slight wilting symptoms developed third day after inoculation in velvetleaf plants inoculated with both isolates A and S (8 wilted plants inoculated with isolate A, 5 with isolate S). Lesions developed on basal stem parts, and some stems were broken (4 plants in each isolate). Size of spots on the stem and the number of wilted and lodged plants increased every day. Five days after inoculation lower leaves in most plants began falling of. Youngest leaves were still green, but lost their turgor. Lower stem parts were dry and bleached. One plant inoculated with isolate A remained without any symptoms till the end of experiment, while all the others were completely dry and lodged. In isolate S two plants developed lesions and wilted, but did not lodge. From the diseased stems no full-stop. *S. sclerotiorum* was re-isolate on PDA. This trial showed that *S. sclerotiorum* is causal agent of velvetleaf decay. Size of lesions on the stems are shown in Fig. 2.

In both sunflower hybrids first symptoms were recorded on the second day after inoculation. Small lesions developed on inoculation site, usually only on one side of the stem, but plants did not wilt yet. All the plants inoculated with isolate A had lesions, but only 4 in each hybrid inoculated with isolate S. Lodging occurred on the third day after

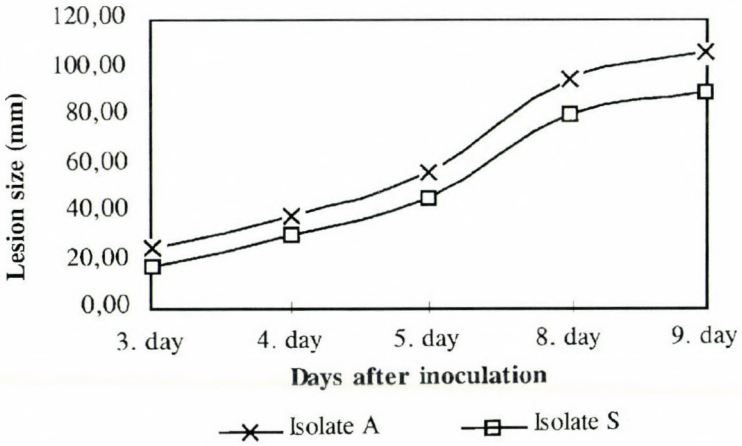

Fig. 2. Mean value of lesion size on velvetleaf stems after inoculation with isolates A and S of *S. sclerotiorum*

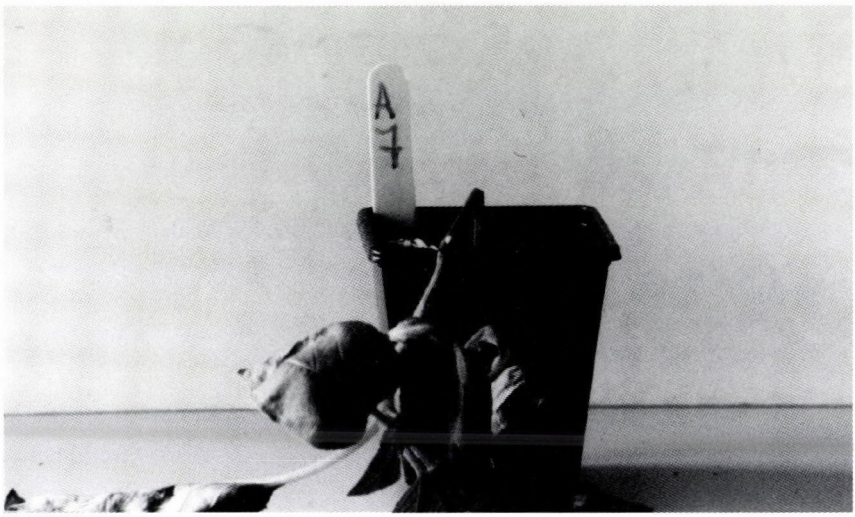

Fig. 3. Lodged sunflower plant (hybrid Fakir) artificially infected with *S. sclerotiorum* isolate A

inoculation (Fig. 3). Seven plants in hybrid Sunce and 8 in hybrid Fakir were lodged when inoculated with isolate A. With isolate S, only 1 plant in hybrid Sunce and 3 in hybrid Fakir lodged. Sclerotia initials were recorded on the fourth day. With isolate A, all plants but one were lodged, with lesions usually up to cotyledons and stems dry and bleached. On the same day, 6 plants in hybrid Sunce and 3 in Fakir were lodged when

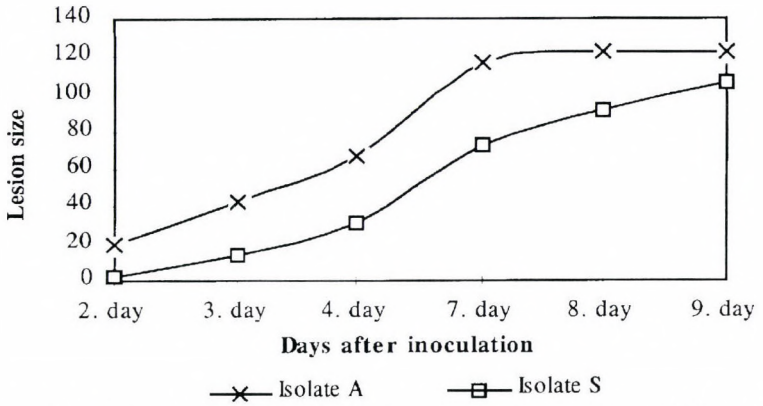

Fig. 4a. Mean value of lesion size of sunflower (hybrid Sunce) stems after inoculation with isolates A and S of *S. sclerotiorum*

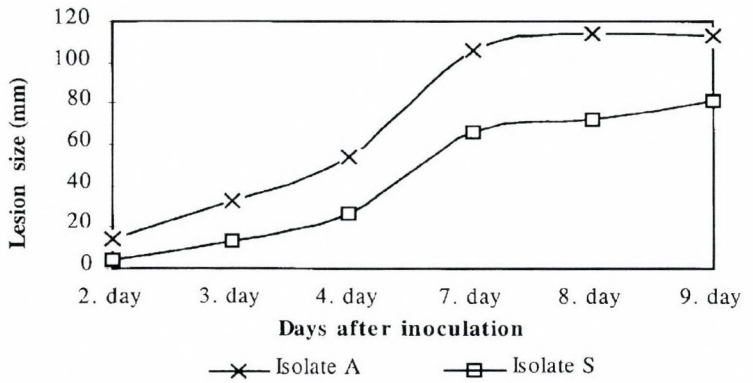

Fig. 4b. Mean value of lesion size on sunflower (hybrid Fakir) stems after inoculation with isolates A and S of *S. sclerotiorum*

inoculated with isolate S. Development of wilting and lodging continued following days. Mean values of lesion size on stems of sunflower plants are shown in Fig. 4a and 4b. On ninth day, only one plant (out of 20) inoculated with isolate A was not lodged, although it had a lesion on the stem. All the others were completely dry. With isolate S, one plant of hybrid Sunce was without any symptoms, others were wilted and lodged, but only four plants completely dry. In hybrid Fakir, three plants dried completely, four lodged but had some green parts, and three plants were without symptoms. Symptoms developed quicker and were more severe in plants inoculated with isolate A, which suggests that this isolate is more virulent for sunflower than isolate S.

There were no symptoms on control velvetleaf and sunflower plants.

Our preliminary research on *S. sclerotiorum* in velvetleaf showed that symptoms on disease in this host correspond with basal stem rot and white rot of upper stem parts in sunflower. In sunflower, soybean and several other hosts *S. sclerotiorum* can be very destructive. Diseased plants wilt and often die before the end of vegetation. Tissues of infected stems decay, stems break and lodging occurs. Velvetleaf plants with such severe symptoms have not been recorded so far.

Significance of weed species as alternative hosts of fungal parasites of arable crops is still insufficiently investigated. However, papers mentioned above as well as papers by Hepperly et al. (1980), Krupinsky (1987), Abbas et al. (1995), Sackston and Wylmore (1990) show that weeds, due to their widespreading, can be important source of inoculum.

Conclusion

For the first time in Croatia, velvetleaf is found to be the host of *S. sclerotiorum*. This parasite could help in reducing the population density of velvetleaf. But, it could cause much more problems since diseased velvetleaf plants are important inoculum source for crops, such as sunflower, soybeans, oil seed rape, etc. Results of our preliminary research showed that isolate of *S. sclerotiorum* from velvetleaf could be even more virulent for sunflower hybrids than isolate of the same parasite from sunflower.

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Reproduction Characteristics of *Pardosa agrestis* (Westring) (Araneae, Lycosidae) Based on Pitfall Trapping in Winter Wheat

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Pitfall trapping was carried out in winter wheat at the Gödöllő University of Agricultural Sciences experimental farm, from the end of March, until harvest in mid-July, 1994.

Dominant spider species of the survey was *Pardosa agerstis* (Westring). Out of the 1373 adult *P. agrestis* collected, there were 222 females. Of these 51 carried cocoons. Average number of eggs per cocoon was 72,43. Average number of larvae or pre-hatched spiderlings per cocoon (74,75) suggests that there is practically no mortality until the larval stage. Frequency distribution of the number of eggs per cocoon shows two peaks at the values of 69 and 84–85 eggs. The number of eggs per cocoon decreases from March until July. It is probable that *P. agrestis* produces two egg sacs from spring until harvest of winter wheat in Hungary.

Studies of predatory arthropod communitites of winter wheat are an important area of research of the Department of Plant Protection, Gödöllő University of Agricultural Sciences. Reports on most important preadators (Araneae, Carabidae, Staphylinidae, Cantharidae, Coccinellidae) and pests (Oulema spp.) (Kiss et al., 1993), on abundance and habitat relation of carabids (Kiss et al., 1994) have been published.

Pardosa agrestis is the dominant wolf spider of Hungarian agroecosystems (Samu et al., 1996; Tóth et al., 1996). Despite the potential role of this species in pest control, only a few publications deal with the so far less known phenology and life history (Samu et al., 1997; Tóth and Kiss, 1997), or methodological problems like density estimation of *P. agrestis* (Kiss and Samu, 1996).

Materials and Methods

The survey was carried out by pitfall trapping in winter wheat at the Gödöllő University of Agricultural Sciences experimental farm (Józsefmajor), 20 km to the north east of Gödöllő, from the end of March, until harvest in mid-July, 1994. Area of the winter wheat field was 61 hectares. Width of the margin was 4–5 m. There was fungicide application on 9 May: Kolfugo 25 FW (carbendazim: 0.92 l/ha) and Microthiol Special (sulphur: 3.7 kg/ha).

Twenty pitfall traps were placed in four parallel rows. One row was placed in the margin and three in the field at increments of 20, 50 and 250 metres from the margin.

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Diameter of the traps was 10 cm. The traps contained a 2% formaline solution to kill and preserve the spiders and a drop of detergent to decrease the surface tension. The traps were emptied weekly.

The cocoons or newly hatched spiderlings were normally separated in the traps, making determinations of the cocoons and the spiderlings difficult. Of the spider species caught, only wolf spiders (Lycosidae) and *Pisaura mirabilis* (Pisauridae) carry their cocoon. Because of their size and special shape, cocoons of the genus *Pardosa* are easily distinguishable from the cocoons of other genera. Except in one case, where two different *Pardosa* females were observed, cocoons or newly hatched spiderlings belonged to females of *P. agrestis*.

Cocoons were dissected and eggs or larvae within were counted. The data were supplemented by counts of the newly hatched spiderlings that, being carried on the back of their mother, had fallen into the pitfall traps.

Results

Out of the 1373 adult *P. agrestis* collected in Józsefmajor, there were 222 females. Of these 51 carried cocoons. The first males were caught at the beginning of April and the first females without cocoons appeared a week later. Females carrying cocoons were first trapped at the end of April. Male and female peak activity occurred near the end of April and the beginning of May. This was also the period when the highest number of cocoons was found. Hatching was first detected in the beginning of June. Adults peak of both sexes was preceded by the peak of subadults (Fig. 1).

There were only 4 non-viable eggs in the 51 dissected cocoons. Average number of eggs per cocoon was 72.43 ($n = 43$; $sd = 16.03$). Average number of larvae or prehatched spiderlings per cocoon was 74.75 ($n = 8$; $sd = 16.05$). Based on these findings, cocoons with larvae or spiderlings, and cocoons containing only eggs are considered equivalent ($p_\alpha = 0.67$; t-test).

Number of eggs per cocoon are represented in Fig. 2. For each value of the number of eggs in the horizontal axis there is a sliding interval of 11 units. The represented x value is in the middle of this interval. Number of cocoons within this interval are represented on the vertical axis. The curve shows two peaks: at the values of 69 and 84–85 eggs.

The number of eggs per cocoon decreases from March until July (Fig. 3). The correlation between date and the number of eggs per cocoon is significant ($r = -0.48$; $df = 50$; $p < 0.001$.

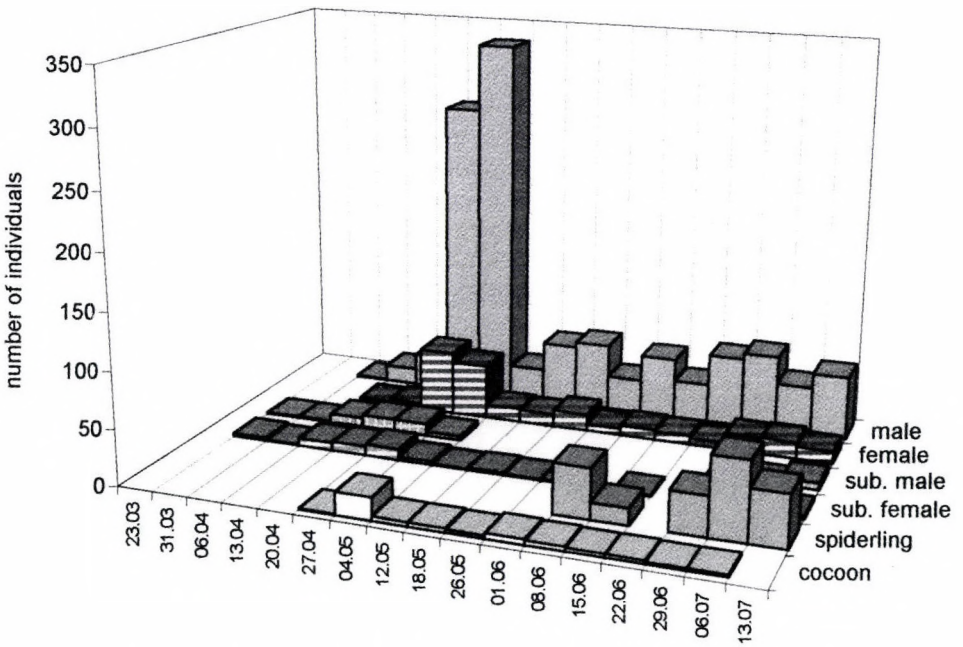

Fig. 1. Phenology of *Pardosa agrestis*, winter wheat, pitfall trapping, 1994

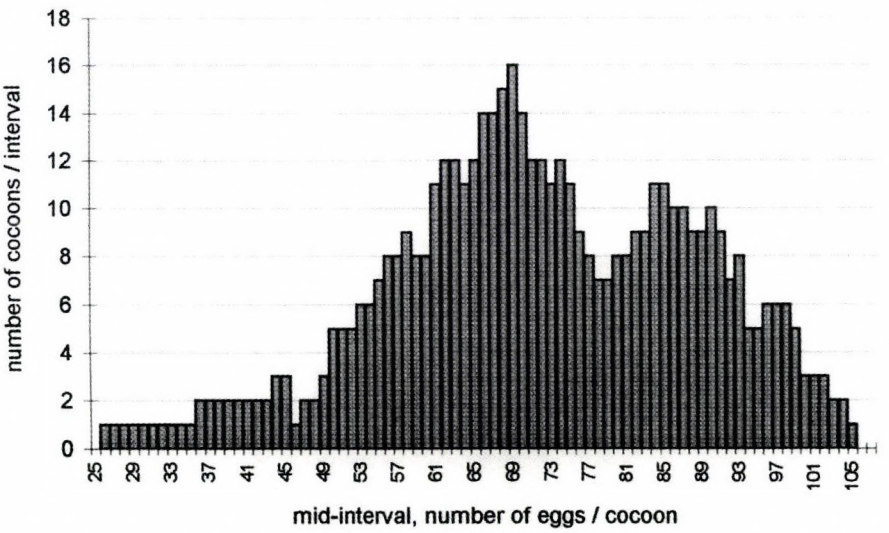

Fig. 2. Frequency distribution of the number of eggs per cocoon, 1994. (width of interval = 11)

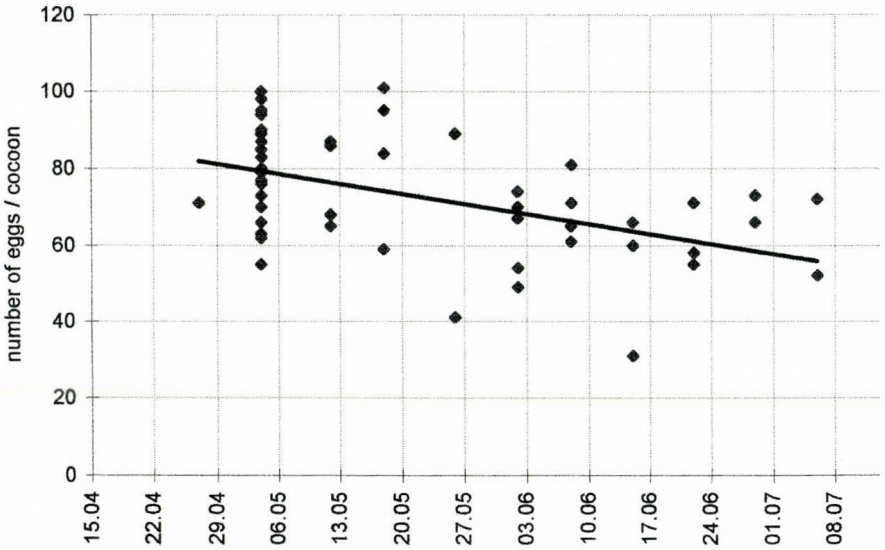

Fig. 3. Number of eggs per cocoon in relation of trapping date, 1994. ($r = -0.48$; $p < 0.001$)

Discussion

There was only a slight difference between average number of eggs and larvae within the cocoons. Thus in-cocoon mortality was not significant during the trapping period in spring and early summer. Besides the first peak of activity in May–June, *P. agrestis* in Hungary has a second peak in August as well (Samu et al., 1997). Balázs Kiss (unpublished data) has observed that mating in late summer must be less successful compared to the mating in spring, because autumn cocoons are more apt to be sterile, and even the number of spiderlings that hatched from the fertile ones is smaller.

In-cocoon development in a temperature range from 20–25 °C of various *Pardosa* species takes about 20–25 days (Kessler, 1971, 1973b), which in somewhat shorter than our field data suggest (ca. one month). Normally the first cocoons contain significantly more eggs than the second (Kessler, 1973a). Therefore, it is possible that our cocoons belonged to the first and then the second brood of *P. agrestis* in the study area.

The frequency distribution curve (Fig. 2) of the number of eggs per cocoon looks like a superposition of two curves of normal distribution. This phenomenon can be explained by the unified presentation of the two consequent broods. However, there is an other possible explanation. On the basis of measuring the carapace dry weight of the females of various *Pardosa* species, the coexistence of two size groups was observed in

the Netherlands. Whether a female hatched from a first or a second cocoon of the previous year determines this paramter, which positively correlates with the number of eggs (Kessler, 1973a).

As a conclusion we formulated a hypothesis that *P. agrestis* produces two egg sacs from spring until harvest of winter wheat in Hungary.

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Light Trapping of Turnip Moth (*Scotia segetum* Schiff.) Connected with Continuance Length of Time and Changes of Péczely Type Macrosynoptic Weather Situations

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The light trapping success of turnip moth (*Scotia segetum* Schiff.) connected with continuance length of time and all the possible changes of Péczely type macrosynoptic weather situations was examined in our present paper using the collecting data of Jermy type light-trap network operating in Hungary. The value of relative catch (RC) was calculated for each observing stations and generations using the catching data.

There was made a comparison between the relative catch values and the Péczely type code number belonging to the date. After it the relative catch values were averaged in all the 13 macrosynoptic situations separated daily according to their continuance time. We compared the difference of the averaged relative catch value of each case with the averaged ones of the sum of all other cases. The significance levels were calculated by t-test. The collecting results connected with the changes of macrosynoptic situations also were examined in the same way.

We can conclude from our results, the significant changing of weather increases the flight activity of examined individuals of species.

Introduction and survey of literature

For determining the mass ratio and swarming time of nocturnal insects, the light-trap is the most widely used sampling device. In Hungary the introduction of the light-trap network, unprecedented even at world standards, was started in 1953 by professor Jermy. At the beginning, regular and scientific sampling was done by research institutions, then the initial problems of technology and operation being solved, the installation of a system of uniform Jermy type light-traps was started in 1958 and 1959 in every country plant protection station, and in 1961 the observation sites of the Forestry Research Institution. In the following years the number of observing stations grew suddenly, because the regional light-traps were organized by plant protection stations. The nationwide light-trap network works at present time.

The Hungarian light-trap network has provided priceless scientific material in the last decades for entomology basic research and plant protection prognostication. We have to know the light-trap would collect always the same part individuals of species being in the environment only in that case if it would work always in same circumstances. Certainly we do not know in this ideal case, which distance the imagos of each species arrive from, whether they are members of biocoenosis in the environment or not, and how much the real mass of this population is. The number of caught insects always would

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show the same proportion of those insects which can react to the trap stimulus, because of this more catching of insects would mean more mass of present insects in the environment.

Still, even the same light-trap cannot work under identical circumstances during two different nights, nor at two different times of the same night, as catching insects by light-traps is affected, apart from the biotic factors, by a number of abiotic ones. A part of their keeps changing in space and time, such as weather phenomena, another part changes, first of all, in time, but in space changes only slightly, such as moonlight and geomagnetism. Again others, such as the flora of the location, transform rather slowly in time, but can be extraordinarily different in space. Because of this the great quantity data collected about each species gave not so much practical advantage for plant protecting forecast till now. The situation would be much more favourable if we were able to recognize the factors that influence the resultativeness of trapping, and to reveal their effect. No wonder that a number of researchers are engaged either in Hungary or all around the world in studying the role of different environmental factors, first of all weather and moonlight. Sorry the new informations can be utilized hardly by the plant protecting prognostic in working out of forecast. One of the reasons of it is, the several environmental factors – though the modifying influence of them are well-known better and better – influence the prevailing catching result not only alone, but also with all the other factors simultaneously and in interaction. The primary aim of the large-scale examinations is to know as many effects of abiotic factors modifying the catches as possible. When the role of the majority of the most effective factors has been recognized, we will have the possibility to correct the prevailing trapping results of the species involved. This being done, the corrected catch datum, parallel to our knowledge growing, will approach a value that will proportionately represent the real amount of population in any case.

Insect flight activity – and similarly, the effectiveness of their light-trap collection – are considerably modified by weather, together with a number of abiotic factors. Unfortunately, a decisive majority of the catch results provided by the light-trap network cannot be examined in connection with the particular weather elements, as most observation sights are situated far from meteorological stations, and those operating the traps did not take any meteorological measurements. Therefore, we revealed the connenction between weather and effectiveness of collecting with a light-trap using a different method. For the purposes of our investigations we found those Péczely's macrosynoptic weather situations to be suitable which express complex weather conditions simultaneously existing and pertaining to the whole area of the Carpathian Basin.

The macrosynoptic typifying which can be considered as pertaining to the area of the Carpathian Basin was elaborated by Péczely (1957 and 1983). The daily macrosynoptic weather situations which were determined on the basis of the baric field at ground level were classified into 13 types and characterized by him. Since 1983, typifying has been continued and the daily code numbers are published by Károssy (Károssy, 1987 and 1994).

The data interpretation period for each type is 24 hours belonging to a calendar day. The one single criterium for coding is the definition of the type which pertains for a

longer period of time during a day, so the type-shift may as well differ ± 12 hours from the time of the change of the calendar date. The progression of the changes in time, as well as the tendency of particular types to endure and the empiric frequency of the occurrence of situations replacing each other differ significantly.

Following Péczely's work in the field of typifying macrosynoptic weather situations (1957 and 1983), his collaborators elaborated on the particular weather situations with regard to some weather elements and included a detailed data-base. In the following, with the continuity of the typifying ensured, the examinations of the element-sets relating to the macrosynoptic situations were also performed.

In the last few years the examination of the connection between the flight activity of harmful insects and the various macrosynoptic weather situations has become an important and determining trend in the above-mentioned research. During this research we examined the effectiveness of trapping in connection with the macrosynoptic weather situations pertaining to the trapping time of harmful insects flying at dawn or in the first part of night. We extended our investigations to the cockchafer (*Melolontha melolontha* L.), which swarms in spring, to the winter moth (*Operophthera brumata* L.), which flies late in autumn, and to two species of moths, which although insignificant from an economical point of view, are easy to trap in autumn,winter and spring, too, the common chesnut (*Conistra vaccinii* L.) and the satellite moth (*Eupsilia transversa* Hfn.) (Károssy et al., 1994). When investigation species which are active all night, we employed a different method since macrosynoptic situations pertain to one calendar day only, so in those cases when one macrosynoptic type pertained to the date of the evening and another to that of the dawn, we had to examine the formation of the flight activity during the periods when the changes occurred. We contracted the 13 macrosynoptic situations typifield by Péczely on the basis of their characteristic wind patterns into 6 types. The contraction was necessary, although we had great number of observing data, but they would be small to examine all of possible changes. The changes of these types form 36 transitional types, so far uncharacterised even from climatological points of view. We examine the effectiveness of light trapping the turnip moth (*Scotia segetum* Schiff.), the fall webworm moth (*Hyphantria cunea* Drury) and the gipsy moth (*Lymantria dispar* L.) in relation to these 36 types (Károssy et al., 1990 and 1992; Nowinszky et al., 1995). The results of our publications connected with this theme were published in summary study in recent past (Károssy et al., 1994). Recently the collecting results of heart-and-dart moth (*Scotia exclamationis* L.) were examined connected with the determined situations for a smaller territory surrounding Budapest using the data of six observing stations in operation at the mentioned territory (Károssy et al., 1996).

In the literature we have not found publications – except ours – which examine the effectiveness of light trapping in connection with macrosynoptic weather situations. The light trapping success of turnip moth (*Scotia segetum* Schiff.) was examined connected with continuance length of time and the all possible changes of Péczely type macrosynoptic weather situations used the newest numerous collecting data in our present paper.

Materials

A short characterization of the 13 marcrosynoptic weather situations is given in the Appendix. The code-number expressing the marcrosynoptic weather situation for the nights studied are taken from Péczely's catalogues (1957 and 1983).

The Jermy type light-trap is a modified version of the Minnesota type, which the guide-sheets have been removed from. The light source is a 100 W normal light bulb at 2 meters above the ground, colour temperature: 2900 K, the killing material is chloroform. The traps of the plant protection institutions worked from 1st April to 31st October, while the forestry ones all the year round, independently of the time of sunrise and sunset, every night from 6 p.m. to 4 a.m. All time data are given in universal time (UT). The insects trapped during one night were stored in one bottle, so the whole catch of one night at one observational site is interpreted as one observational datum.

The collecting data of turnip moth (*Scotia segetum* Schiff.) were used for examinations getting from 78 observing stations of national agricultural and forestry network operating between 1957–1990. During 4 566 nights 58 159 individuals were caught by the traps. We used 40 858 observing data in our examination. We mean on observing data the catching data at one night at one observing station independently of caught individuals.

We hereby express our gratitude to Dr. Pál Szontágh, for collecting data coming from light-traps of forestry, Dr. András Vojnits, senior research-fellow, Museum of National Sciences and Györgyné Mohai research-fellow at Budapest Phytopathological and Soil Protecting Station for collecting data coming form light-traps of plant protection stations and agricultural ones.

Methods

To know the supposed modifying influence of marcrosynoptic weather situations we had to use all of the collected and available data belonging to each species getting from the data of national light-trap network. We could reach, the effect of not examined factors get on less from the simultaneously existent numerous abiotic factors. There were favourable and also unfavourable values in the examined period according to light trapping, that is why their modifying influence had indifferent effect for the results, because of the gigantic number of data. If we want to use contracted the catching results coming from different observing stations and different generation, we cannot use the real number of caught insects. The quantity of caught insects were modified significantly by biotic and abiotic factors.

The environmental factors are not the same at all places and in all times of trapping, because of this it is sure, catching of the same number of individuals at two different observing stations or in two periods mean other proportion of examined populations. To solve the problem, from the catch data we calculated relative catch (RC) values for

Table 1

The average values of relative catches of the turnip moth (*Scotia segetum* Schiff.) according to the continuance length of time of Péczely type macrosynoptic weather situations

Codes	Continuance length of time of Péczely type marcrosynoptic weather situations									
	1	2	3	4	5	6	7	8	9	10
mCc	0.913	1.082	**0.857**	0.956	**0.807**					
(1)	(732)	(235)	(128)	(67)	(24)					
AB	0.993	**0.925**	**0.900**	1.085	1.136	**1.268**	1.595	0.799	1.112	0
(2)	(837)	(402)	(210)	(144)	(110)	(89)	(14)	(14)	(13)	(1)
CMc	**1.124**	**1.258**	**1.146**	1.002						
(3)	(287)	(104)	(43)	(30)						
mCw	1.030	**0.798**	**0.932**	1.188	0.200					
(4)	(769)	(325)	(122)	(25)	(5)					
Ae	1.027	***0.794***	0.965	***1.389***	1.016	1.126	0	0		
(5)	(799)	(403)	(199)	(96)	(53)	(5)	(3)	(3)		
CMw	0.899	***0.663***	1.096							
(6)	(496)	(89)	(15)							
zC	0.946	***0.728***	***0.405***	***0.381***	**0.890**					
(7)	(418)	(165)	(42)	(21)	(20)					
Aw	0.982	1.026	0.943	***0.821***	0.943	***1.644***	**1.289**			
(8)	(2078)	(879)	(273)	(102)	(45)	(33)	(16)			
As	0.979	**1.218**	0.539	0						
(9)	(278)	(46)	(8)	(3)						
An	0.978	0.972	1.001	**0.914**	***0.809***	***0.715***	***0.587***	***0.675***	1.133	
(10)	(1506)	(701)	(492)	(263)	(152)	(91)	(56)	(49)	(27)	
AF	0.957	**1.238**	**1.126**	***1.410***	***1.360***	**1.336**	0.749			
(11)	(616)	(358)	(210)	(149)	(103)	(53)	(42)			
A	1.084	0.986	1.010	1.116	1.067					
(12)	(1244)	(297)	(194)	(107)	(38)					
C	**1.107**	1.724	0	0						
(13)	(87)	(7)	(2)	(2)						

Significance levels are shown with bold numbers (if more than 95%), italic and bold ones (if more than 99%), normal underscored ones (if there are significant differences, but it does not mean important difference from trade point of view) and normal ones (if there are not significant differences). The number of observing data are given in parentheses.

observation sites, species and generations. RC is the quotient of the number of individuals caught during the sampling interval (1 night or 1 hour), and the mean values of the number of individuals of one generation counted for the sample interval. In this way, in the case of expected mean number of individuals, the value of relative catch is 1.

There was made a comparison between the relative catch values and the Péczely type code number belonging to the date. After it the relative catch values were averaged in all the 13 macrosynoptic situations separated daily according to their continuance time. We compared the difference of the averaged relative catch value of each case with the averaged ones of the sum of all other cases. The significance levels were calculated by t-test. The collecting results connected with the changes of marcrosynoptic situations also were examined in the same way. Because of using very numerous data, there was a danger to show significant differences in those cases, when the differences are relatively small and not very significant from trade point of view. Therefore only those significance difference were accepted as significant ones, when they differ from one another with at least 10%.

Results

The relative catch average values of turnip moth (*Scotia segetum* Schiff.), the number of observing data and the significance levels connected with continuance length of time of Péczely type marcrosynoptic weather situations are shown in Table 1 and according to the changing type of marcrosynoptic situations are given in Table 2.

Discussion

If certain situations remain during some days the collecting is favourable on the first day or from the beginning of the first day only at the cases of CMc (3) and C (13) situations, but it is unfavourable in CMw (6) situation. There are not significant difference between the number of caught individuals and the average (expectable) value in all the other weather situations on the first day. Generally the favourable catching results (As [9], AF [11]) and the unfavourable (mCc [1], AB [2], mCw [4], Ae [5], zC [7]) ones are found only from the second or third day or exceptionally later (An [10]) from the fifth day. There are two situations (Ae [5], Aw [8]) when the unfavourable catching result on previous days later change for favourable. According to our suppose in these cases after the decrease of insect's flight activity during some days, the insects have to conform to the unfavourable conditions. The imago has short life, that is why it cannot interrupt the ripening nourishment, the copulation or the oviposition for a long time, because the absence of these ones would endanger the continuance of species. It is sure the zC (7) situation is unfavourable, but the AF (11) one is favourable for catching from the second day. It is remarkable the central anticyclon (A [12]) have not any influence

Table 2

The average values of relative catches of the turnip moth (*Scotia segetum* Schiff.) in the period of Péczely type macrosynoptic weather situations changes

In the evening	Péczely type macrosynoptic weather situations: At daybreak												
	mCc 1	AB 2	CMc 3	mCw 4	Ae 5	CMw 6	zC 7	Aw 8	As 9	An 10	AF 11	A 12	C 13
mCc 1	–	***0.785*** (231)	**0.655** (26)	1.062 (238)	0.521 (15)	0.966 (169)	0.987 (84)	0.991 (842)	**0.874** (50)	***1.456*** (113)	***0.319*** (38)	***0.784*** (56)	***1.219*** (67)
AB 2	**0.884** (111)	–		0.964 (33)	0.989 (1)	**1.138** (58)	1.050 (27)	***0.742*** (292)	**0.657** (26)	1.102 (347)	0.997 (232)	**0.924** (134)	**0.169** (15)
CMc 3	0 (1)	1.097 (176)	–	***0.693*** (102)	0 (1)	1.032 (140)	***0.542*** (88)	1.099 (231)		0.977 (73)	0.895 (31)	***0.327*** (36)	0.440 (4)
mCw 4	0.961 (996)	**1.304** (77)	0.553 (10)	–	**1.258** (57)	1.052 (235)	1.008 (198)	**0.923** (626)	**0.422** (25)	1.124 (36)	**0.499** (30)	1.088 (16)	0.876 (109)
Ae 5	***1490*** (92)	**0.856** (94)	***1.707*** (18)	0.998 (504)	–	**0.870** (283)	**0.810** (69)	1.010 (680)	***0.632*** (56)	***1.248*** (181)	**0.831** (30)	***1.385*** (120)	***2.232*** (19)
CMw 6	1.149 (94)	0.333 (12)	***0.870*** (589)	**1.243** (44)	1.058 (27)	– –	0 (1)	***0.469*** (159)	**1.729** (14)	***0.775*** (166)	**0.807** (34)	***1.330*** (71)	1.067 (320)
zC 7	0.968 (142)	**0.760** (53)		1.063 (150)	1.195 (14)	1.132 (116)	– –	**0.878** (262)	0.921 (65)	**0.904** (36)	***2.533*** (14)	***0.749*** (53)	***0.448*** (34)
Aw 8	0.977 (152)	**0.898** (221)	***0.624*** (42)	**1.157** (358)	1.083 (212)	***1.594*** (180)	**0.926** (281)	– –	***0.829*** (224)	1.097 (719)	1.011 (91)	0.976 (1640)	0.973 (20)
As 9	**0.755** (50)	***0.496*** (37)		1.055 (164)	**1.178** (56)	***1.668*** (30)	***1.487*** (34)	***0.643*** (129)	– –	0.683 (10)	0.400 (5)	**0.923** (142)	0.200 (5)
An 10	***0.682*** (70)	1.092 (135)		**1.136** (392)	1.052 (734)	**0.861** (116)	2.183 (3)	***1.549*** (255)	0.470 (14)	– –	0.940 (291)	1.003 (446)	1.116 (24)
AF 11	0.691 (15)	**0.839** (83)	0.704 (14)	**1.201** (56)	0.929 (118)	***0.612*** (47)		0.929 (55)		1.007 (411)	– –	1.617 (18)	0.161 (11)
A 12	1.097 (69)	1.025 (165)		**1.106** (379)	1.024 (936)	**1.265** (81)	**1.200** (91)	***1.182*** (456)	**1.184** (176)	1.093 (331)	***1.828*** (34)	– –	**1.488** (13)
C 13	***0.859*** (164)	**0.838** (39)	0.922 (150)	***1.321*** (58)		***1.216*** (101)	**0.756** (20)	***1.270*** (68)	0.479 (8)	1.036 (42)		**1.212** (28)	– –

Significance levels are shown with bold numbers (if more than 95%), italic and bold ones (if more than 99%), normal underscored ones (if there are significant differences, but it does not mean important difference from trade point of view) and normal ones (if there are not significant differences). The number of observing data are given is parentheses.

for success of collecting during the whole continuance. If this situation changes at dawn, the number of caught insects will also increase in seven cases. We can conclude the quiet situation is not favourable according to the collecting success, but the changes are favourable for it.

High and low catching results also belong to changing weather situations. After examining these situations we cannot declare clear regularity, although it is remarkable at changing the meridional northern situations there are more small catching results, than high ones. This establishment is also true, if the meridional northern situations change to any other ones, but it is mainly true, if any other weather situations change to meridional northern ones. It is typical of the meridional northern directions situations, their appearance often belong to passing of cold weather fronts. At that time the weather is cool, windy, cloudy and frequently there is a rainfall. After these circumstances the low flight activity of insects can be understood. We could often find high collecting result if there were those changing situations when any other situations changed for meridional southern one. There were some cases when the zonal western situation changed for meridional southern one, or the central anticyclon changed for meridional southern situation, the cloudless, dry weather was followed by cloudy and rainy one. Probably the increase of collecting is caused by prefrontal influence.

Relatively the number of those changing situations are less when the catching results do not differ significantly from the expectable values. This is a proof for that fact, mainly the number of caught moths increase in weather changings. Sometimes when significant low or high catching results do not belong to the changing situations, seeing the high number of observing data we can be sure in this time the flight activity neither increases, nor decreases. Probably in other cases the modifying influence cannot be proved, because of the relatively less collecting results. Sometimes we found strong significant differences at that case when the number of observing data were less then 20. May be such kinds of changing weather situations modify significantly the catching results, we think the practical importance is very small, because it is very infrequent.

We can conclude from our results, the significant changing of weather increases the flight activity of examined individuals of species. This fact does not mean favourable weather conditions for the flying of insects. The low values of relative catch mean those weather situations in all cases, when the flight activity of insects decreased, but the meaning of high values are not so equivalent. The significant environmental changes cause physiological changes in the organism of insects. The life of imago is short, the unfavourable weather endangers not only the continuance of individual but also the continuance of the total species. According to our supposition the individuals can use two kinds of strategies to prevent the hindering influences of normal function in phenomenon of life. First is the increased activity. It means the growing of intensity in flying, copulation and oviposition. The second strategy is to hide and ride out in passivity the unfavourable situation. Seeing the above-mentioned facts, according to our present knowledge high light trapping results can belong to both favourable and unfavourable situations.

Seeing that the Péczely's marcrosynoptic situations are valid simultaneously in the whole Carpathian Basin, our results can be utilized not only in Hungary, but also in

one part of territory in neighbouring countries for the purpose of making plant protecting prognosis. We can declare is spite of that case we cannot give the correct explanation of high or low catching results in all the changing situations according to our knowledge. Further agrometeorological researches are necessary to find how can be modified the insect's comfort feeling and flight activity by different type changing situations.

Using the Péczely's marcrosynoptic weather situations offers a possibility for investigating the insect's life-phenomena in connection with weather also in those cases where the measuring of certain elements for some reasons comes up against difficulties. The collecting data of the national light-trap network, which is invaluable for science, has also become employable to insect ecological and etological investigations. On the basis of our work it is also proved that Péczely's macrosynoptic situations are reliable not only from the point of view of climatological typization, but also with regard to agrometeorological research. We think it essential to elaborate a similar typization for other geographical regions, and other harmful species of insects as well.

Appendix

A short characterization of the 13 marcrosynoptic weather situations is given in the following:

MERIDIONAL, NORTHERLY ORIENTED SITUATIONS

mCc (1) Cold front from the meridional situations

A situation with meridional direction and northern stream. Hungary belongs to the rear cold front current system of the cyclone, which stays east or nort-east of it, over the Balticum or the Ukraine. This situation causes changeable, windy and wet weather in the Carpathian Basin. In summer a version without a cold front may also arise, when a termic depression effect from South-West Asia spreads over South-East Europe. In summer, this situation is favourable for forming local showers, thunderstorms, in winter snowstorms are frequent. In summer the temperature is above average, in winter it is below average, in spring the deviation is not significant. Cloudiness surpasses the average level, visibility is good, in winter the tendency for fog is smaller. Air pollution is usually insignificant. Typically, the northerly and the north-westerly winds are strong while the westerly and south-westerly winds are strong beyond the Tisza river. There is more precipitation in the eastern half of the country. Atmospheric temperature layers are stabile, the lower layers are warmer. The daily temperature fluctuation is small and aperiodic.

AB (2) Anticyclone over the British Isles

This is a meridionally directed situation with northerly current. Partly because of the Azores cyclone moving to the north, partly because of the anticyclones moving from the arctic basins to the south, high-pressure air masses develop over the British Isles or the North Sea. Its appearance in the Carpathian Basin is usually connected to the passing of a cold front, and results in intensive north-, north-westerly air currents in our region.

When the above situation stabilizes in summer, the baric gradient is a lot lower over Central Europe; on such occasions dry, prolonged warm weather evolves in the Carpathian Basin. It is a misty situation in autumn, winter and spring as well. During the greater part of the year it is characterized by colder air masses of arctic origin and average cloudiness, with higher degrees of cloudiness in summer. There is a strong tendency for fog in winter. There is a north-westerly, westerly wind; over the Tisza river it is westerly, south-westerly, and relatively strong. The temperature-stratification of the air is stabile.

CMc (3) Cold front arising from a Mediterranean cyclone

A situation with meridional direction and northern current. It is the current-system of the back-side of the cyclone. The situation emerges by way of a Mediterranean cyclone moving towards the Balcan peninsula or the region of the Black Sea, so the Carpathian Basin falls in the rear, cold front current system of the cyclone. The movement of air is in a northern, north-west direction. Its speed – mainly in the Transdanubia – may even reach storm intensity. Especially in summer, precipitation may increase, in different amounts at various locations. Snow showers are frequent in winter, storms in spring. Cloudiness is definitely extensive, especially in the summer half of the year. Air pollution is low, the tendency for fog is also low in winter. The temperature is lower in spring and autumn, and higher in winter than on the days preceding this weather situation. The daily fluctuation of the temperature is aperiodic.

MERIDIONAL SITUATIONS WITH A SOUTHERN DIRECTION

mCw (4) Warm front arising from a meridional cyclone

This is a situation of meridional direction, with flow toward the south; it is the frontal current system of the cyclone. The current over the Carpathian Basin is directed by a cyclone with its centre either in the region of North-Western Europe or in Western Europe. Hungary's territory is under the effect of the cyclone's warm front, or falls into its warm sector. In autumn it is cooler, in winter and spring milder than the average temperature of the given season. Cloudiness is more extensive, mainly in spring and autumn. Prolonged, slow rains and snowfalls are equally frequent from autumn to spring. Visibility is bad, the frequency of fog is high in winter. In summer it is characterized by sultriness and high degree of air pollution. The southern air current brings considerable precipitation, especially in the winter half of the year.

Ae (5) Anticyclone located east of the Carpathian Basin

A meridional situation with southern current. A dry, southerly, or south-westerly air current dominates in an anticyclone located east of Hungary with its center over the Ukraine. The weather fronts range west of the Carpathian Basin. This situation is characterized by dry, warm, bright weather in summer, and in winter, after snowy days by bitter cold, frequent rime and fog. In autumn and spring, temperature fluctuation is large with a strong rise in temperature. In the cold season the range of the Eastern Carpathians often modifies the direction of the izobars, and in this way the cold, surface level air masses

invade the territory of the country passing round the Southern Carpathians (Kossava effect). It is characterized by a temperature surpassing the average prevalent during the greater part of the year. Cloudiness, mainly in summer, is smaller and dry, droughty weather is frequent at this time. In accordance with the weak, southerly current, the amount of precipitation is small, visibility is bad, and air pollution is considerable. The air shows inverse temperature stratification.

CMw (6) Warm front arising from a Mediterranean cyclone

This situation has a meridional direction and southerly current. The cyclone's frontal system of current asserts itself in Hungary. The system is defined by a cyclone which arises over the central part of the Mediterranean Sea and moves toward the Adriatic region. Its warm front passes over the Carpathian Basin causing substantial rains in the winter and spring months, as well as snowfalls in winter. In summer its temperature is lower than the national average temperature. Visibility is low, cloudiness strong, and the fluctuation of the temperature is aperiodic.

ZONAL SITUATIONS WITH WESTERN DIRECTION

zC (7) Zonal cyclone

There is a zonal, westerly flow. While it prevails the European stretch of the frontal zone ranges near the 50° latitude. The air flow is westerly. Northern Europe is affected by fast moving cyclones. The weather is windy and changeable. The temperature, characteristically, is cool in autumn, mild in winter, and in summer it is colder than the average for that season. In spring the fluctuation in temperature is low. Cloudiness is strong, especially in the spring and autumn months. The yield of precipitation is larger at the beginning of autumn and in winter. The lower air strata are warmer. Colder, arctic air strata flow in the higher layers.

Aw (8) Anticyclone located west of the Carpathian Basin

It has zonal current with a western direction. When the Azores cyclone travels north (mainly in summer), its protrusion advances as far as the Central-European region. Its formation usually takes place in connection with a cold front which passes through and results in an intense westerly or north-westerly current in the Carpathian Basin. It is characterized by pleasant, warm and bright weather which however, is misty in autumn and spring, and mild, misty and foggy in winter. In winter it is colder than the temperature typical for that season. Its cloudiness is average, yet it is overcast in summer. Visibility is good, air pollution is low. The lower stratum of air is usually warmer than the one over it, in which there is a cold air current.

As (9) Anticyclone located south of the Carpathian Basin

This situation has a zonal, western current. The northern fringe of the anticyclone situated over the basin of the Mediterranean Sea protrudes into the Carpathian Basin. The northern edge of the frontal zone moves upward, so the cyclone moves along a more northern trajectory, and their frontal system does not effect Hungary. During the greater

part of the year this situation-type is warmer than the average and is characterized by a lower degree of cloudiness. In winter, autumn and spring the bright, warm days are followed by mild nights. In winter cloudiness is somewhat stronger, and the frequency of fog is higher. In summer it brings about sultry weather. The air flow is weak, and precipitation is low. The lower stratum of air is colder than the upper, however the opposite may also occur.

ZONAL SITUATION EASTERN DIRECTION

An (10) Anticyclone located north of the Carpathian Basin

This situation has an eastern, zonal current. The anticyclone stays north of Hungary over the Balticum or Poland, and forms a high-pressure ridge from the British Isles as far as Eastern Europe. In summer it is warmer than the temperature typical for that season. It causes a strong fall in temperature in autumn and in spring, but after the cold night a rise in temperature follows about midday. It is characterized by clean air and northern winds. In winter it is connected with the invasion of very cold air masses. On such occasions it is easy to observe how the Carpathian ranges modify the movement of ground level cold air masses and their passage through mountain passes. Many times characteristic, embracing izobars develop along the Carpathians, and the cold invasion from either side sometimes may result in and occlusion front inside the Basin. The weather is windy and foggy even in winter with average cloudiness, and a sky which is a bit more overcast in the spring and autumn months. Sometimes air pollution is high. The air-flow is typically of north-eastern direction. The stratification of air characterized by warmer lower and colder higher strata.

AF (11) Anticyclone located over the Scandinavian peninsula

This situation has a zonal eastern air-flow. The characteristic orientation of the longitudinal axis of the anticyclone which stays in the Fenno-Scandinavian region has a north-easterly diretion. This weather situation brings about a northern or north-eastern flow in Hungary. During it s existence, the weather, especially in autumn, winter and spring is bright and clear, but the air is very cold. It is characterized by northerly winds, wide fluctuation in temperature, average cloudiness, and little precipitation. The Icemen (the three chilly days in May) are usually connected to this marcrosynoptic type.

CENTRAL ANTICYCLONE

A (12) Anticyclone located over the Carpathian Basin

The whole region of Central Europe is dominated by a centrally situated anticyclone which rises above the Carpathian Basin. It can be of smaller size, even just a few hundred kilometres in diameter, but it can also be a so-called intermediate anticyclone, which moves fast separating other cyclone systems. In most cases, however, it remains for a longer period over the Carpathian Basin. Its duration gets prolonged in winter by a cold air-cushion stuck on the bottom of the Basin (inversion). Its prolonged existence ensures undisturbed radiation weather. In winter it is accompanied by a strong fall in the temperature, and considerable inversions of temperature, and in summer by a great rise in

temperature, heat waves and thunderstorms. One frequent feature is an air-flow in diverse directions which originates from the centre. During the greater part of the year it can be characterized by a temperature of radiation effect – i.e. warm during the day and in summer, cold during the night and in winter. The weather is warm and pleasant either in spring or in autumn, while it is foggy, frosty and rimy in winter. Temperature fluctuation is great. Cloudiness is slight. It is a bit more overcast in winter, and brighter in summer. Precipitation is small, showing large regional variability. Visibility is bad. There is a high frequency of fog, and air pollution may be strong. The air is usually dry. The wind has no uniform or characteristic direction.

CENTRAL CYCLONE

C (13) Cyclone located above the Carpathian Basin

The centre of the cyclone is located over the Carpathian Basin. In a great majority of cases, Mediterranean cyclones which pass over Hungary from this type. There may, however, be cases when a cyclone develops having local, orographic causes along a front that has grown stagnant. A sharp contrast in temperature evolves in Hungary. The north-western parts of the country fall in the rear flow system of the cyclone, so the temperature there is much lower than in the eastern part of the country, which fall into the frontal flow system. In the western, north-western and south-western regions of the country, because of what was said above, the frequency of fronts is higher than in the rest of the country. When this type is present, in winter the temperature is higher, in summer it is lower than during the preceding days. In autumn this type is characterized by cold, windy, overcast and rainy weather, and in winter by stormy weather. In spring it is characterized by rainy weather. In all three seasons temperature fluctuation is small. Cloudiness is greater in summer, smaller in winter. Visibility is bad, and air pollution is low. A strong field of flow is characteristic, although its direction is not homogeneous. Precipitation is markedly large.

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Light Trapping Success of Heart-and-Dart Moth (*Scotia exclamationis* L.) Depending on Air Masses and Weather Fronts

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The Jermy type light-trap network – unprecedented even at world standards – has been working all over Hungary since 40 years. The collecting success is influenced by a number of abiotic factors. We examined from these factors the influences of the weather fronts, air masses, the height of tropopause and the microbarographic oscillations to make a useful prognosis by using these gigantic data. We got the meteorological data measuring hourly in Budapest by National Meteorological Service. We categorized the weather fronts, discontinuity surfaces, the surface and upper air masses after Berkes (1961). We determined the upper air masses according to the measuring of radiosondes giving informations about the cross-section in time. We used for our examinations the data of the heart-and-dart moth (*Scotia exclamationis* L.) adults getting from the light-trap network in Hungary. We chose the data taking from 6 observing stations working surroundings Budapest.

For determining the mass ratio and swarming time of nocturnal insects, the light-trap is the most widely used sampling device. In Hungary, the introduction of the light-trap network, unprecedented even at world standards, was started in 1953 by professor Jermy.

The Hungarian light-trap network has provided priceless scientific material in the last decades for entomological basic research and plant protection prognostication. If we want to use the catch data for prognostication, we must accept as basic principle that in so far as the light-trap works under identical circumstances all the time, the number of catches is identically proportionate in any case to the population density in the surroundings. Only on this basis can it be assumed that a larger catch means a larger number of insects present in the surroundings. Still, even the same light-trap cannot work under identical circumstances during two different nights, nor at two different times of the same night, as catching insects by light-trap is affected, apart from the biotic factors, by a number of abiotic ones.

The situation would be much more favourable if we were able to recognize the factors that influence the efficiency of trapping, and to reveal their effect. No wonder that a number of researchers are engaged either in Hungary or all around the world in studying the role of different environmental factors, first of all weather and moonlight. The primary aim of the large-scale studies is to know as many effects of abiotic factors modifying the catches as possible. When the role of the majority of the most effective factors has been recognized, we will have the possibility to correct the prevailing trapping results

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of the species involved. This being done, the corrected catch date, parallel to our increasing knowledge, will approach a value that will proportionately represent the real density of populations in any case.

The frontal passages and changes of air mass cause the sudden and fundamental changes of living creatures' physical environment. The organism of a person or animal answer with front sensitivity symptoms for all the simultaneous changing of weather factors. The behaviour of insects and their flying activity were examined connected with variant front types and air masses by a few researchers in contrast to wide medical meteorological researches. The effect of weather fronts on light trapping of insects was examined by Wéber (1959) in Hungary. In his opinion the research of the front changing effect is hindered by a number of factors (fronts can follow one another with one or two hours differences, weather fronts can pass through without changing of air masses, the intensities of some kinds of fronts can be different). Because of these problems he did not try to determine general regularities in his works. Justed of this he proved the modifying influences for catching of weather fronts with graphic method after the examination of some concrete, typical events. The influences of air masses were also examined by Wéber (1957). He made certain the number of insects caught is significantly the highest at the arrival of subtropic air masses. Járfás (1979) preferred to examine each weather factor instead of weather fronts because of mentioned problems. Kádár and Szentkirályi (1991) showed that the flight activity of ground beetles (Coleoptera, Carabidae) decreases at arrivals of cold fronts, but it increases at arriving warm fronts. They used the front- and air mass calendar issued by the Hungarian National Meteorological Service. These two kinds of front types were determined in this calendar. The light trapping results were examined in New Zealand in relation to cold fronts by Helson and Penman (1970). They experienced ion activity maxima short fly before their arrivals. We did not find fundamental publications in the literature about connections with weather fronts and light trapping.

The great number of collecting data getting from our national light-trap network is suitable to study the influence of weather fronts with mathematical-statistical methods, too. That is why we had to expand our research work in this direction to get suitable results in plant protection forecasting.

Materials

We got the meteorological data from measuring hourly in Budapest by the National Meteorological Service. We used the hourly observations for determination of frontal passages and discontinuity levels. We considered also temperature, atmospheric humidity, wind direction and strength, cloudiness, visibility and air pressure data. We used the categories of weather fronts and discontinuity levels determined by Berkes (1961). The classification of the surface and upper air masses were also made following Berkes (1961). We determined the upper air masses examined the so-called cross-sections in time using the informations of radiosondes that took off at Budapest-

Szentlőrinc. There were two takings off daily. We hereby express our gratitude to the management of the National Meteorological Service for furnishing these data. The taking off were at 0 and 12 o'clock in universal time (UT). The data of temperature, atmospheric humidity, air pressure, wind direction and strength were measured by the ballon radiosonde and relayed on sender to the observing station. The points indicating changes, so-called "marked points" are given in forms of telegraphic code. Several meteorological data can be counted using these informations and a special calculating rule (similar to the slide-rule), for example equipotential temperature. The used method saves "conservative characteristics" of the air. This means whether the temperature or the atmospheric humidity change, the "heat content" of air fragment is shown by the several combinations even at 9 km height.

The values of equipotential temperature drawing one over the other, result in get a curved line, which can change from day to day or even hour by hour according to the weather situations. We determined in the cross-sections characteristic data of "main" air masses using "typehomologs" according to Schinze (1932) from Germany. In this way we got the following types: polar cold, polar warm and subtropical ones. It was clear from the early sixties, that the upper division of air types in Carpathian Basin are different from the "Central European" model. That is why we calculated for every month the basis values of temperature, relative humidity, specific humidity equivalent and equipotential temperature for the 1000, 850, 700, 600, 500, 400 and 300 hPa pressure surfaces using the aerological data measured in Budapest during 6 years. The typehomologs were determined for polar, cold, temperate, warm and subtropical air masses using the informations. The continental and maritime air masses were also separated. If the actual equipotential temperature curved line shows the changings of these air masses, examining the barometric data we can determine the belonging heights. If the boundaries of distinct air currents are registered, thus the air masses being above one another can be separated. Examining the vertical cros-ssection of the tropopause is the apart air current between the lower air masses (troposphere) and the upper air masses (stratosphere) can be determined. The height of troposphere is variable, because at the time of very cold polar air it is only 5 km, but at subtropical air mass it can be even 16 km. Sometimes there are 2–3 tropopauses above one another. In this case we can wait for strong weather changes or we find cloudless, anticyclonic weather situation.

We worked with the following air masses in our examinations: *1. mA* maritime polar air, coming from the area of Greenland and Spitsbergen on polar axis through the North Sea, *2. cA* continental polar air, coming through the European territories of Russia on ultrapolar axis, *3. mK* maritime cool air, coming from the area of Iceland and Scotland on polar axis, *4. rK* returning maritime cool air from SW direction, *5. cK* continental cold air, coming from North Siberia on ultrapolar axis, *6. r'K* returning polar (cK) air from SE direction, *7. mM* maritime air coming from the area of 50° north latitude, *8. m'M* Mediterranean air, *9. m"M* Black Sea air, *10. cM* continental air coming from the area of 50° north latitude, *11. mW* maritime warm air, *12. m'W* Mediterranean warm air from SW direction, *13. m"W* warm air coming from the Black Sea, *14. rW* returning maritime warm air, *15. r'W* returning warm air (m"W or TM) from NE direction, *16. cW* continen-

tal warm air, *17. mT* air coming from the Azores with W and WSW direction, *18. m'T* Saharian air coming through the Mediterranean Sea, *19. m"T* Saharian air coming through the Black Sea, *20. TM* subtropical air, *21. cT* continental subtropical air coming from the Near-East with SE direction, *22. S* Saharian (cT) air (observable only at upper levels).

The weather data of fronts and apart air currents exactly fit into the vertical cross-sections, even they show the boundaries of colder or warmer air masses in upper air masses. This is independent of the front types near the surface. The different air masses and apart air currents were also classified following Berkes (1961): *1. W* surface warm front, *2.* W_h upper warm front, *3.* O_w warm occluded front, *4.* W_u unstable warm front, *5.* W_m masked warm front, *6. C* surface cold front, *7.* C_h upper cold front, *8.* O_c cold occluded front, *9.* C_u unstable cold front, *10.* C_m masked cold front, *11.* C_p paradox cold front, *12. O* neutral occlusion, *13.* O_{OR} orographic occlusion, *14.* S_{ww} stationary front that arrives and leaves as warm front, *15.* S_{cc} stationary front that arrives and leaves as cold front, *16.* S_{wc} stationary front that arrives as a warm front and leaves as a cold front, *17.* S_{cw} stationary front that arrives as a cold front and leaves as a warm front, *18. D* subsidence in general, *19. D'* descending motion between two cold fronts, *20. S* destroying high pressure system, accompanied by following falling barometric tendency. The weather fronts were numbered following Berkes (1961), and we used the 0, 1 and 2 exponents for characterization of their power.

We also used the data of microbarographic oscillation for our examinations. The instrument used for this measurement was made in the years before World War II. The air was exhausted from a 30 liter flask jointed to a very sensitive barometer. The time is registered on a 10 minutes graduation, the hourly registrations are about 20 mm and a sinus graph is written on the paper of relative graduation hourly. If there are weather changes or front passages the values change. There are 3 different classes. The class 0 is if the frequency writes one sinus wave and its amplitude is 2 mm. In class 1 the frequency of waves is between 3–5 and the amplitude is 5 mm. In class 2 the frequency is above 5 and the amplitude is above 5 mm. These are in relation to the long-wave electromagnetic radiation according to German literature (Daubert, 1955).

We used for our examinations the data collected by six light-traps operating in the surroundings of Budapest. We worked with the catches of heart-and-dart moth (*Scotia exclamationis* L.). We could not make a comparison between the meteorological data and the collecting results coming from all of the observing stations, because the validity territories of air masses and weather fronts are uncertain, and the territory studied does not extend to the whole country (Csizsinszky, 1964). We chose this species, because it swarms in two generations yearly and it can be caught in great numbers generally between May and September.

The Jermy type light-trap is a modified version of the Minnesota type one, from which the guide-sheets have been removed. The light source is a 100 W normal light bulb at 2 meters above the ground, colour temperature: 2900 K, the killing material is chloroform. The traps of plant protection institutions worked from 1st April to 31st October, while those of the forestry all the year round, independent of the time of sunrise

and sunset, every night from 6 p.m. to 4 a.m. The insects trapped during one night were stored in one bottle, so the whole catch of one night at one observational site is interpreted as one observational datum. We got data from the observing stations as below: Budakeszi (1962–1970), Budatétény (1960–1967), Budapest-Rókushegy (1959–1967), Nagytétény (1959–1976), Martonvásár (1960–1961) and Érd (1977–1979). We used altogether the data of 1762 nights, 7239 individuals and 3461 observations. We consider catching data of each night at one observing station independently of caught individuals.
We hereby express our gratitude to Dr. Pál Szontágh, Doctor of Agricultural Science for collecting data from forestry light-trap, Dr. András Vojnits (Museum of Natural Science) and Mrs. Gy. Mohai (Budapest Phytopathological and Soil Protecting Station) for collecting data from light-trap of plant protection.

Methods

The number of the individuals trapped at different observation sites and times cannot be compared to each other even in the case of identical species, as each trap works in a different environment, and the environmental factors vary constantly according to time as well. To solve the problem, from the catch data we calculated relative catch (RC) values for observation sites, species and generations. RC is the quotient of the number of individuals caught during the sampling interval (1 night or 1 hour), and the mean values of the number of individuals of one generation counted for the sample interval. In this way, in the case of expected mean number of individuals, the value of relative catch is 1.

The research on influence of air masses were made according to more points of view. We separated those cases at examinations on the influence of air masses near the surface when the same air masses were in the surrounding of the selected light-trap during the night and those ones where air masses changed during the night. The same air mass spends longer time in upper air-layers then in lower ones. Because of this here we could separate the collecting results belonging to the arriving days and following ones. Using this method we could examine whether the influence of arriving air masses and staying for a long time ones on flight activity are the same or not.

We examined separately the success of trapping at those nights when the air masses near the surface and when the air masses in the upper air-layer there are same. We also made an examination for the success of collection in those cases when the air masses were mixed with one another not far from the surface.

We calculated the success of light trapping connected with nocturnal averages of heights of tropopause and also in the cases of multiple tropopause. We worked up separately the catch data of those nights in the examinations for supposed influence of weather fronts and discontinuity levels when there was only one front effect and when the weather fronts changed one another. Finally we compared the successes of light trapping when the microbarographic oscillations changed during the night.

We summarized and averaged the results of relative catches coming from several observing stations for each nights in the flying period of heart-and-dart moth (*Scotia*

exclamationis L.) connected with the air masses, weather front types, height values of tropopause and microbarographic oscillation. The supposed influence of multiple tropopause in modifying the catch result was examined compared with light trapping results of preceding and following days. We compared the differences of the averaged relative catch values of each case with the averaged ones of the sum of all other cases. The significance levels were calculated by *t*-test.

Results

Our results are shown in Tables 1–9.

Discussion

A few numbers of individuals were caught by the light-trap if the same cold air mass was near the surface (1. mA, 2. cA, 4. rK, 5. cK). This conclusion is also right at that time, when there are 1. mA or 2. cA air masses both near the surface and in upper level. The catch is also small, if 7. mM air mass is near the surface or 10. cM one in upper part. The collecting is successful if there are warm air masses above the surface (9. m″M, 11. mW, 12. m′W, 13. m″W, 14. rW, 15. r′W, 16. cW, 17. mT, 18. m′T, 19. m″T and 21. cT) and if 9. m″M, 13. m″W, 15. r′W and 20. TM air masses are present near the surface and also in the upper air-layer. These results support our knowledge, the flight activity of insects is low in cold air and it is high in warm one. We got interesting results by examining the collecting success of arriving days belonging to different air masses and compared with following days. We found lower collecting in several cases on arriving days and higher light trapping results on following days with the same air masses (1. mA, 11. mW, 19. m″T, 20. TM and 21. cT). We noticed sometimes opposite phenomena (2. cA, 9. m″M, 14. rW, 15. r′W and 22. S). We found the effectiveness of subtropical air masses in increasing flight activity and of course light-trap catching, especially the 17. mT, 18. m′T and 22. S air masses on arriving days, and 20. TM and 21. cT on following days. This three kinds of air masses show strong biological activity in human biometeorology as well. It was suprising that the catch decreased on the arriving day and following ones of 19. m″T air coming from the Black Sea.

The surface 1. mA and 2. cA air are unfavourable for light trapping, but if there is 17. mT above the surface 1. mA, the activity of moths increases. We found the same in the activity when above the Mediterranean warm air (12. m′W) arrives bringing fresh subtropical air from the Azores (17. mT). The 20. TM air mass do not cause development in collecting, but arriving above 17. mT or 18. m'T means increase in the catch. May be because of the strong atmospherical electricity the effectiveness of light trapping is high if Saharian air comes above maritime temperate air (7. mM). This hypothesis is found on results of spherics measuring made by Sulman (1976) in Israel.

Table 1

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest connected with unchangeable air masses during the night

No. and code of air masses		Unchanging during the whole night near the surface		Unchanging during the whole night in upper air layers				Same near the surface and in upper air layers	
				on arriving day		on following days			
		RC	Sign. (%)	RC	Sign. (%)	RC	Sign. (%)	RC	Sign. (%)
1	mA	0.740	99	*0.922*	95	*1.116*	95	0.863	95
		(204)		(154)		(94)		(72)	
2	cA	0.659	95	*0.991*	–	*0.791*	95	0.464	90
		(41)		(49)		(40)		(12)	
3	mK	0.969	–	0.851	99	0.815	99	0.912	90
		(378)		(167)		(84)		(78)	
4	rK, m′K	0.727	95	0.915	90	0.124	–	1.322	–
		(29)		(35)		(7)		(6)	
5	cK	0.470	95	0.577	–	–	–	–	–
		(15)		(3)					
6	r′K	–	–	0.370	–	0.540	–	0.370	–
				(8)		(3)		(8)	
7	mM	0.941	95	0.922	95	0.934	90	1.023	–
		(370)		(331)		(179)		(135)	
8	m′M	0.993	–	0.965	90	1.031	–	0.900	–
		(68)		(84)		(27)		(7)	
9	m″M	1.544	99	*1.318*	95	*1.088*	–	1.643	95
		(34)		(32)		(21)		(12)	
10	cM	1.012	–	0.976	–	0.878	90	0.722	95
		(193)		(196)		(79)		(35)	
11	mW	1.328	99	*0.696*	99	*1.279*	99	0.861	–
		(60)		(138)		(59)		(8)	
12	m′W	1.137	90	1.116	90	0.995	–	1.011	–
		(131)		(195)		(95)		(42)	
13	m″W	0.907	90	1.698	90	–	–	–	–
		(30)		(13)					
14	rW	1.834	99	*1.075*	–	*0.833*	95	–	
		(35)		(99)		(44)			
15	r′W	0.907	90	*1.486*	95	*0.916*	–	1.021	
		(30)		(23)		(19)		(9)	
16	cW	1.207	95	1.064	–	1.077	–	0.927	
		(55)		(136)		(67)		(9)	
17	mT	1.106	90	1.140	95	1.001	–	0.954	
		(145)		(221)		(296)		(101)	
18	m′T	1.121	95	1.114	95	0.953	95	0.888	95
		(311)		(143)		(244)		(135)	
19	m″T	1.512	99	0.311	95	0.873	95	0	
		(60)		(10)		(20)		(3)	
20	TM	1.033	–	*1.053*	–	*1.262*	99	1.618	95
		(65)		(86)		(154)		(51)	
21	cT	1.202	95	*1.078*	–	*1.530*	99	1.307	
		(26)		(64)		(63)		(6)	
22	S	–	–	*1.428*	99	*1.068*	–	–	
				(41)		(111)			

Notes: The number of observing data are given in parenthesis under values of relative catch. The differences in the averaged relative catch value were compared in each case with the averaged ones of the sum of all other cases. The significance levels were calculated with *t*-test. Significance levels are also shown by italics at upper air layers when the values of relative catch on arriving days and following ones differ more than to 95%.

Table 2

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest when the air masses near the surface and higher are not same

Air masses near the surface	Upper air masses	Relative catches	No. of data	Air masses near the surface	Upper air masses	Relative catches	No. of data
1 mA	3 mK	**0.596**	32	8 m'M	17 mT	*0.847*	13
1 mA	7 mM	**0.657**	31	8 m'M	18 m'T	**1.823**	23
1 mA	10 cM	**0.587**	28	8 m'M	20 TM	*0.878*	17
1 mA	11 mW	**0.746**	22	10 cM	2 cA	**1.646**	24
1 mA	16 cW	**0.475**	16	10 cM	3 mK	**0.839**	22
1 mA	17 mT	**1.477**	24	10 cM	7 mM	1.068	35
2 cA	1 mA	**0.671**	14	10 cM	8 m'M	**1.335**	22
2 cA	10 cM	**0.393**	12	10 cM	11 mW	**1.307**	12
3 mK	1 mA	1.005	46	10 cM	14 rW	**0.394**	16
3 mK	4 rK	1.008	59	10 cM	16 cW	**0.675**	11
3 mK	7 mM	*0.907*	119	10 cM	17 mT	0.991	28
3 mK	10 cM	**0.847**	43	10 cM	18 m'T	**0.607**	19
3 mK	11 mW	**0.305**	21	10 cM	20 TM	**0.884**	14
3 mK	12 m'W	1.066	14	11 mW	17 mT	**0.723**	18
3 mK	14 rW	1.013	20	11 mW	18 m'T	**2.018**	14
3 mK	16 cW	1.041	22	12 m'W	8 m'M	**0.520**	16
3 mK	17 mT	**0.675**	36	12 m'W	10 cm	1.122	12
3 mK	18 m'T	**0.769**	17	12 m'W	17 mT	**2.029**	12
3 mK	20 TM	**0.787**	19	12 m'W	18 m'T	**0.756**	14
7 mM	1 mA	**0.699**	14	16 cW	17 mT	**1.550**	17
7 mM	3 mK	**0.800**	40	16 cW	18 m'T	**1.500**	16
7 mM	8 m'M	**1.242**	38	17 mT	7 mM	**0.577**	24
7 mM	10 cM	0.990	36	17 mT	11 mW	**0.780**	18
7 mM	11 mW	**0.676**	52	17 mT	18 m'T	0.967	14
7 mM	12 m'W	*0.941*	14	17 mT	20 TM	**1.549**	22
7 mM	14 rW	*0.858*	26	17 mT	22 S	0.922	17
7 mM	16 cW	**0.773**	26	18 m'T	7 mM	**0.404**	12
7 mM	17 mT	**1.246**	92	18 m'T	8 m'M	**1.313**	49
7 mM	18 m'T	**0.692**	40	18 m'T	10 cM	0.992	12
7 mM	20 TM	**0.904**	16	18 m'T	12 m'W	1.084	16
7 mM	21 cT	**0.542**	15	18 m'T	17 mT	*1.111*	39
7 mM	22 S	**1.562**	18	18 m'T	20 TM	**1.314**	26
8 m'M	7 mM	**1.758**	14	18 m'T	22 S	1.028	43
8 m'M	10 cM	*0.700*	12	21 cT	10 cM	*1.425*	11
8 m'M	12 m'W	**0.719**	17	21 cT	18 m'T	*0.839*	11

Notes: Only those air masses are shown in the table which were frequent and at the time of simultaneous occurrence the values of relative catch have significant difference compared with the relative catch values of all the other observing data. The significance levels are shown with bold numbers (more than 99%) and italic ones (more than 95%).

Table 3

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest when the air masses near the surface change one another during the night

Previous air masses	Following air masses	Relative catches	No. of data	Previous air masses	Following air masses	Relative catches	No. of data
1 mA	3 mK	*0.826*	24	7 mM	16 cW	*1.509*	11
3 mK	1 mA	**0.715**	70	7 mM	18 m'T	1.134	17
3 mK	7 mM	**0.594**	31	8 m'M	7 mM	**0.596**	17
3 mK	10 cM	**1.514**	32	10 cM	7 mM	**0.494**	23
4 rK	3 mK	**1.848**	11	11 mW	7 mM	**0.708**	23
7 mM	3 mK	**0.718**	100	17 mT	7 mM	1.018	25
7 mM	8 m'M	*1.200*	24	18 m'T	7 mM	**2.598**	14
7 mM	11 mW	*0.396*	12	18 m'T	17 mT	*0.784*	16

Notes: Only those data are shown in the table which are frequent or the values of relative catch have significant difference examined the relative catch values belonging to all the other observing data. The significance levels are shown with bold numbers (more than 99%) and italic ones (more than 95%).

The number of caught individuals is low on those nights when there are different air masses in the surfacial and upper air-layers and we can find 1. mA, 2. cA, 3. mK, 7. mM, 8. m'M, 11. mW, 12. m'W and 17. mT ones near the surface except when there are simultaneously warm air masses high above in the air (17. mT, 18. m'T, 20. TM, and 22. S). We observed hight catch in most cases, if there were warm air masses near the surface (16. cW and 18. m'T). We could find both high and low collecting results connected with surface air masses (10. cM and 21. cT). The catching is very high if on the surface there is continental temperate air (cM) – which has neutral effect in human biometeorology – and continental polar air arrives above it, which used to come with cold fronts.

We found high catch in that cases, when the arriving cold front brings temperate maritime air (7. mM) in place of Saharian air coming through the Mediterranean Sea (18. m'T) which is with the strong activity of spherics (electromagnetic radiation).

During the nights we could determine almost all of the combinations of mixed or very changeable air masses near the surface. High and low light trapping results can also belong to these ones. Although high numbers of trapping data were available no significant differences could be established in the combinations of air masses present. We cannot come therefore to final conclusions based on a number of trapping data.

The explanation of insects' behaviour is hindered, because the examinations of concrete weather characteristics belonging to the mixed air mass and the mentioned changings are not yet finished.

We found close positive correlation between the height of tropopause and the number of specimen for light trapped moths. Our results getting with the examination of influences of air masses are confirmed by this fact. The low tropopause has a connection

Table 4

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest in the frequent cases of mixed air masses near the surface

First arriving air mass	Later arriving air mass	Relative catches	No. of data	Significance level (%)
1 mA	3 mK	0.675	35	99
2 cA	1 mA	1.327	12	95
3 mK	1 mA	0.804	32	99
3 mK	7 mM	0.888	81	99
3 mK	10 cM	0.746	43	99
7 mM	1 mA	0.483	10	90
7 mM	2 mK	0.783	86	99
7 mM	10 cM	0.667	18	95
7 mM	11 mW	1.399	19	99
7 mM	16 cW	0.865	16	95
7 mM	17 mT	0.723	37	99
7 mM	18 m'T	0.847	73	99
10 cM	1 mA	1.179	16	90
10 cM	3 mK	0.904	65	95
10 cM	7 mM	1.815	24	99
11 mW	7 mM	0.475	26	99
11 mW	17 mT	0.602	12	95
12 m'W	8 m'M	1.619	12	95
12 m'W	18 m'T	0.399	15	95
16 cW	11 mW	1.362	15	95
16 cW	7 mM	1.162	25	90
16 cW	8 m'M	1.978	11	95
16 cW	17 mT	0.544	13	95
17 mT	7 mM	0.689	26	99
18 m'T	12 m'W	0.466	14	95
21 cT	18 m'T	1.796	12	95

Notes: Only those frequent mixed air masses are shown in the table, when the relative catch values differ significantly from the relative catches of all the other observing data.

with presence of cold air masses and high tropopause with warm ones. Depending on in which part of the day the multiple tropopause was observable, we found differences in catching. The multiple tropopause generally anticipates energetic weather changes. If it was noticed during the day time the success of light trapping already decreased significantly during the same night. If the multiple tropopause was observed only in the evening or at night the decrease of collecting was only noticed during the next night. The unfavourable catch result remained still during next nights in all three cases. The height of tropopause above 13 kilometres shows influx of subtropical air mass in great height and it has strong biological effectivity. The above-mentioned weather changes also show in

Table 5

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest connected with the average height of tropopause at night

The average height of tropopause at night (km)	Relative catches	No. of data	Significance levels (%)
8.2	**0.607**	34	99
9.0	**0.775**	83	99
9.5	**0.874**	94	95
10.0	**0.902**	401	95
10.5	**0.880**	355	99
11.0	1.006	960	–
11.5	1.055	484	–
12.0	1.066	802	–
12.5	**1.087**	178	–
13.0	**1.277**	143	99
14.1	**1.250**	81	99

Notes: The differences of the averaged relative catch value of each case were compared with the averaged ones of the sum of all other cases. The significance level is shown with bold number if it is more than 95%. The value of correlation coefficient (0.966) is significant at 99.9% level. This value was calculated using the height of tropopause and the relative catch.

Table 6

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest examined at the nighbourhood of days with plural tropopause

Days	By day		In the evening		At night	
	RC	N	RC	N	RC	N
–2	1.081	69	**0.953**	44	1.039	59
–1	*1.047*	72	**1.115**	43	**0.947**	61
0	***0.705***	68	***1.181***	43	***1.038***	73
1	**0.845**	68	***0.692***	43	***0.639***	59
2	**0.955**	67	***0.910***	41	***0.910***	60
3	**1.153**	65	**1.188**	40	***1.237***	59

Notes: Bold numbers show, if the difference of relative catch from the average of all the other observing data is at least 95%. The same significant difference between the relative catch of two neighbouring nights are shown with italic numbers. N = means the number of observing data.

Table 7

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest connected with the weather fronts and discontinuity levels (there was only 1 kind of front during the night)

		Relative catches	Significance levels (%)	No. of data
Weather fronts				
1.	W^0	*1.587*	99	105
	W^1	*0.713*	95	29
2.	W_h	1.016		61
3.	O_w	1.296	95	69
4.	W_u	–	–	–
5.	W_m	0.600		8
6.	C	0.935	95	423
7.	C_h	1.082		226
8.	O_c	0.854	95	101
9.	C_u^{0-1}	*0.980*		115
	$C_u^{>2}$	*1.341*	99	39
10.	C_m	0.890	95	51
11.	C_p^{0-1}	*0.676*	99	22
	$C_p^{>2}$	1.258	95	17
12.	O	0.243		6
13.	O_{OR}	1.623	95	33
14.	S_{ww}	1.006		9
15.	S_{cc}^{0-1}	*0.485*	99	78
	$S_{cc}^{>2}$	*1.095*		42
16.	S_{wc}	1.045		76
17.	S_{cw}	0.864	90	49
Discontinuity levels				
18.	D^0	*1.027*		189
	D^{1-2}	*0.519*	90	19
19.	D′	0.761	90	30
20.	S	2.319	95	12
Without any front		1.106	95	185

Notes: The difference of the averaged relative catch value of each case were compared with the averaged ones of the sum of all other cases. The significant differences belonging to the same fronts but different in intensity ones are shown with italic numbers.

the catching result noticed after the multiple tropopause took place at different times of the day. The increase of caught moth's number on the third following day can be attributed to the subtropical tropopause. We can conclude from these results, the atmospherical electric factors also have major part, mainly at the time of influx of upper subtropical air. At this time the impulse number of 3 Hz spherics decreases, but the suncosmic radiation shows increase (Örményi, 1984). The atmospherical ions also can have major influence

Table 8

Relative catch (RC) the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest connected with the weather fronts and discontinuity levels changing one another during the night

Previous fronts	Following fronts	Relative catches	No. of data	Previous fronts	Following fronts	Relative catches	No. of data
1 W	7 C_h	1.085	29	8 O_c	18 D	**1.260**	56
1 W	18 D	**1.280**	21	9 C_u	6 C	*1.142*	47
2 W_h	7 C_h	**0.505**	17	9 C_u	7 C_h	**1.335**	28
2 W_h	18 D	1.165	24	9 C_u	10 C_m	*0.504*	10
3 O_w	6 C	1.010	12	9 C_u	15 S_{cc}	1.388	14
3 O_w	7 C_h	1.066	24	9 C_u	18 D	1.229	15
3 O_w	18 D	**0.680**	40	15 S_{cc}	6 C	**0.572**	21
6 C	3 O_w	**0.806**	29	15 S_{cc}	7 C_h	*0.733*	11
6 C	7 C_h	**0.702**	27	15 S_{cc}	18 D	1.056	19
6 C	8 O_c	**0.455**	39	16 S_{wc}	6 C	**0.722**	18
6 C	15 S_{cc}	**1.224**	38	16 S_{wc}	7 C_h	**0.540**	14
6 C	16 S_{wc}	**0.804**	11	16 S_{wc}	8 O_c	1.102	12
6 C	17 S_{cw}	1.040	12	16 S_{wc}	15 S_{cc}	*1.380*	12
6 C	18 D	*1.127*	71	16 S_{wc}	18 D	**0.431**	16
7 C_h	1 W	*0.888*	13	18 D	1 W	**1.345**	42
7 C_h	2 W_h	**0.361**	12	18 D	2 W_h	**0.585**	23
7 C_h	6 C	*0.911*	56	18 D	3 O_w	**0.892**	35
7 C_h	10 C_m	*1.339*	10	18 D	6 C	1.094	42
7 C_h	15 C_{cc}	**1.476**	29	18 D	7 C_h	**1.249**	76
7 C_h	18 D	1.051	35	18 D	8 O_c	**0.534**	21
7 C_h	19 D'	*1.110*	28	18 D	15 S_{cc}	*1.192*	29
7 C_h	20 S	1.024	20	18 D	16 S_{wc}	**0.707**	16
8 O_c	6 C	**0.505**	35	18 D	17 S_{cw}	*0.930*	11

Notes: Relative catches belonging to the frequent front changes are shown in the table. Significance levels are shown with bold numbers (more than 99%) and italic ones (more than 95%).

(Örményi, 1967). The predominance of negatively charged ions measured in the polar air cause decrease in activity, but the predominance of positively charged ions being in subtropical maritime air mass (mT) can be increasing flight activity.

The light warm fronts (W^0), the strong unstable cold fronts (C_u^2), surface cold fronts (C), the strong paradox cold fronts (C_P^2), the warm occluded fronts (O_w), orographic occlusion fronts (O_{OR}) and the apart air current process from discontinuity levels are favourable for light trapping of the examined heart-and-dart moth. The cold front (C), the masked cold fronts (C_M), the light paradox cold fronts (C_P^{0-1}), the stationary fronts that arrive and leave as cold fronts (S_{ww}^{0-1}), the stationary fronts that arrive as cold fronts and leave as warm fronts (S_{cw}), the strong subsidence in general (D^{1-2}) and the descending motion between two cold fronts (D′) are unfavourable. The number of caught individuals

Table 9

Relative catch (RC) of the heart-and-dart moth (*Scotia exclamationis* L.) using the data of light-traps surrounding Budapest connected with the microbarographic oscillations

Unchangable microbarographic codes	Relative catches	Significance levels (%)	No. of observing data
0	0.898	90	323
1	1.039		1334
2	0.986		440
The microbarographic code changes in one direction	**Relative catches**	**Significance levels (%)**	**No. of observing data**
$0 \rightarrow 1$	1.218	95	125
$1 \rightarrow 0$	1.148	95	316
$0 \rightarrow 2$	*0.673*	95	37
$2 \rightarrow 0$	*1.330*	95	42
$1 \rightarrow 2$	0.904	90	291
$2 \rightarrow 1$	0.850	95	301
The microbarographic code changes several times	**Relative catches**	**Significance levels (%)**	**No. of observing data**
$0 \rightarrow\leftarrow 1 \rightarrow\leftarrow 2$	0.868	90	29
$0 \rightarrow\leftarrow 1$	1.385	95	57
$1 \rightarrow\leftarrow 2$	1.147		47

Notes: The difference of the averaged relative catch value of each case were compared with the averaged ones of the sum of all other cases. Significance levels are shown with italic numbers (at least 95%) used the data of changes in opposite direction.

was modified oppositely in some cases by the light and the strong fronts. The (W^0) and the (D) increase the catch, but both the strong ones strongly decrease the collecting. We found the opposite influence with the unstable cold fronts (C_u), the paradox cold fronts (C_p) and S_{cc}. There were favourable and also unfavourable collections on those nights when the weather fronts came after one another. The trapping results reflect therefore the effect of the air masses arriving later.

We could observe frequently increases in the number of caught insects connected with different types of cold fronts coming after other cold fronts. This increase means the biological effectiveness in this event. We could find some other effective front changes: subsidence in general (D) comes after the cold or warm fronts (C or W); stationary front, that arrives and leaves as a cold front (S_{cc}) comes after stationary front that arrives as a warm front and leaves as a cold one (S_{wc}), which is often connected with

effective cyclon situation; upper cold front (C_h) or stationary front, that arrives and leaves as a cold front (S_{cc}) comes after subsidence in general (D).

After finding that high and low catches can belong to both warm and cold fronts we can conclude that there can be among insects individuals sensitive to cold or warm fronts. The weather front sensitivity symptoms belonging to the insects appear in changes of flying activity. The further examinations of this problem, the proving of above-mentioned hypothesis and determination of ratio of individuals sensitive to cold and warm fronts would be very important both for entomological basic research and plant protection practice.

The number of caught moths is lower, if the microbarographic oscillation can be characterized with level 0 and they are unchanged during the night. It is favourable from the point of view of collecting if the code of oscillations change between 0 and 1 in any direction or it changes from 2 to 0. It is unfavourable if the change is between 1 and 2 in any direction, it increases from 0 to 2 or it is modified in different directions between 0, 1 and 2.

The low values of relative catches indicate in all cases those weather situations when the flying activity of insects decreased, but the interpretation of the high values is not so simple. The significant environmental changes cause physiological changes in the organism of insects. The imaginal life is short, the unfavourable weather endangers not only the life of the individual but also the continuance of the total species. According to our supposition the individuals can use two kinds of strategies to prevent the hindering influences of normal functions in life. First is the increased activity. It means the growing of intensity in flying, copulation and oviposition. The second strategy is to hide and ride out in passivity the unfavourable situation. Seeing the above-mentioned facts, according to our present knowledge high light trapping results can belong to both favourable and unfavourable situations.

Our present work means the beginning of those examinations to study the vital functions of insects connected with air masses, height of tropopause and its changes, the weather fronts and microbarographic oscillations. It is not well known which are the favourable and unfavourable weather influences for the insects at the time of several kinds of weather fronts, air masses and mainly at the time of their changes and combinations. We think to examine more these events to use the results for plant protection prognosis because changes in weather fronts are frequent and the air masses change very often in swarming time of insects.

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Differences in the Rate of Larval Development, Head Capsule Width and Diapause Induction in Populations of *Pieris brassicae* L. (Lepidoptera, Pieridae) Reared at Different Temperatures and Photoperiods

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In laboratory experiments conducted in 1994 and 1995, larvae of *Pieris brassicae* L. were reared on cabbage leaves at three temperatures (23, 25 and 28 °C, respectively) and at five photoperiods (ranging from LD 13 : 11 to LD 17 : 7). Observations on the rate of larval development and the incidence of pupal diapause were carried out. Under the same rearing conditions widths of larval head capsules were also measured in the first four stages.

Shortdays (LD 13 : 11) induced pupal diapause in 100% of individuals both at 23 °C and 25 °C but only in 60% in a population kept at 28 °C. Rearing larvae at LD 14 : 10 photoperiod evoked diapause merely in 50% of insects and at 23 and 25 °C only. With the increase of temperature larvae exhibited faster development and a similar reaction was observed also in connection with the prolongation of daylength. At higher temperatures and shortdays (LD 13 : 11 and LD 14 : 10) smaller size of larval head capsules could be measured.

The influence of photoperiod and temperature on the development of *Pieris brassicae* was studied by Danilevskii and Geispits (1948), Danilevskii and Goryshin (1960), Danilevskii (1965, 1968). Goryshin (1964) as well as Bünning and Joerrens (1959) studied the influence of the intensity and wavelength of light. Chernysh (1973) investigated the effect of geographic latitude and temperature. L'Helias (1960) dealt with the diapause of *P. brassicae* from the viewpoint of insect physiology. Saunders (1976) summarized knowledges on the pupal diapause of this species.

Data on head capsule widths of different larval stages were published by Klein (1932), David and Gardiner (1972), Eassa (1963) and Shrihari (1972).

In experiments conducted in 1994 and 1995, our aim was to study the influence of photoperiod and temperature on the diapause induction and larval development of *P. brassicae*. In addition, measurements were also carried out to reveal the effect of these ecological factors on the head capsule widths of larvae.

Materials and Methods

Larvae of the large white butterfly, *Pieris brassicae* L. (a laboratory strain from the Agricultural University, Wageningen, The Netherlands) were reared, starting with freshly hatched insects, at three temperatures (23, 25 and 18 °C, respectively) and five

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photoperiods ranging from LD 13 : 11 to LD 17 : 7. The catterpillars were kept in hygrostats (closed glass jars with an atmosphere of controlled humidity) and fed regularly with fresh cabbage leaves.

Twenty-five larvae per experimental variant were used. Later, the proportions of individuals entering pupal diapause were checked. Larval mortality was also registered.

For the evaluation of eventual effects of temperature and photoperiod on the head capsule width, capsules shed at moult were routinely collected. Their widths were measured with a stereomicroscope equipped with ocular micrometer.

After measurements, it was checked if the so-called "Dyar's rule" can be applied to *P. brassicae*. According to Dyar (1890, in Watzl, 1947), there is a constant value characterizing the ratio of the head capsule widths of consecutive larval instars ($L_2 : L_1$, $L_3 : L_2$ etc.).

Results and Conclusions

a) Effect of temperature and photoperiod on the development of P. brassicae

Table 1 contains also data published earlier (Papiewska-Csapó, 1996). Along with the total duration of larval development. Table 1 shows the time of development of particular larval stages. This makes easier also the evaluation of the effect of ecological factors (temperature, photoperiod) regarding consecutive instars.

From data presented in Table 1 it is obvious that with the increase of temperature larval developed faster at all five photoperiods. An exception can be noted az 28 °C where at photoperiods LD 15 : 9, 16 : 8 and 17 : 7 the time of development of larval was unusually long. This can be explained by the unsuitable rearing conditions inducing some kind of disease and considerable mortality at this high temperature and long illumination. Data also show that at photophases of 13 and 14 hours the decrease of total time of development of larvae was more pronounced when the temperature was changed from 25 to 28 °C than in the case of a shift from 23 to 25 °C. This positive influence of temperature on the rate of larval development was most significant at short daylength (LD 13 : 11) and in early larval stages.

The effect of photoperiod on the developmental time was especially clear at 23 °C. At higher temperature (25 °C), L_5 larvae proved to be considerably sensitive to the increase of daylength.

Analysing the data on the percentage of diapause induction (Fig. 1), it is clear that 100% of pupae entered diapause at 23 and 25 °C and at LD 13 : 11, while at LD 14 : 10 photoperiod only 50% of the population diapaused. At the highest temperature (28 °C) only 60% of the larval population reared at LD 13 : 11 produced diapausing pupae. However, at LD 14 : 10 the high temperature eliminated the effect of photoperiod and 100% of individuals developed without diapause.

With longer periods of illumination (15, 16 and 17 hours), 100% of the population developed uninterruptedly into adults, regardless to the temperature.

Table 1

The effect of photoperiod and temperature on the rate of larval development

Photoperiod	Average duration of larval development (days)																	
	23 °C						25 °C						28 °C					
	L_1	L_2	L_3	L_4	L_5	L_1–L_5	L_1	L_2	L_3	L_4	L_5	L_1–L_5	L_1	L_2	L_3	L_4	L_5	L_1–L_5
LD 13 : 11	4.16	2.72	3.16	3.40	5.44	18.88	2.96	2.56	2.68	2.76	7.52	18.48	2.32	2.00	2.20	2.44	5.16	14.12
LD 14 : 10	3.76	2.12	2.16	2.48	5.32	15.81	3.28	2.44	2.52	2.84	5.76	16.84	3.04	2.04	1.80	2.08	4.96	13.92
LD 15 : 9	3.56	2.16	2.36	2.76	4.92	15.76	2.80	2.84	2.16	2.32	5.12	15.23	3.56	2.88	2.60	2.26	6.18	17.48
LD 16 : 8	3.36	2.52	2.16	2.72	4.88	15.64	3.36	2.08	2.08	2.56	4.96	15.04	3.36	2.80	2.72	2.52	5.44	16.84
LD 17 : 7	3.00	2.20	1.30	3.08	4.80	14.38	2.76	2.56	1.80	2.36	4.40	13.88	4.00	3.20	2.04	2.58	4.80	16.62

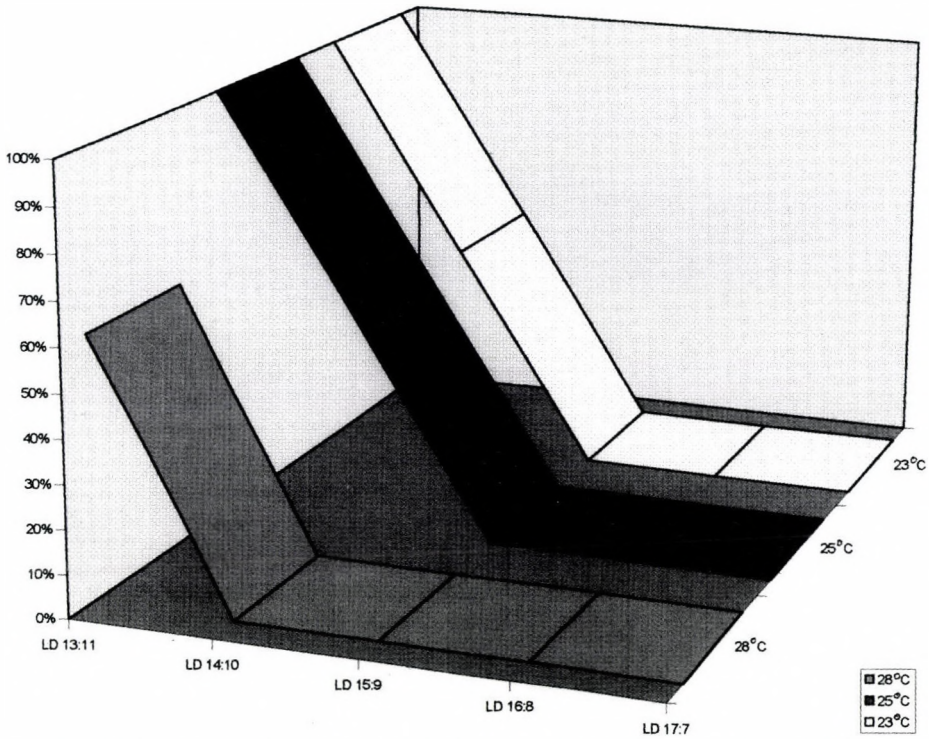

Fig. 1. Induction of pupal diapause in populations reared at different temperatures and photoperiods

Table 2

Larval mortality in consecutive instars under different rearing conditions

Temperature	Photoperiod	Larval mortality (%)					
		L_1	L_2	L_3	L_4	L_5	L_1–L_5
23 °C	LD 13 : 11	12	4	0	4	4	24
	LD 14 : 10	8	0	0	0	4	12
	LD 15 : 9	4	0	0	0	0	4
	LD 16 : 8	4	4	0	0	0	8
	LD 17 : 7	0	4	0	0	0	4
25 °C	LD 13 : 11	0	0	0	0	0	0
	LD 14 : 10	0	0	0	0	16	16
	LD 15 : 9	0	0	0	0	0	0
	LD 16 : 8	0	0	0	0	4	4
	LD 17 : 7	0	0	0	0	0	0
28 °C	LD 13 : 11	0	0	0	0	8	8
	LD 14 : 10	0	0	0	0	4	4
	LD 15 : 9	0	20	4	8	48	80
	LD 16 : 8	0	8	8	12	12	40
	LD 17 : 7	0	4	16	4	40	64

Table 3

Head capsule widths in consecutive larval stages reared under different conditions

Photoperiod	Average width of head capsule (mm)											
	L_1			L_2			L_3			L_4		
	23 °C	25 °C	28 °C	23 °C	25 °C	28 °C	23 °C	25 °C	28 °C	23 °C	25 °C	28 °C
LD 13 : 11	0.451	0.431	0.433	0.695	0.692	0.682	1.166	1.109	0.953	1.756	1.811	1.691
LD 14 : 10	0.448	0.392	0.442	0.678	0.623	0.677	1.124	1.021	1.062	1.741	1.651	1.649
LD 15 : 9	0.441	0.448	0.426	0.661	0.697	0.695	1.136	1.077	0.849	1.769	1.742	0.673
LD 16 : 8	0.451	0.478	0.452	0.727	0.712	0.663	1.156	1.124	0.014	1.725	1.714	1.644
LD 17 : 7	0.452	0.437	0.429	0.735	0.712	0.659	1.136	1.164	1.073	1.748	1.741	1.647

Larvae kept at different temperatures and photoperiods showed various mortality rates (Table 2). According to present investigations, the best conditions for *Pieris larvae* can be created by keeping them at 25 °C. First instar larvae had mortality at 23 °C only. At the highest temperature, as the larvae advanced in their development, their mortality increased.

Table 4

Dyar's rule: Evaluation of its validity
on the basis of data on head capsule widths

Larval stages	Ratio of head capsule widths		
	23 °C	25 °C	28 °C
$L_2 : L_1$	1.558	1.566	1.555
$L_3 : L_2$	1.631	1.597	1.451
$L_4 : L_3$	1.529	1.531	1.601

Insects affected by adverse conditions also ate less. Dead pupae (e.g. dried ones) were also common in these populations. The symptoms indicated viral or bacterial infection, or in some cases, the presence of fungi (sp. Paecilomyces order Moniliales).

b) Head capsule width of P. brassicae *reared at different photoperiods and temperatures*

According to Klein (1932), David and Gardiner (1962), head capsule width of larvae is in direct ratio to their body length. Another aim of our experiments was to investigate the influence of temperature and photoperiod on the head capsule.

Analysing the results presented in Table 3 it can be observed that the narrowest head capsules were measured in larvae (all stages) reared at 25 °C and LD 14 : 10 photoperiod. Furthermore, in most cases the keeping of larvae at LD 14 : 10 photoperiod resulted in smaller head capsules in comparison to the corresponding data obtained at LD 13 : 11.

If we approach the problem from the action of temperature, another tendency can be also stated: the higher the temperature was, the smaller were the head capsules in all larval stages.

Concerning the applicability of Dyar's rule, it was found (Table 4) that despite the artifical conditions of insect rearing, the head capsule index proved to be more or less constant. Again, the most diverse values were measured in the population reared at 28 °C.

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Effect of Neem Azal-F on *Tetranychus urticae* and Three Predacious Mites of the Family Phytoseiidae

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Neem Azal-F a commercial preparation of neem seed kernel extract was tested for its repellency, toxicity and oviposition deterence against the phytophagous mite *Tetranychus urticae* Koch. Significant reduction in oviposition, which correlated with Neem Azal-F concentrations were recorded, with a significant increase in repellency. Leaf discs treated with increasing concentrations of the product showed increased mortality of *T. urticae* and redcution in the total number of eggs laid, but the percentage of egg hatchability was 100%. The effect of Neem Azal-F on the predacious mites, *Amblyseius barkeri* (Hughes), *Amblyseius swirskii* Athias-Henriot and *Amblyseius zaheri* yousef and El-Borolossy was studied, too. Neem Azal-F decreased the food consumption rate at the two concentrations used for all the predacious mites, as well as egg laying at the two concs. in *A. swirskii* only. Neem Azal-F was highly toxic to *A. swirskii*. In contrast, the two tested concentrations were considered to be safe for *A. barkeri* and *A. zaheri*.

The widespread use of synthetic, broad-spectrum pesticides is being viewed with concern because of the possible hazardous effects on the environment and on human health (Pimentel et al., 1992). In recent years, the attention has been focused to use the natural pesticides. Among of them, azadirachtin, a mixture of several structurally related tetranortriterpenoids isolate from the seeds of the neem tree [*Azadirachta indica* A. Juss. (Meliaceae)] has attracted greatest attention (Rembold, 1989; Govindachari et al., 1992). Azaderchtin has deterrent, antiovipositional, antifeedent, growth disrupting, fecundity and fitness reducing properties on insects (Saxena, 1986; Koul et al., 1990). While there is ample information on the effect of neem seed kernel extracts on pest insects, there are very few detailed studied focusing on their effects on mites and their natural enemies. Considerable reports regarding the biological activity of neem-used formulation on mites were recorded by (Schauer and Schmutterer, 1981; Mansour et al., 1987, 1993; Dimetry et al., 1993). Solvents in neem seed kernel extracts (Mansour and Ascher, 1983; Mansour et al., 1986, 1987) and formulation additives (Mansour et al., 1993; Dimetry et al., 1993) are reported to influence the mortality, fecundity and repellency on mites. However, this is the second report from Egypt concerning the effect of neem seed extracts on the predacious mites of the family Phytoseiidae. The first report by (Dimerty et al., 1994) demonstrated that Neem Azal-S and Margosan-O decreased eggs laying as well as the food consumption rate of the two predacious mites *Amblyseius berkeri* (Hughes) and *Typhlodromus richteri* karg.

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The present work was carried out to assay the effect of the neem formulation, namely Neem Azal-F on the predacious mites *A. barkeri, A. swirskii* Athias-Henriot and *A. zaheri* Yousef and El-Borolossy as well as the phytophagous mite *Tetranychus urticae* Koch in the laboratory.

Materials and Methods

Maintenance of mite stock cultures

The stock culutres of *T. urticae* were collected from infested lima bean (*Phaseolus vulgaris* L.) in the laboratory at N.R.C. Cairo. The predacious mite *A. swirskii* was collected from an apple orchard and reared on eggs and immature stages of *T. urticae* from the laboratory colony. *A. zaheri* and *A. barkeri* were found on leaves of egg-plant and were fed *T. urticae* in the laboratory. The mites were reared in a controlled climate room at 25–27 °C and 60 ± 5% R.H.

Commercial products

Neem Azal-F is commercial neem seed extract obtained by courtesy of Prof. Hubertus kleeberg (Germany): Different concentrations of the product were prepared and tested against the adult females of *T. urticae*. Two concentrations were selected (0.2 and 0.05%), prepared and tested for the phytoseiid mites.

Treatment

REPELLENCY AND TOXICITY TEST PROCEDURE
FOR ADULT FEMALES OF *T. URTICAE*

Raspberry leaf discs (4 cm in dia.) were placed with the lower surface up wards in a Petri dish lined with moist cotton wool. One half of the lower surface of each disc was treated separately with selected concentrations, while the other half served as control. Ten adult females of *T. urticae* were then placed on the centre of each leaf disc, using a fine camel's-hair brush, ten replicates leaf discs were used per concentration. At 2, 24 and 48 h after treatment, the number of mites that moved to the treated and control half-discs [repellency (mites which had left the treated discs were considered as repelled)], the number of eggs laid on each half and mortality of mites were recorded. The repellency was calculated according to (Lwande et al., 1985).

TOXICITY AND BIOLOGICAL EFFECTS OF NEEM
AZAL-F ON ADULT FEMALES OF *T. URTICAE*

Newly emerged female mites were placed singly on raspberry leaf discs treated on both halves with different concentrations. Twenty replicate leaf discs were used per concentration and a similar number of discs treated with water only as control. The total

number of eggs laid on the treated discs were recorded over a period of 7 days. The mortality of the mites and % hatch. of eggs were also recorded. The deterant indices were calculated as reported by (Lundgren, 1975).

DIRECT EFFECT ON SOME PREDACIOUS MITES

Newly emerged and mated females of 3 predators species (*A. barkeri, A. swirskii* and *A. zaheri*) were treated with both concentrations and transfered singly to the under surface of raspberry leaf discs previously treated with the prepared concentrations of Neem-Azal-F. Untreated nymphs of *T. urticae* were offered as food to the predators. A control treatment was included in each test. Observations were taken daily on consumption, reproduction, mortality and sex-ratio of the progeny for 7 successive days.

The % redcution in food comsumption was calculated according to (Samsoe-Petersen, 1983). All the experiments reported herein were carried out in the laboratory at 27 ± 2 °C and 70–75% R.H.

Results and Discussion

Repellency, mortality and oviposition deterrence

Percent repellency gradually increased with Neem Azal-F concentration (Table 1). At the 2 h period, the formulation proved to be completely deterrent for *T. urticae*, but over 48 h. period deterrency of Neem Azal-F decreased more rapidly than that after 48 h period. After 48 h period, mortality were noted when concentrations of Neem Azal-F increased. The average number of eggs laid by females of *T. urticae* after 48 h varied according to the concentrations (Table 1). Jacobson (1978) found that 1.0% of the hexane extract of neem kernels significantly deterred feeding by *Panonychus citri* (McGregor). Mansour and Ascher (1983) revealed that neem seed kernels prepared from various solvents strongly repelled the females *Tetranychus cinnabarines* (Boisd), from treated leaves and egg laying was reduced.

Concentration effects on reproduction and mortality

Significant reduction in the total number of eggs laid during 7 days period were found for all the conc. tested (Table 2). Fecundity was severely reduced as the concentration of Neem Azal-F increased. These observations are in agreement with Dimetry et al. (1993) and Sundaram and Sloane (1995).

The depression in total number of eggs with high concentrations could be attributed to feeding inhibition and irritant effects of the formulation, causing depression of reproductive activity. The number of eggs which hatched during the 7 days, interval was not influenced by the different concentrations. Hatchability was 100% at all conc. In contrast, Dimetry et al. (1993) and Sundaram and Sloane (1995) demonstrated that hatchability greatly decreased especially at the highest concentrations. However % mor-

Table 1

Concentration effects of Neem Azal-F on Percent mortality and oviposition deterrence of adult female *T. urticae*, 48 h after treatment with Neem Azal-F

Conc. %	% Distribution of mites on treated leaf part after			% M after 48 h	Avrg. No. eggs/ female after 48 h		% Repellency
	2 h	24 h	48 h		T	C	
0.4	0	0	18	4	0.16	4.9	96.73
0.2	0	0	26	2	0.2	3.62	94.48
0.1	0	2	36	0	0.84	3.22	73.91
0.05	0	4	42	0	0.92	3.0	69.33
0.025	0	6	50	0	1.36	2.96	54.05

T = Treated
C = Control
M = Mortality

Table 2

Concentration effects of Neem Azal-F on reproduction, hatchability and mortality of adult female *T. urticae* placed on treated raspberry leaf discs for 7 days

Conc. %	No. eggs/female/7 days	Hatchability %	Deterrent index	% Mortality
0.4	8.4	100	36.79	45.46
0.2	9.1	100	61.36	36.26
0.1	10.1	100	58.00	27.47
0.05	10.3	100	57.35	12.5
0.025	14.9	100	43.67	9.1
Control	38	100	0	0

L.S.D. 0.05 : 6.017
0.01 : 7.957

tality was significantly concentration – dependent. It was 45.46% and 9.1% at 0.4 – 0.025% (Table 2). Mansour et al. (1993) reported that Margosan-O and Azatin were not toxic to *T. cinnabarinus*, but RD9 – Repelin was highly toxic to the mite.

Table 3

Effect of Neem Azal-F on the food consumption of *A. barkeri*, *A. swirskii* and *A. zaheri*

% Conc.	Total prey/female/7 days	Total prey /Female/day	% Reduction in food consumption
	A. barkeri		
0.2	83.1	11.87**	40.65
0.05	112.5	16.07**	19.65
Control	140.0	20.2	–
0.05 : 1.741			
L.S.D.			
0.01 : 2.386			
	A. swirskii		
0.2	13	1.86**	88.13
0.05	28.05	4.01**	74.41
Control	109.7	15.67	–
0.05 : 0.930			
L.S.D.			
0.01 : 1.273			
	A. zaheri		
0.2	56.7	8.1**	41.56
0.05	72.7	10.39**	25.04
Control	97.0	13.86	–
0.05 : 1.110			
L.S.D.			
0.01 : 1.520			

** High significant

Effect of Neem Azal-F treatments on predacious mites

ON FOOD CONSUMPTION

Results from Table 3 show that a significant lower consumptions were recorded at 0.2% on treated females of *A. barkeri*, *A. swirskii* and *A. zaheri* when exposed to nymphs of *T. urticae* formerly kept together on plant leaves treated with Neem Azal-F (more than 40%). At low concentration (0.05%), the percentage reduction in the food consumption decreased to 19.65, 74.41 and 25.04 for *A. barkeri, A. swiriskii* and *A. zaheri* (Table 3). A significant lower consumption was recorded on *A. gossipi* El-Badry when exposed to treated prey with fenugreek and canna extracts (Dimetry and Amer, 1992) and also on *A. barkeri* when kept on treated leaves with lupin extract, Margosan-O and Neem Azal-S (Momen and Amer, 1994; Dimetry et al., 1994).

Table 4

Effect of Neem Azal-F on the reproduction, mortality and sex-ratio of the progeny of treated females of *A. barkeri, A. swirskii* and *A. zaheri*

% Conc.	Total No. eggs/ female/7days	No. eggs/ female/day	% M after 7 days	% Hatching	Sex.-ratio Females : Males
		A. barkeri			
0.2	12.9	1.84**	20	96.90	1 : 0.79
0.05	17.0	2.43	0	97.06	1 : 0.57
Control	18.4	2.63	0	97.83	1 : 0.44
0.05 : 0.340					
L.S.D.					
0.01 : 0.464					
		A. swirskii			
0.2	1.95	0.28**	60	64	1 : 1.67
0.05	2.6	0.37	45	80.77	1 : 1.33
Control	15.5	2.21	0	100	1 : 0.43
0.05 : 0.205					
L.S.D.					
0.01 : 0.280					
		A. zaheri			
0.2	8.5	1.21**	10	97.01	1 : 1.07
0.05	13.4	1.91	5	97.94	1 : 0.86
Control	13.9	1.99	0	100	1 : 0.74
0.05 : 0.400					
L.S.D.					
0.01 : 0.548					

** High significant

ON MORTALITY AND EGGS PRODUCTION

Results from Table 4 show that Neem Azal-F appeared to be harmless *for A. barkeri* and *A. zahri* as mortalities ranged between (5–20%) after 7 days treatment. In contrast, the two concentrations used proved to be slightly to moderately harmful for *A. swirskii* (45–60%). In previous studied, Neem Azal-S and Margosan-O proved to be harmless for *A. barkeri* at conc. (0.2–0.05%) but were slightly harmful to *Typhlodromus richteri* Karg (Dimetry et al., 1994). RD9-repelin was highly toxic to *Typhlodromus athiasae* Porath and Swirski but no toxic effect was recorded in case of Margosan-O and Azatin to the predator (Mansour et al., 1993).

As to the effect on the egg production, Table 4 shows that at high conc. (0.2%) a significant reduction in the average number of eggs laid/♀/day during 7 days period for all predators. At low conc. a significant reduction in fecundity was recorded in case of *A.*

swirskii only: It seems to be that Neem Azal-F was more toxic to *A. swirskii* but safe for *A. barkeri* and *A. zaheri*.

Kleeberg (1992) stated that the effect of neem ingredients on useful insects are negligible. Also Sanguanpong (1992) indicated that Azadirachtin had only minor potential for the control of tetranychid, and extracts at concs. up to 1% were harmless to *Phytoseiulus persimilis* Athias-Henriot. Momen and Amer (1994) recorded that *A. barkeri* suffered a drop in reproduction when fed on prey kept on leaves treated with lupins, turnip and fenugreek extracts. There would be some reasons explaning this phenomenon on, may be the direct influence of the substance on the female ovaries or it is possible also that contact application of any substance on the cuticle may disturb the production of phermones. Herout 1970 listed many examples of disturbing insect development process by natural products.

ON HATCHABILITY AND SEX-RATIO

The number of eggs which hatching during the 7 days interval was influenced to some extent at the two concs. in case of *A. swiriskii* (Table 4). The % hatch ranged from 64–81. The results also revealed that egg hatchability was not significantly affected in *A. barkeri* and *A. zaheri* as it averaged between 96–97% which was similar as control. The sex-ratio of the progeny resulted from the eggs of *A. barkeri* and *A. zaheri* was nearly unaffected at the two conc. (Table 4). In *A. swirskii* the sex-ratio was tended to be in favour of males.

On scrutinizing our results it will be shown that Neem Azal-F is satisfactory as regards relatively both high mortality for *T. urticae* and low reduction of fecundity for *A. barkeri* and *A. zaheri* only. *A swirskii*, a phytoseid which is an important predator of *T. urticae* (Momen and El- Sawy, 1993), was affected by Neem Azal-F even at the lowest dose used. In some situations the anti-feedant activity could be sufficient to control the pest by itself, but in most cases additional control measures are likely to be necessary. It worth mention that the use of a botanical pesticid that is relatively non-toxic to natural enemies could increase the effectiveness of natural predation.

More intensive studies are needed in order to find out the legendary use of Neem in mite control programs and its pesticidal properties, its potential as a systemic pesticide to control other mite pests in green house programs.

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Parasitoids Attacking Genus *Aleurolobus* (Homoptera: Aleyrodidae) in Egypt

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A survey of the genus *Aleurolobus* Quaintance and Baker was carried out in Egypt during the period from Aug. 1995 to Aug. 1996. The following five indigenous parasitoids were recorded: *Encarsia elegans* Masi, *E. lutea* (Masi), *Eretmocerus* nr. *haldemani* Howard, *Eretmocerus* sp. and *Euderomphale* sp.

A monthly samples of these species were carried out in three localities in Egypt, Sharqiya (lower Egypt), Assiut (upper Egypt), and El-Arish (northeast Sinai), representing various bioclimatic regions. *E. elegans* was the dominant parasitoids of *Aleurolobus niloticus* with parasitism rates of 72.0% in July 1996, at Sharqiya governorate, and 56.1% in Nov. 1995, at Assiut governorate. The same species recorded also in Dec. 1995 at El-Arish region associated with *Aleurolobus olivinus* with maximum parasitism rates 12.8%. Total parasitism of parasitoids associated with *A. niloticus* reached a maximum during Nov. 1995 and July 1996 with parasitism rates of 83.3% and 89.6% at Sharqiya and associated governorates, respectively. Parasitoids associated with *A. olivinus* reached a maximum during Oct. 1995 with parasitism rates of 27.8% in El-Arish region.

Over 47 species of the genus *Aleurolobus* Quaintance and Baker are well known in the world. They attack 99 plants including, citrus, olive, nabk, orenementals plants and weeds (Mound and Halsey, 1978; Bink-Moenen, 1983). In Egypt, this genus in represented by two species, the nabk whitefly, *Aleurolobus niloticus* Priesner and Hosny (1940) and the olive whitefly, *Aleurolobus olivinus* Silvestri, attacking 10 plants including nabk, henna, sycamore fig, Egyptian balsam and olive (Abd-Rabou, 1996).

Numerous parasitoids of the genus *Aleurolobus* were recorded from many parts of the world, these are *Amitus mineruae* Silvestri (Platygastridae), *Azotus delhiensis* lal, *A. pulchriceps* Zehntner (Aphelinidae), *Encarsia bifasciafacies* Hayat, *E. elegans* Masi, *E. isaaci* Mani, *E. lutea* (Masi), *E. macroptera* Viggiani, *E. olivina* Silvestri (Aphelinidae), *Eretmocerus aleurolobi* Ishii and *Er. haldemani* Howard (Aphelinidae) (Silvestri, 1911; Fulmek, 1943; Hayat, 1989; Polaszek et al., 1992; Nguyen et al., 1993). However, in Egypt, Priesner and Hosny (1940) recorded *E. elegans* associated with *A. niloticus*.

In order to reduce future damage of this pest species in Egypt, a study of the possibilities for its biological control has been undertaken. In the present work a survery carried out to study the role played by this indigenous parasitoids in controlling the two species of the genus *Aleurolobus* in Egypt.

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Materials and Methods

A preliminary survey was carried out, in practically all *Zizyphus spinachersti* and *Olea* sp. of some localities of Egypt, in order to determine the parasitoids of *A. niloticus* and *A. olivinus*.

A. niloticus and *A. olivinus* second, third and pupal stages were sampled from *Z. spinachersti* at Sharqiya and Assiut governorates and from *Olea* sp. at El-Arish region. Leaves of *Z. spinachersti* and *Olea* sp. were collected monthly during Aug. 1995 until Aug. 1996 (30 leaves per sample) and transferred to the laboratory. *A. niloticus* and *A. olivinus* eggs and first larval stages were eliminated, as well as any other insect species. The second and third larval and pupal stages were recorded per leaf. Each leaf was stored in well-ventilated emergence glass tubes and monitored daily for parasitoids emergency.

Percent parasitism was calculated using the formula: Percent parasitism = (total number of parasitized immature per leaf)/ (total number of susceptible whitefly immature stages per leaf). Susceptible stages considered as the third and fourth instar and the early pupal stages with unpigmented eyes (Vet et al., 1980).

Results and Discussion

1. Aleurolobus niloticus Priesner and Hosny

Four species of parasitic hymenoptera were reared from samples of *A. niloticus* on *Z. spinachersti*. In the present study, these are listed below in alphabetical order.

– *Encarsia elegans* Masi
– *Encarsia lutea* (Masi)
– *Eretmocerus* nr. *haldemani* Howard
– *Euderomphale* sp.

Parasitoids which emerged from samples of *A. niloticus* on *Z. spinachersti* varied according to the two regions of Egypt. In lower Egypt (Sharqiya governorate). *A. niloticus* was parasitized by *E. elegans, Er.* nr. *haldemani* and *Euderomphale* sp. with and average rates of 43, 11.2 and 1.2%, respectively (Table 1).

Peak parasitism of 89.6% occurred in Sharqiya during July 1996 and lowest parasitism of 18.5% occurred during Jan. 1996. In Assiut (upper Egypt) the average parasitism were 27% by *E. elegans* and 8.3% by *E. lutea*. Total rate of parasitism in Assiut reached a maximum of 83.3% during Nov. 1995, when *E. elegans* was responsible for 56.1% of the total parasitism (Table 2), while the minimum parasitism reached 10% during March 1996. Priesner and Hosny (1940) recorded *E. elegans* as a parasitoid of *A. niloticus*. In the present work *E. lutea, Er.* nr. *haldemani* and *Euderomphale* sp. were reared for the first time from *A. niloticus* in Egypt. The two surveyed regions were distinctive in their locations as well as their weather. *E. elegans* was the most abundant parasitoid in all the regions of this study. It is considered an effective aphelinid parasi-

Table 1

Percent parasitism of *A. niloticus* on *Z. spinachersti* by different aphelinid an platygastrid parasitoids in Sharqiya governorate, in relation to weather factors

Date	Immature stages of whitefly	Percent parasitism			Temperature		RH
		E. elegans	*Er.* nr. *haldemani*	*Euderomphale* sp.	Max	Min	
Aug. 95	213	54.5	30	0	35:3	22:5	64
Sep.	198	50	24.7	1	33.7	21.4	64
Oct.	201	39.8	18.4	3	29.4	17.9	63.6
Nov.	167	34.1	11.4	1.2	24.1	12.6	61
Dec.	160	28.8	5	0	21	9.5	71.3
Jan. 96	103	14.6	3.9	0	20.3	9.4	65.3
Feb.	97	19.6	3.1	1	21.6	9.7	62
Mar.	53	26.4	1.9	0	23.1	10.6	59.6
April	73	41.1	1.4	0	26.3	13.8	56.6
May	67	47.8	1.5	0	33	18.6	54
June	113	68.1	8	2.7	33.9	20.1	58
July	125	72	13.6	4	35.2	22.4	57.3
Aug.	187	61.5	22.4	2.1	35.2	22.5	64

Table 2

Percent parasitism of *A. niloticus* on *Z. spinachersti* by different aphelinid parasitoids in Assiut governorate, in relation to weather factors

Date	Immature stages of whitefly	Percent parasitism		Temperature		RH
		E. elegans	*E. lutea*	Max	Min	
Aug. 95	320	32.5	8.1	36.2	22.8	39
Sep.	312	31.1	10.3	35.9	20.9	38.6
Oct.	288	47.9	13.9	30.1	17.1	45.6
Nov.	173	56.1	27.2	23.2	10.1	41
Dec.	135	50.4	20.7	20.1	6.7	57.6
Jan. 96	100	28	7	20.9	6.4	46.3
Feb.	112	12.5	3.6	22.9	8.5	42.5
Mar.	70	5.7	4.3	24.7	10.9	36
April	87	9.2	1.1	29.7	13.7	31.3
May	89	11.2	2.2	37.1	20.4	24.6
June	135	20	1.5	35.8	20.8	31.3
July	203	19.7	3.9	36.5	22.4	36
Aug.	255	27.1	3.9	37.1	22.7	36

Table 3
Percent parasitism of *A. olivinus* on *Olea* sp. by different aphelinid prasitoids in El-Arish region, in relation to weather factors

Date	Immature stages of whitefly	Percent parasitism		Temperature		RH
		E. elegans	*Eretmocerus* sp.	Max	Min	
Aug. 95	48	10.4	12.5	31.7	21.4	72.6
Sep.	57	7	8.8	30.6	19.9	69.6
Oct.	54	18.5	9.3	27.5	15.7	70.3
Nov.	50	12	4	20.1	7.3	74.6
Dec.	39	12.8	0	23.5	10.7	60.3
Jan. 96	20	5	0	19.3	7.6	69.6
Feb.	14	7.1	0	20.6	9.2	65.3
Mar.	8	12.5	0	21.4	9.9	64
April	11	9	0	23.2	11.7	66.3
May	21	4.8	4.8	28.3	16.0	68
June	38	2.6	7.9	29.3	17.6	72
July	45	8.9	11	31.0	21.3	72
Aug.	60	10	15	31.4	21.1	73.3

toids associated with *A. niloticus* in Egypt. The present work observed the peak parasitism of *E. elegans* occurred in July 1996 (72.0%) at Sharqiya and in Nov. 1995 (56.1%) at Assiut.

Er. nr. *haldemani* and *Euderomphale* sp. collected from Sharqiya were not reported in Assiut. Sharqiya governorate is located in lower Egypt and can be characterized by mild temperature. This may refelct on the efficacy *A. niloticus* parasitoids. *E. lutea* was recorded for the first time associated with *A. niloticus* in Assiut and not reported in Sharqiya. Higher temperature and low relative humidity in Assiut could to be responsible for the occurrence of *E. lutea* in Assiut only.

2. Aleurolobus olivinus Silvestri

Two species of aphelinid parasitoids were reared from samples of *A. olivinus* on *Olea* sp. These are listed below in alphabetical order:

– *Encarsia elegans* Masi
– *Eretmocerus* sp.

El-Arish, the average parasitism were 9.3% by *E. elegans* and 5.6% by *Eretmocerus* sp. Peak parasitism of 27.8% occurred during Oct. 1995 (Table 3). *Eretmocerus* sp. were reared here for the first time in Egypt as a parasitoids of *A. olivinus, E. elegans, E. olivinus* and *A. minervae* were recorded as a parasitoids *of A. olivinus* in Italy and other countries (Mound and Halsey, 1978). El-Arish region is located in northeast Sinai and

can be characterized by colder and longer winter. Also El-Arish is isolated from the other locations by a vast desert region of Sinai, which may be reflected on the occurrence of *A. olivinus* and its parasitoids in this area only.

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Abundance and Habitat Preference of Some Adult-overwintering Ground Beetle Species in Crops in Western Hungary (Coleoptera: Carabidae)*

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The abundance of ground beetles in ten various crops and two adjacent habitats (bank side and a field for bustards) was investigated, in spring and summer in 1991 and 1992, in a Transdanubian agricultural region of Hungary. More than 25 000 carabids representing 68 species were collected in pitfall traps. Results of statistical analyses of data of the five most abundant adult-overwintering species (*Poecilus cupreus, P. sericeus, P. punctulatus, Platynus dorsalis* and *Brachinus explodens*) showed that the abundance of carabids varied between crops, and each species was significantly more abundant at least in one crop compared to the others, reflecting a crop effect. The different catches indicated that the five abundant species showed preferences for winter crops and early sown spring crops rather than late sown crops.

Ground beetles are well known as important components of the epigeal fauna in agricultural areas. There are several papers on their occurrence and importance (see e.g. reviews by Thiele, 1977; Allen, 1979; Luff, 1987). Carabids are usually predators, but they are also prey for numerous animals, among others e.g. various birds. In an earlier Hungarian investigation it was observed that Coleoptera served as an important diet source for wild fowls (e.g. partridge, pheasant, bustard) and carabids formed a large proportion of the Coleoptera-prey (Faragó, 1989).

The present paper is based on pitfall trapping of carabid beetles in various crops in a western Hungarian agricultural region, the same area where the previously mentioned birds were investigated. The aim was to answer the questions:

1 Which ground beetle species were the most abundant in the area within the studied time periods?

2 Whether the relative abundance of dominant carabids reflects habitat preference in various crop types?

Materials and Methods

Study area, time periods and crop type. The study area was situated at Mosonszolnok, ca. 160 km west of Budapest, in Transdanubia (Hungary). The soil is chernozem soil with a gravel layer at a variable depth. The precipitation is 500 mm, the annual aver-

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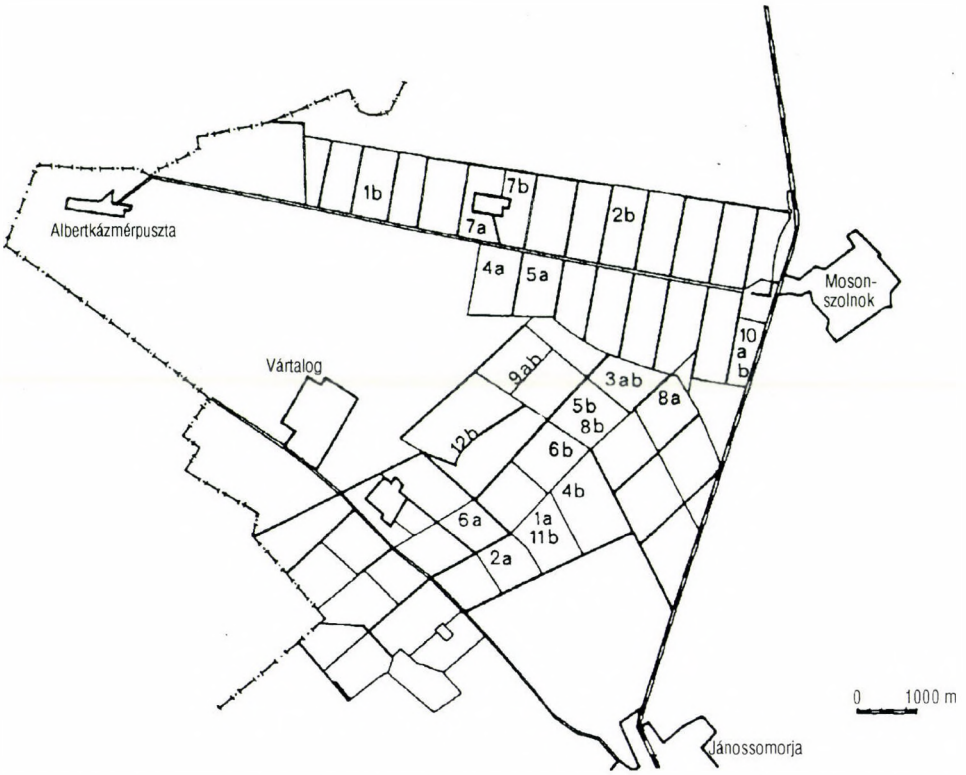

Fig. 1. Distribution of the investigated plots at Mosonszolnok (in 1991 and 1992) 1 = winter wheat, 2 = winter barley, 3 = alfalfa, 4 = pea, 5 = potato, 6 = sugar beet, 7 = rape, 8 = maize, 9 = field for bustards, 10 = grassland, 11 = spring barley, 12 = bank side; a = in 1991, b = in 1992

age temperature is 9.6 °C, the climate is continental (Faragó, 1989). The period of study was 1 May–25 July (1991) and 1 May–25 June (1992). Figure 1 shows the crop types, distribution and sizes of the studied crop plots (fields) and adjacent habitats. Total cultivated area was 2809 ha, and 10–15 different crops were included in the crop rotation. There were winter crops (winter wheat, winter barley and rape), early sown spring crops (spring barley and pea), late sown crops (maize, sugar beet and potato) and others. The average size of cultivated fields was 52 ha. Cultivation, fertilization and pest control was performed according to conventional methods.

Sampling method. Five plastic traps (80 mm diameter, 85 mm depth) containing 5% formalin were installed in an approximately central line in each of the study plots at 5 m distance from each other. The traps were emptied at 2 week intervals.

Data analyses. The examined periods (i.e. May–June) coincided with the main locomotory activity period of the adult-overwintering carabids, because single carabid

species have peaks in activity (in relation to their reproduction rhythms) either in early summer or late summer (e.g. Larsson, 1939; Lindroth, 1945; Thiele, 1977; Niemelä et al., 1989; Den Boer and Den Boer-Daanje, 1990; and others), therefore only adult-overwintering species were selected for analyses. Natural logarithmic transformation was performed on the catch data to stabilize the variance. In cases where variances were homogeneous, we performed one-way ANOVA to examine the effects of crop types. When the results of ANOVA indicated a significant effect, we tested pairs by *t*-test. To test for differences among data whose variances were heterogeneous, Kruskal-Wallis nonparametric ANOVA and posterior Wilcoxon–Mann–Whitney test were applied (Sokal and Rohlf, 1981; Siegel and Castellan, 1988). Pooled data were used for analyses when there were several plots in a given crop. Where the catch was zero or very low (<10 individuals) the data were not taken into account.

Analyses were also performed on 1992 data obtained by trapping at different plots within a crop type such as winter wheat (5 plots), winter barley (2 plots) and alfalfa (2 plots) to examine the effects of some disturbances (i.e. pesticide treatment in winter wheat and barley, and cutting in alfalfa).

Results

During the two periods of the study ca. 25,000 individuals belonging to 68 species were trapped. The most abundant species were: *Brachinus explodens* Duftschmid, *Harpalus rufipes* (De Geer), *Platynus dorsalis* (Pontoppidan), *Poecilus cupreus* (Linnaeus), *P. punctulatus* (Schaller), *P. sericeus* (Fischer). *P. cupreus* was found to be the most frequent species. Moderately abundant carabids were: *Amara similata* (Gyllenhal), *Calathus fuscipes* (Goeze), *Calosoma auropunctatum* (Herbst), *H. albanicus* Reitter and *Pterostichus melanarius* (Illiger). Further discussion below will be limited to five of the most common adult-overwintering species.

Individual numbers of the five species in ten various crops and two adjacent habitats (bank side and a field for bustards) and statistical analyses of catch data are given in Table 1. ANOVA showed significant differences in relative abundances among the crops within species ($p < 0.05$) except for *P. punctulatus* in 1991 and *Br. explodens* in 1992. Posterior pair tests showed significantly higher catches in at least one crop compared to the others in one of the years (see Table 1): the catches of *P. cupreus* in winter wheat in 1991 ($t \geq 2.46$) and in pea in 1992 ($t \geq 2.09$), *P. sericeus* in pea in 1992 ($t \geq 3.28$), *P. punctulatus* in pea in 1992 ($t \geq 2.54$), *Pl. dorsalis* in pesticide-treated winter wheat in 1992 ($z \leq -2.06$) and *Br. explodens* in rape in 1991 ($t \geq 2.28$) were significantly higher ($p < 0.05$) than in other crops ('t' and 'z' values are the results of *t*-test and Wilcoxon–Mann–Whitney tests, respectively; see Materials and Methods). Generally, the relative abundance of the selected species was high in winter wheat (except *P. sericeus*) and pea (except *Br. explodens*).

In 1992, there was no significant difference in the catches neither in pesticide-treated and untreated winter barley plots nor between the cut and uncut alfalfa plots. The

Table 1

Catches of five carabid species in various crops at Mosonszolnok (Hungary) in 1991 and 1992

Crop type	Catch / 5 traps				
	P. cupreus	*P. sericeus*	*P. punctulatus*	*Pl. dorsalis*	*Br. explodens*
1991	F (8,38) = 8.64 p < 0.05	F (7,34) = 2.75 p < 0.05	F (2,11) = 0.09 p > 0.05	F (8,38) = 2.39 p < 0.05	F (4,21) = 4.57 p < 0.05
Winter wheat	764 d	17 a	3 –	220 c	37 a
Winter barley	161 bc	11 a	10 a	95 bc	10 a
Alfalfa	33 a	13 a	18 a	70 abc	0 –
Pea	1179 cd	29 a	15 a	120 abc	6 a
Potato	382 bc	33 a	2 –	141 abc	0 –
Sugar beet	16 a	23 a	1 –	17 a	0 –
Rape	166 bc	5 –	2 –	127 abc	331 b
Maize	21 a	16 a	0 –	6 –	1 –
Field for bustards	100 b	103 b	1 –	29 a	0 –
Grassland	1 –	1 –	0 –	15 a	6 a
Total	2823	251	52	840	391
1992	F (8,49) = 24.33 p < 0.05	F (8,49) = 16.69 p < 0.05	F (5,38) = 6.07 p < 0.05	H (9,62) = 35.23 p < 0.05	F (3,28) = 0.31 p > 0.05
Winter wheat 1	448	51	92	144	127
Winter wheat 2*	3154	166	59	479	139
Winter wheat 3*	1938	42	37	462	80
Winter wheat 4*	1983	109	42	558	242
Winter wheat 5	2053	84	105	236	271
Pooled data (1–5)	9576 d	458 d	335 b	1879 c	859 a
Winter barley 1	77	40	0	60	0
Winter barley 2*	132	73	3	49	0
Pooled data (1–2)	209 a	113 cd	3 –	109 ab	0 –
Alfalfa 1	22	8	27	61	0
Alfalfa 2**	65	6	7	92	1
Pooled data (1–2)	87 a	14 a	34 a	153 b	1 –
Pea	4291 e	419 f	232 c	6 –	0 –
Potato	332 bc	39 cd	0 –	25 ab	0 –
Sugar beet	23 a	23 bc	0 –	62 ab	0 –
Rape	748 cd	101 de	12 a	35 ab	280 a
Maize	6 –	1 –	0 –	103 abc	0 –
Field for bustards	123 ab	32 bc	49 b	154 b	117 a
Spring barley	436 c	172 e	20 a	16 a	7 –
Bank side	9 –	1 –	0 –	184 bc	144 a
Total	15840	1373	685	2726	1408

F = ANOVA's *F*-value; H = Kruskal-Wallis ANOVA's *H*-value; Individual number within columns followed by the same letter are not significantly different ($p < 0.05$; *t*-test and/or Wilcoxon–Mann–Whitney test); – = not taken into account; * = treated by pesticide; ** = cut; (see text for further explanation)

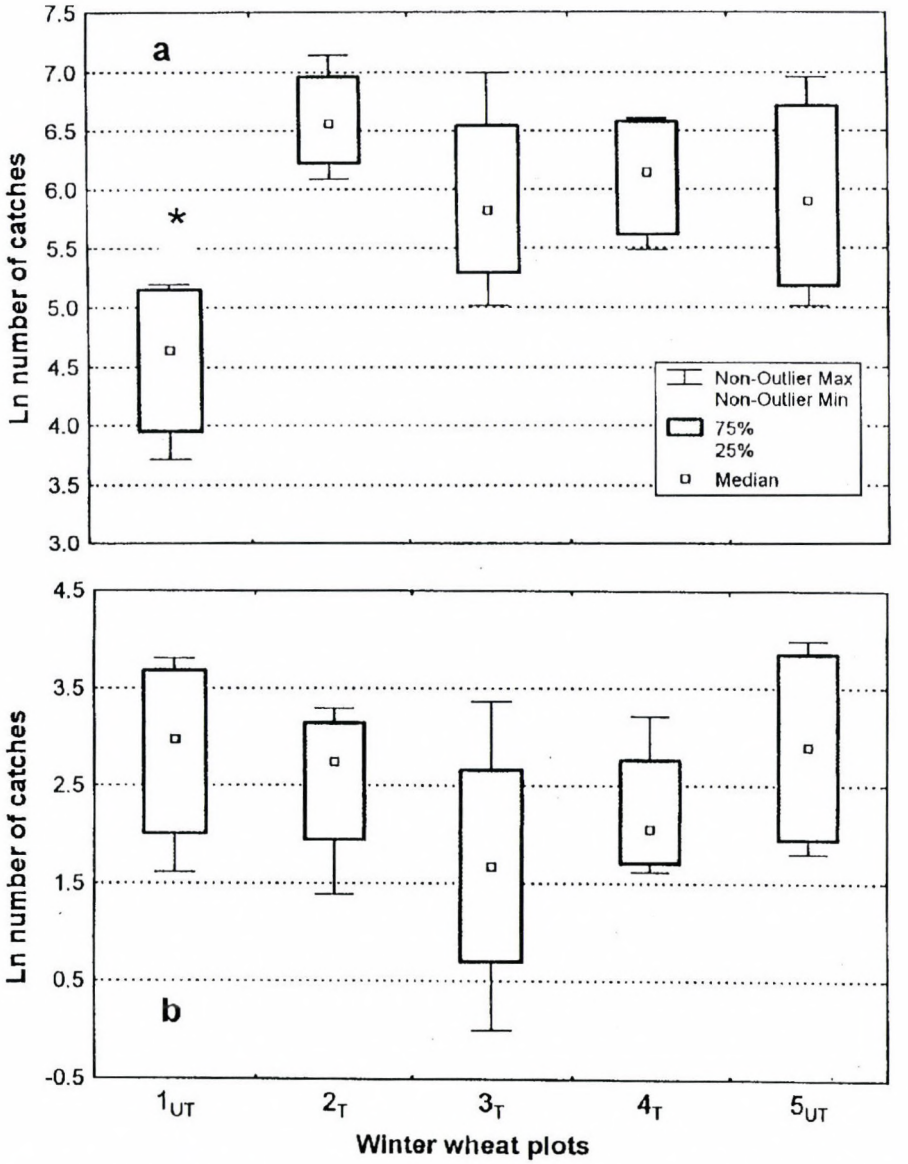

Fig. 2. Box plots of catches of *Poecilus cupreus* (a) and *P. punctulatus* (b) in pesticide-treated (2_T, 3_T and 4_T) and untreated (1_{UT} and 5_{UT}) winter wheat plots at Mosonszolnok in 1992. Asterisk represents significant difference ($p < 0.05$). See text for details

catches of *P. punctulatus* (see Fig. 2/b) and *Br. explodens* were not significantly different in the five winter wheat plots ($F = 0.92$ and 1.78, $p > 0.05$) while in the abundance *of P. cupreus* (see Fig. 2/a), *P. sericeus* and *Pl. dorsalis* there were significant differences ($F = 4.43$, 6.23 and 5.73, respectively, $p < 0.05$). In the latter species abundances were higher on most of the treated plots (marked by * in Table 1) than on the untreated ones. But, Fig. 2/a shows that effect of this treatment is not obvious even in the case of a single species, because there is no overlapping between the untreated plots either (see plots marked by 1_{UT} and 5_{UT} in the Figure).

Discussion

The majority of the abundant species commonly occur in European agricultural regions (see e.g. Luff, 1987; Lövei and Sárospataki, 1990). But for example, *P. sericeus* and *Calosoma auropunctatum* are abundant in Eastern Europe only (Thiele, 1977; Lövei and Sárospataki, 1990).

Of the three *Poecilus* species, *P. sericeus* in the one we have the least information about. In Hungary, the winter wheat field is a suitable habitat for *P. sericeus* (Kiss et al., 1993). This study, however, has shown pea as an even more favoured crop. *Br. explodens* seems to be a special case in that the larvae of the Brachinini group are ectoparasitoids, e.g. on water beetle pupae (Erwin, 1979), therefore, it can be expected that the abundance of the host will regulate the population size of this species. Kromp and Steinberger (1992) showed seasonal fluctuations in the catches of *Br. explodens* as indicated by migrations between a wheat field and its field margin in Austria. Kiss et al. (1994) found significantly more adult in the field margin than in the winter wheat field in 1992 during spring and early summer. *Br. explodens* was recorded as an abundant species in Hungary only in an abandoned apple orchard (Fazekas et al., 1992), where together with *Pl. dorsalis* they were the dominant species.

Based on our results our conclusions are in agreement with statements of Rivard (1966), Hance et al. (1990) and Cárcamo and Spence (1994) that the relative abundances of carabids are influenced by crop types. Rivard (1966) pointed out that effect is not because of the nature of crop. Cárcamo and Spence (1994) demonstrated that the physical nature of the crop alone did not affect the habitat choices of *Pt. melanarius*.

Numerous niche dimensions influencing abundance have been postulated, such as humidity (Rivard, 1966), prey (Barney and Pass, 1986) or microclimate (Honek and Martinková, 1991) and light intensity (Varis et al., 1984). There are also important factors such as treatments, management, cultivation and agronomical practices (Hokkanen and Holopainen, 1986; Booij and Noorlander, 1992; Fan et al., 1993; Cárcamo and Spence, 1994; Cárcamo et al., 1995).

Generally, the results of the present study have confirmed the conclusion drawn by Jensen et al. (1989), Hance et al. (1990), Booij and Noorlander (1992) and Cárcamo and Spence (1994), namely that it is apparent that several carabid species which overwin-

ter as adults show a preference for winter crops (winter wheat, rape) and early sown spring crops (e.g. pea) rather than late sown crops such as sugar beet and maize.

The results are possibly influenced (to a great extent) by the dispersion of the carabid species studied. This is supported by the findings of Sotherton (1984) and Hance et al. (1990). Sotherton (1984) proved that distribution and abundance of carabid predators was partly determined by the distribution and abundance of suitable overwintering habitats (e.g. field margins). Furthermore, Hance et al. (1990) showed that the carabid fauna is influenced by the neighbouring habitats.

It may also be due to their dispersion that there was not an unambigous effect of pesticide treatment on their abundance in wheat. The following data also support the above. Rapid redistributions of some species were experienced by Gordon and McKinlay (1986) following an insecticide treatment in field investigations. Catches of two *Pterostichus* spp. were usually lower immediately after pesticide treatment in sprayed as compared with unsprayed plots, and higher a week or two later in experiments of Dixon and McKinlay (1992). In the study of Los and Allen (1983) one of four abundant ground beetle species was more abundant in untreated fields, whereas other three carabids were more abundant in insecticide-treated fields. Therefore, it seems that the varied effects of pesticide treatments on carabids cannot be measured by pitfall trapping alone (Dixon and McKinlay, 1992).

To clarify the actual relations further extensive field investigations are necessary.

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Mosquitos of the Lake Balaton and their Control

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This paper gives a survey of the mosquito research in Hungary carried out around Lake Balaton in the past 50 years. In the authors' 25-years work 33 species were found to occur around the Lake. From the area of Hungary a total of 44 species are known. Chemical control by helicopter began in 1976. Larva control with BTI was started in 1986. At present the control takes place with biological (BTI) and chemical (deltametrin) methods. Species most frequently occurring: *Mansonia richiardii* Fic., *Aedes annulipes* Meig., *Aedes vexans* Meig., *Culex modestus* Fic.

Lake Balaton is Hungary's natural resource of high priority requiring increased protection and appreciation. Almost the whole society of Hungary follows with great concern the changes occurring in the lake and its environment. It is not by chance that the conservation of the "Hungarian sea", the improvement of the quality of its water, the gradual infrastructural development of the region are managed and supervised by a government commissioner. To avoid earlier unfavourable effects on the lake, any human intervention concerning Lake Balaton and its environment must be preceded in the future by scientific examinations. Since protection against mosquitos is a yearly recurring task, the above requirement increasingly applies to the mosquito control which often arouses hot discussion among people.

Although the mosquito-bite is more or less painful (depending on species and individual sensitiveness), the human race have been compelled for thousands of years to live together with the harm caused by the mosquitos (like other vexatious insects), and regarded it as an unavoidable natural distress. No doubt, mosquitos multiplying here and there in large numbers can cause heavy economic losses, too, since they make e.g. forest cultivation impossible, but they may even play a role in destroying the game population, first of all, the young animals, moreover, similarly to the horse-flies they may greatly reduce the milk output of cattle, etc.

However, in the course of the development of the medical science it has come to light that apart from the pain caused by biting the mosquitos belong to the most important vector insects spreading numerous human and animal diseases. It was toward the end of the last century that the role of mosquitos was discovered in the transmission of malaria which mainly in the tropical and subtropical regions carried away millions; the researchers' attention was therefore called at once to this insect. The question has been studied by Hungarian authors, too (Makara and Mihályi, 1943).

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History of mosquito research in Hungary

Intensive studies on the mosquito problem came into prominence first of all from a public health point of view in Hungary, too. Investigations aimed at throwing light on the distribution of malaria mosquitos (Anopheles sp.) and on their role in causing malaria in Hungary were started in 1934 by Ferenc Lőrincz, head of the Parasitological Section of the National Institute of Public Health. From 1937 to 1944 György Makara directed the work in which Ferenc Mihályi, Béla Lovas and Sándor Székely also took part. In the course of their exploratory work they found two major areas infected by endemic in Hungary at that time, one in the nort-east (mainly in Szabolcs-Szatmár county), the other in the south-west (first of all in Baranya and Somogy counties along the river Dráva). The residential buildings and stables of the heavily infested regions were treated for 3–4 years with DDT already available at that time, whereby the mosquito density was successfully reduced to minimum, and the disease (supposedly due mostly to the control) almost completely repressed (Lőrincz and Mihályi, 1937a, 1937b, 1938; Makara and Székely, 1940).

In order to prepare a skillful protection against the mosquitos, besides studying the malaria mosquitos investigations aimed at acquiring a thorough knowledge of the Hungarian fauna began in 1938. It was for this purpose that the Balaton Mosquito Research Station was established in 1938 in Tihany, right at Lake Balaton, within the Hungarian Biological Research Institute. Ferenc Mihályi, who made the first purposeful study gave a general outline with two years of work of the composition of the mosquito faune of the lake, and laid down the major directives of control (Mihályi, 1939, 1941).

The work interrupted by World War II was continued in the early 1950s by a team (F. Mihályi, Á. Soós, M. Sztankay-Gulyás, N. Zoltai) under the sponsorship of the Hungarian Academy of Sciences. They explored the local mosquito fauna, cleared up the way of life of the different species and the role they played in the mosquito damage, and disclosed the growing places of the larvae (Mihályi and Soós, 1952; Mihályi and Zoltai, 1956a; Mihályi et al., 1952a, 1952b, 1953a, 1953b, 1954, 1956b). They prepared a detailed proposition on the possibilities of control covering almost the whole coastal sector (Mihályi and Soós, 1952), which was, however, hardly if at all used by the local magistrates, in spite of the fact that parallel with a rapid upswing of the Balaton tourism the demand for preventing or at least reducing the mosquito damage gradually increased.

Changes in the mosquito fauna of Lake Balaton

For some decades Lake Balaton and its environment countable from the point of view of mosquito reproduction have considerably changed, many growing place shave disappeared while new ones have come into existence. Yet, the mosquito damage has not decreased, on the contrary, supposedly in connection with the deterioration of the quality of water it has even increased. Due to the surveys made at the beginning of the 1950s we can form a conception of the major changes too that have taken place in the mosquito fauna (Kecskeméti and Tóth, 1981). As we can see from a comparison of Figs 1 and 2

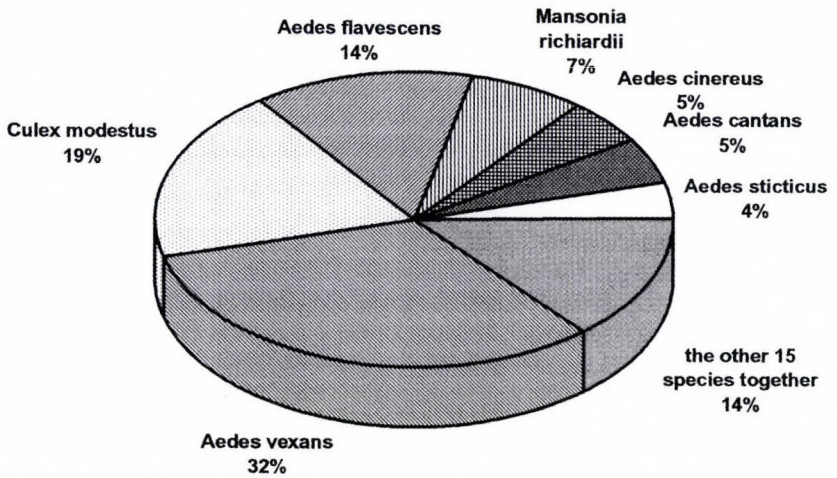

Fig. 1. Quantitative composition of mosquitos collected while biting around Lake Balaton in 1950–1951

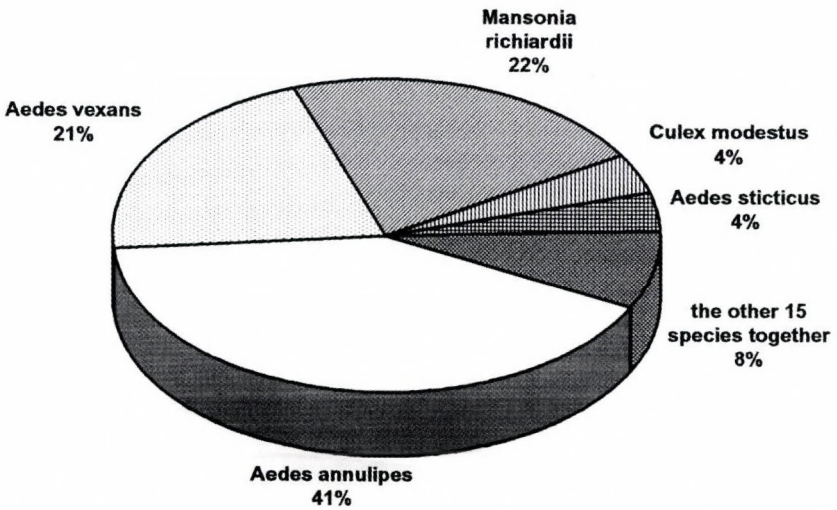

Fig. 2. Quantitative composition of mosquitos collected while biting around Lake Balaton in 1996

four of five decades ago the species diversity was higher, more taxa played important role in the mosquito damage. It is a remarkable difference that the *Mansonia richiardii* Fic., or even more the *Aedes annulipes* Meig. were present at the coast of Lake Balaton in much lower individual number than they are now. Only the *Aedes vexans* Meig. has retained almost all along its dominance of about 30% (in 1996 it was only 21%).

The beginnings of mosquito control

From the second half of the 1960s, first of all under the pressure of those on summer holiday, mainly the larger hotels, rest-houses, campings ordered one after the other mosquito control. This work was done with ground machines, mostly wihout the proper professional background and not always by using the right chemical. In a large proportion of the cases the control was not effective, but at the same time often caused immeasurable damage to the environment. These unfavourable experiences also contributed to a change-over to the more successful up-to-date aerial control which satisfies better the aspects of nature conservation.

The Executive Committee of Balaton, the host of the lake (possessing the licence of the Ministry of Health), in cooperation with the Airplane Service of the Ministry of Agriculture and Food organized the ever since existing aerial mosquito control in the Balaton region first in 1976. The fixed-wing aircraft equipped with ULV nozzle, earlier widely used in plant protection, later only the better manoeuvring helicopter distributed 0.4 l/ha Malathion insecticide (active ingredient: phosphorous acid ester) recommended by, the WHO (World Health Organization of the UNO) in places marked in the map for the pilot. On consideration of environment protection, machines doing the work of spraying must keep at least 50 meter off the shore. However, even with the strict observance of this prescription it cannot be perfectly excluded that the air current carries sometimes minor quantities of the chemical into the water of Lake Balaton.

Examination of the effect of mosquito control

For some days following spraying Malathion ensured an almost complete freedom from mosquitos (the degree of efficiency reached 90–97%). Since, however, this drastic insecticide was not selective at all, it caused very great losses among other insects in the zone treated. Investigations to this end in 1977 pointed out that the destruction of a single biting mosquito with Malathion cost the life on nearly 200 various insects (first of all diptera developing with the largest individual numbers by the lake) (Kölüs and Tóth, 1979; Sáringer, 1980, 1983a, 1983b, 1988; Sáringer et al., 1984; Tóth, 1979).

Considering the harmful effects of mosquito control the Environment- and Water Protection Special Committee of the Executive Committee of Balaton ordered in 1976 the establishment of a committee of experts. Its task is to study the effects of mosquito control (within this to size up the mosquito fauna, folow the trend of the mosquito density and of other arthoropodal fauna before and after the treatments, keep an eye on the hydrobiological changes under the influence of chemicals occasionally getting into the water etc.), and to make proposal on the time and area of treatments. The committee took special care not to carry out mosquito control at the time of a higher rate swarming of Chironomidae, which are of outstanding importance from the point of view of making the lake free from phosphorus. Further, the expert committee examined how the arthropodal fauna of the chemical treated zone was replaced following the spraying. It was found that during the 2–3-week period between two sprayings the individual- and species

number of the fauna gradually renewed, by the end of the period it almost reached the original level. Beside the individuals freshly hatched at the place important role is played in this by immigration from the surrounding untreated areas. To ensure the possibility of immigration the treated zone should not be continuous (Tóth, 1979). The treated area (and the number of sprayings) has recently been so much reduced, partly of financial reasons, that this problem has practically ceased to exist, and other sources threatening the fauna, water pollution in the first place, have come into prominence.

Biological control

Because of its drastic effects, after some years Malathion was replaced at Lake Balaton too by other colicides containing deltametrin and permetrin as active agent (K-OTHRIN 1 ULV, UNITOX 14 ULV, RESLIN-SUPER ULV). To some extent these are of selective effect, and for this very reason cause less damage to the environment. But at the same time they kill the mosquitos with lower efficiency, of 70–85% even under optimum conditions. The reduction of individual number in other arthropodal species is 60% on the average of many years.

Owing to the harmful effects of chemicals attempts were even earlier made to solve the preventive mosquito control. The so-called juvenile hormone preparation which hinder the development of larvae (e.g. ABATE 1 SC, active agent: difenfosz; VIODAT 10 MG, active agent: metopren) were commercially available as early as in the mid-seventies, but for mosquito control they were used in a restricted measure only (Erőss, 1988), partly because they were not selective; besides the mosquito larvae they caused damage to other aquatic insects, too. With the favourable experiences gained in Hungary and adopted from abroad the protection against mosquitos with biological methods began in 1986 in the region of Lake Balaton, too. The *Bacillus thuringiensis* var. *israelensis* H-14 serotype preparations (TEKNAR HP-D, SKEETAL, VECTOBAC) developed for this purpose, applied at a concentration depending on the depth of water, larva density and degree of water pollution kill only the mosquito larvae. It has the incomparable advantage of preventing the development of mosquitos from the larvae, so if everything turns out well there is no need (at least theoretically) to carry out chemical control against the adults (Entwistle et al., 1993).

The Negev desert, as the cradle of BTI

It must be mentioned that the discovery of *Bacillus thuringiensis* var. *israelensis* (abbr.: BTI) is owed to the Hungarian origin Prof. Dr. Yoel Margalith. The well-known expert of mosquito living in Israel found the bacillus in question in 1976 in mosquito larvae collected in a small puddle of Negev, a desert in the southern part of Israel; from that bacillus was the preparation developed. Many variations of the preparation are produced in factories and used today against mosquito larvae successfully almost all over the world. Millions, mainly in the malaria attacked regions of the tropics owe their lives to the researcher.

On the 20th anniversary of the sensational discovery, in honour of Professor Dr. Yoel Margalith, in Israel, near Jerusalem (Shoresh) the Ben-Gurion University organized the second BTI World Conference between 12 and 16 August, 1996, which was attended by some 60 researchers (including many mosquito experts) from Brasil through the United States, Europe, Asia to Japan, mainly those interested in the production and utilization of BTI.

The efficiency of the BTI is good according to both Hungarian and international experiences. In the course of treatments carried out at Lake Balaton an average of 80% of the larvae were destroyed at the sites examined. The efficiency of the larvicide is however, influenced by numerous factors. The time of the treatment must be carefully chosen, because the older, fourth stage larvae are less, if at all responsive to BTI, while on the pupae it is totally ineffective. According to our examinations the result greatly depends on the purity, depth and temperature of water, on the larva density, and particularly on the extent to which the growing place is covered by vegetation.

From the point of view of the biological control great difficulties are caused at Lake Balaton by the larvae of *Mansonia richiardii* Fic., which owing to their peculiar way of life are – according to laboratory examinations in Hungary (Tóth, 1991) – less sensitive than the other species' larvae. The larvae of *Mansonia uniformis* Theob. in South-East Asia are killed by SPHERMOS FC, a larvicide containing *Bacillus sphericus* Neide as active agent. According to examinations we carried out in spring 1997 at Lake Balaton, this larvicide was ineffective on *Mansonia richiardii* Fic. larvae both in laboratory and in the field.

As it is clear from the above, at present the mosquito problem of Lake Balaton cannot be exclusively solved by the larva control; if the region is to be made acceptably free from mosquitos the two methods must be alternately (occasionally parallel) employed.

Biting mosquito fauna at Lake Balaton and in its immediate environment

The environment of Lake Balaton belongs to the most mosquito stricken areas of Hungary. Some two-third of the 44 biting mosquito species identified so far in Hungary occur at the lake too.

(1) *Anopheles algeriensis* Theobald, 1903
(2) *Anopheles atroparvus* (Van Thiel, 1927)
(3) *Anopheles claviger* (Meigen, 1804)
(syn.: *Anopheles bifurcatus* Meigen, 1918)
(4) *Anopheles hyrcanus* (Pallas, 1771)
(5) *Anopheles maculipennis* (Meigen, 1818)
(6) *Anopheles messeae* Falleroni, 1926
(7) *Anopheles plumbeus* Stephens, 1828
(8) *Uranotaenia unguiculata* Edwards, 1913
(9) *Culiseta annulata* (Schrank, 1776)
(syn.: *Theobaldia annulata* /Schrank, 1776/)

(10) *Culiseta longiareolata* (Maquart, 1839)
(syn.: *Theobaldia longiareolata* /Maquart, 1838/)
(11) *Culiseta morsitans* (Theobald, 1901)
(syn.: *Theobaldia morsitans* /Theobald, 1901/)
(12) *Mansonia richiardii* (Ficalbi, 1889)
(syn.: *Coquillettidia richiardii* /Ficalbi, 1889/)
(syn.: *Taeniorhynchus richiardii* Ficalbi, 1889)
(13) *Aedes annulipes* (Meigen, 1830)
(14) *Aedes cantans* (Meigen, 1818)
(15) *Aedes caspius* (Pallas, 1771)
(16) *Aedes cataphylla* Dyar, 1916
(17) *Aedes cinereus* Meigen, 1818
(18) *Aedes dorsalis* (Meigen, 1830)
(19) *Aedes excrucians* (Walker, 1856)
(20) *Aedes flavescens* (Müller, 1764)
(21) *Aedes geniculatus* (Olivier, 1791)
(22) *Aedes leucomelas* (Meigen, 1804)
(23) *Aedes pulchritarsis* (Rondani, 1872)
(24) *Aedes refiki* Medschid, 1928
(25) *Aedes rossicus* Dolbeshkin, Goritzkaja and Mitrofanova, 1930
(26) *Aedes rusticus* (Rosii, 1790)
(27) *Aedes sticticus* (Meigen, 1838)
(28) *Aedes vexans* (Meigen, 1830)
(29) *Culex hortensis* Ficalbi, 1890
(30) *Culex modestus* Ficalbi, 1890
(31) *Culex pipiens* Linnaeus, 1758
(32) *Culex pipiens molestus* Forskal, 1775
(33) *Culex territans* Walker, 1856.

Most harmful members of the Balaton mosquito fauna

Due to the investigations long since carried on today not only the list of species is wellknown, but the qualitative and quantitative composition of the mosquito fauna, too.

Although among the mosquitos living in the environment of the lake only several species cause major damage, but their mass reproduction often means almost unbearable vexation both to the local inhabitants and to persons on summer holiday. See below the species belonging to this group.

Mansonia richiardii Fic.

The most characteristic mosquito species of Lake Balaton is able to develop in unbelievable masses at places suitable for it (as is the environment of the lake itself). People are agressively attacked by it, in woody, shady places its bite may unbearable

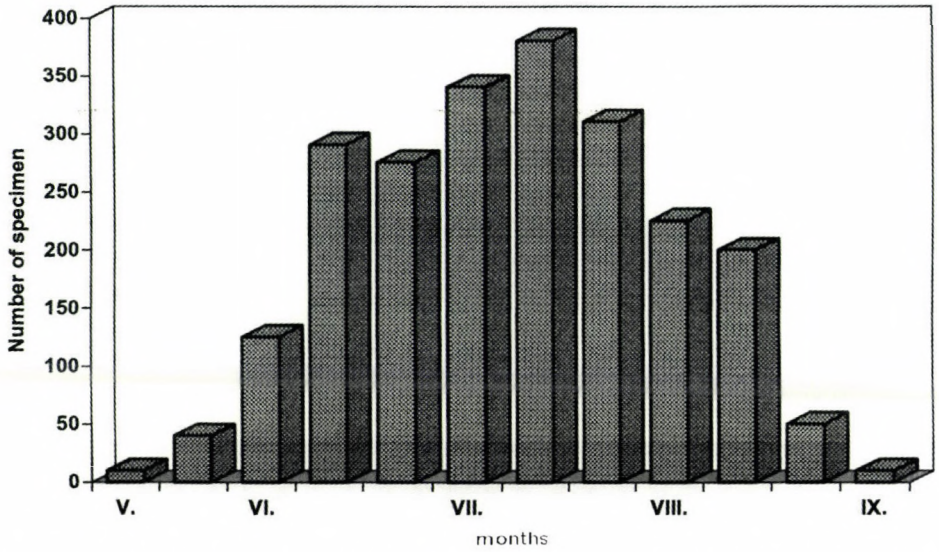

Fig. 3. Flying dynamics of *Mansonia richiardii* Fic. population on the basis of collection data in Hungary

Fig. 4. Fourth stage larvae of *Mansonia richiardii* Fic. on the roots of bulrush

Fig. 5. In the costal region of Lake Balaton the Külső Lake is one of the most important growing places of *Mansonia richiardii* Fic.

even in daytime. The way of life of its larva differs from that of any other mosquito species. Namely, the larva and the pupa do not come up to the surface of water to take air, they drive their breather-pipe into the roots of plants, or less often into other plant parts under the water, and ensure their oxygen requirements from the plant tissues (while living fixed to the plant). Its development takes 9–10 months, it can therefore survive exclusively in permanent waters. It has a single generation a year; the adults fly from the second half of May to mid-September (Fig. 3). As it is shown by its Hungarian name (marsh-mosquito), the larvae live first of all in shallow edges of muddy marshes (of more or less polluted water) covered by reed and bulrush (Fig. 4).

The *Mansonia richiardii* Fic. is a biting mosquito from time to time absolutely dominant in the environment of Lake Balaton (Fig. 5). In some cases it made up 70% of the mosquitos collected while biting by the lake. It occurs in the highest individual number in the neighbourhood of the Tihany-peninsula, in the Badacsonyörs-bay, and in the western basin of the lake (between Balatonederics and Fonyód). Mainly because of the peculiar way of life of the larva itis our most problematic mosquito from the point of view of biological control. Its lower metabolism compared to the other species is also supposed to have something to do with the lower sensitiveness of its larvae to the usual biological preparations.

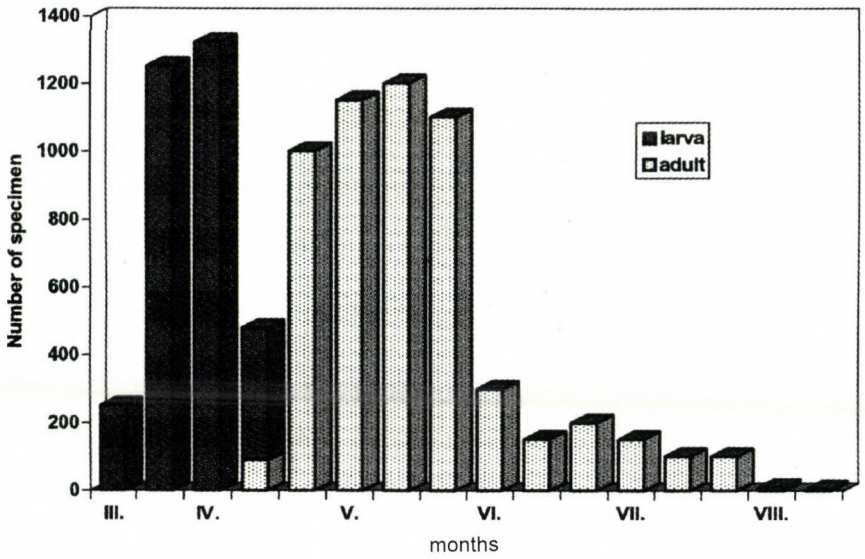

Fig. 6. Dominance relations of *Aedes annulipes* Meig. population on the basis of collecting in 1996 (as broken down to larva and adult)

Aedes annulipes Meig.

It is widely distributed in Hungary, though not everywhere occurs in larger numbers. At Lake Balaton in the early fifties it made up only 0.07% of the local fauna. Recently, however, its individual number has almost steadily increased, it has become one of the dominant species of the environment of Lake Balaton, with its share exceeding 40%. Similarly to the marsh-mosquito it attacks people cruelly, its bite is painful. It has a single generation a year, the larvae may even appear in March (though mostly in the first half of April) depending on the weather, the adults molest people from the beginning of May to the end of July–beginning of August (Fig. 6). Therefore the protection against it at Lake Balaton is of outstanding importance. The larvae prefer the sunny waters and are less particular about the quality of water; they are characteristic of more or less polluted waters, and according to the experiences they feel well in extremely polluted growing places as well (Figs 7 and 8). The biological control of larvae can be efficiently carried out in the middle or second part of April depending on the weather (water temperature).

Aedes vexans Meig.

It is a species typically with several generations; from spring to late autumn generations develop as many times as growing places favourable for the larvae occur. Mass reproduction takes place under the influence of June- and late summer-, autumn rainfalls.

Fig. 7. In the shallow water of the Kerekedi-by first of all the larvae of *Aedes annulipes* Meig. develop in masses

Fig. 8. Moorland in the Tapolca basin beside Szigliget. In spring it is the growing place of *Aedes annulipes* Meig. From the beginning of summer to late in autumn several generations of *Aedes vexans* Meig. may fly up from the shallow puddles

Their typical growing places are stagnant rainwaters left after flood in low grassy places (meadows) of flood-plains of larger brooks and rivers. In lower individual numbers, however, they are continuously present in shallow standing waters, e.g. in the outer edges of Balaton reeds, rush-beds, in occasional puddles, ditches, channels etc. In hot sunny weather the larvae develop even within a week, so the time required for detecting the growing place and organizing the control is very short (3–4 days at the most). The species is inclined to migration, according to some authors' opinion the female is able to migrate to a distance of 5–6 km from its growing place. The species' role in the mosquito damage of Lake Balaton is outstanding, the share of its population is about 30% in the long run, depending on the amount of precipitation.

Beside the *Aedes vexans* Meig. other species with similar way of life must be reckoned with (in yearly changing proportions) at Lake Balaton, too. Among them *Aedes cantans* Meig., *Aedes sticticus* Meig. and *Aedes cinereus* Meig. are of some importance. In particular the population of *Aedes sticticus* Meig. may sometimes be considerable (e.g. in 1992 its share in the mosquito damage was 36%, exceeding any other species).

Culex modestus Fic.

Its generations, several in a year, only strengthen gradually from the middle of summer; therefore its role in the mosquito damage may be of some importance in the second half of summer and in autumn. The larvae develop in the shallow water of the outer edges of Balaton reeds rich in aquatic vegetation. The adult holds to the water, hardly flying farther than 100 m from its growing place. It is a characteristic species of reeds, the adults continually molest the people even in daytime. In areas farther from the water it is of hardly any importance. Its share in the composition of the mosquito fauna of Lake Balaton is 3–5% (at the beginning of the 1950s it was still placed second after the vexing mosquito).

Practical control

The first biological control (depending on the weather influencing the development of the mosquito larvae) may become necessarys in the middle of April, first of all against *Aedes annulipes* Meig. which almost regularly appears in great masses every year, and against some early developing species of minor importance.

A subsequent biological control can be justified in the case of a mass development of larvae of *Aedes vexans* Meig. and of *Aedes sticticus* Meig., a species of subordinate role but similar way of life, developing in temporary puddles occurring after June rainfalls in low grassy places of the coast-line (and even farther off). It is made very difficult by the fact that in the hot summer weather the *Aedes vexans* Meig. larvae develop in 7–8 days, so the time required for detecting the growing places and organizing the treatment is very short. In addition, under the influence of repeated rainfalls further growing places continuously arise.

It may therefore happen that the third biological control must be timed to the second half of the rainy period. In droughty weather the development of *Aedes vexans* Meig. mostly takes place in the shallow fore-shore of reeds.

At Lake Balaton the first chemical control of mosquito adults can be timed to the end of June–beginning of July, after the mass flying of *Mansonia richiardii* Fic. (which otherwise largely coincides with the beginning of the high season of holiday, and at the same time the *Aedes annulipes* Meig. adults too are still present in large individual numbers). The control must by all means be preceded by a thorough professional survey, when increased attention is worth being paid to the flying of (not biting) Chironomidae, which play an extremely important role in the life of Lake Balaton, among others in the phosphorus elimination and as a food for fish. They are sensitive to chemicals in the same way as their biting "relatives" are. It is understandable that a considerable proportion of people cannot make distinction between mosquito and mosquito, many are unreasonably afraid of the harmless Chironomidae. No doubt, the mass flying of Chironomidae may be unpleasant, but relatively seldom occurs a gradation similar to that in 1995, when mainly in the catering trade many people clamoured for their control. However, an organized control of these useful insects would be incorrect, even if only on nature conservation consideration. Justification of a second or further chemical controls and determination of their time are functions of mosquito density (number of bites per hour).

Protection against mosquitos is an extremely complex problem. One must become reconciled to the thought that perfect freedom from mosquitos cannot be expected even from an organized control. This could be approximately achieved only with such drastic insecticides as e.g. once the DDT and later the Malathion were. However, their use would probably cause irreversible destructive processes in the arthropodal fauna of the area treated.

Keeping its own interest in view the local population can to some extent also contribute to the control of mosquitos, e.g. by following growing places to be four around or arising after rainfalls with attention. A biological preparation (CULINEX) containing BTI, which may be suitable for preventing in an ecologically safe way the development of larvae in small pools around the house is already commercially available in the form of tablets.

Our allies in destroying mosquitos are the various predatory Arthropoda (Odonata, Asilidae, Arachnoidea etc.), the singing-birds, and last but not least the frogs, and the Caudata, the diet of which includes beside the adults the larvae and pupae of mosquitos, too. If only for this reason increased appreciation and protection is deserved by amphibious fauna which has recently become endangered in the Balaton region, too (Tóth, 1996).

Mention should be made of the importance of individual defence. Preparations suitable for killing or repelling mosquitos either in closed space or outdoors are at present available in abundance (mosquito-cide sheet, evaporating apparatus, smoking spiral, mosquito-repellent aerosol, various creams etc.).

Finally, in keeping of our blood-sucking summer enemies, the long-since well proved traditional means of protection against mosquitos, the mosquito-net fixed to the openings of the building can be of much help.

Acknowledgement

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Comparative Toxicity of Pesticides to *Typhlodromus athiasae* (Acari: Phytoseiidae)

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Comparative toxicity of four pesticides to the predacious mite *Typhlodromus athiasaé* Porath and Swirski was evaluated.

Fenpropathrin was the most toxic material to the predator, followed in decreasing order by fenvalerate, propargit and tedifol. Females of *T. athiasae* suffered a depression in reproduction when sprayed with fenpropathrin, fenvalerate and propargit (when used concentration equivalent to the LC_{50} of the prey *Tetranychus urticae* Koch), while the number of prey consumed by female was significantly lower in case of all four pesticides.

The members of the family Phytoseiidae are known as the major natural enemies in suppressing the population of spider mites.

The phytoseiid mite *Typhlodromus athiasae* Porath and Swirski is important natural enemies on citrus, mango, apple, guava and eggplant in Egypt. Although few studies have been conducted on *T. athiasae*, the predator has been shown to be an effective predator on tetranychid mites (Swirski et al., 1967; Mansour, 1990; Momen and El-Brolossy, in prep.); on eriophyid mite, *Eriophyes dioscoridis* Solimon and Abou-Awad, the cotton and tomato whitefly *Bemisia tabaci* Genni and on pollen grains of *Ricinus communis* (L.) (Momen, in prep).

Successful biological control with phytoseiids requires the presence or release, of an insecticide – resistant strain of the predator where their population is scarce, and a pest management program that minimizes the use of insecticides, miticides and fungicides that are harmful to the predator (Van De Vrie, 1985). The insect growth regulators triflumuron and fenoxycarb and the fungicide triadimenol caused only slight mortality of *T. athiasae* but a highly significant reduction in fecundity (Mansour et al., 1993). This study was undertaken to provide information on the direct effect of some pesticides on *T. athiasae* in the laboratory and to chose the more selecitve pesticides to be used for integrated phytophagous mite management.

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Materials and Methods

Laboratory stock cultures

The stock cultures of *Tetranychus urticae* were collected from infested lima bean (*Phaseolus vulgaris* L.) in the laboratory at N.R.C. Cairo. Phytoseiid stock culture was initiated from approximately 25 females of *Typhlodromus athiasae* collected from infested eggplant leaves of vegeteable field located at Tobhar village, Faywam Governorate.

Materials tested

The pesticide formulations were diluted in water to the concentration needed.

SPECIFIC ACARICIDES

1. Propargit 75% (2-(p-tert-butyl phenoxy) cyclohexyl-2-propynyl sulfite).
2. Tedifol (dicofol 18.5% + tedion 6%)

PESTICIDES

1. Fenpropathrin 20% (α-cyano-3-phenoxy benzyl 2,2,3,3,-tetramethyl cyclo propane carboxy late).
2. Fenvalerate 20% (cyano (3-phenoxy phenyl) methyl 4-chloroalpha-(1-methyl-ethyl) benzeneacetate.).

Toxicity test procedures

DIRECT EFFECT ON ADULT FEMALES

Adult females of *T. athiasae* were sprayed with different concentrations from every material using a glass atomizer. Females were confined on the lower surfaces of detached raspberry clean leaves (4 cm in dia.) while the upper surfaces were placed on cotton saturated with water. Aring of Tangle foot glue was applied to the cut edges to prevent escape of the mites Each test contained 5 concentrations and each concentration had 4 replicates (20 females/replicate) and each assay was repeated twice. In every test, a water control was included. Mortality was recorded 48 h after application. Corrected mortality counts according to abbott's formula (1925), and were statistically analysed by (Finney, 1952).

SECONDARY EFFECT ON ADULT FEMALES:

The effect of a concentration equivalent to the LC_{50} of the prey *T. urticae* of each pesticide on the reproduction and consumption of the predacious mite was also studied. Newly moulted and mated females of *T. athiasae* were sprayed with different concentrations (1.237, 1.879, 2.483, 7.067 p.p.m. for tedifol, fenpropathrin, fenvalerate and propargit, respectively; Amer, 1992).

Table 1

Toxicity of different pesticides to females of *T. athiasae*

Pesticides	LC_{50} p.p.m	LC_{90} p.p.m	Slope	Toxicity index at		N. of folds compared with Tedifol at	
				LC_{50}	LC_{90}	LC_{50}	LC_{90}
Fenpropathrin	0.403	2.505	1.61	100	100	1286.85	3525.35
Fenvalerate	2.957	27.58	1.32	13.63	9.08	175.38	320.20
Propargit	234.7	1844	1.43	0.17	0.14	2.21	4.79
Tedifol	518.6	8831	1.04	0.08	0.03	1.0	1.0

Twenty treated gravid females of *T. athiasae* on clean discs were provided daily with a sufficient known number of protonymphs of *T. urticae* for 10 days. A controll treatment was included in the experiment. Observation were taken daily on consumption, reproduction, mortality and sex-ratio of the progeny for 10 successive days.

All the experiment reported herein were carried out in the laboratory at 27 ± 2 °C and 70–75% R.H. Calculation and classification of the adverse effect of the different pesticides on *T. athiasae* followed (Overmeer and Van Zon, 1982; Hassan, 1985), while the percentage of reduction in food consumption was calculated according to (Samsoe-Petersen, 1983).

Results and Discussion

The data obtained in Table 1 shows fenpropathrin was the most toxic material to adult females of *T. athiasae* while tedifol was the least. Based on the LC_{50} level of the tested pesticides toxicity were in an ascending order as follows: tedifol, propargit, fenvalerate and fenpropathrin. Amer (1992) indicated that tedifol was the most efficient pesticide on eggs females of the pest *T. urticae* followed by fenpropathrin and fenvalerate. In a field experiment, Mansour et al. (1993) demonstrated that pesticides such as azinphos-methyl 25%, diflubenzuron 25%; thuringiensin 1.5%, teflubenzuron 15% and propargit 30% were less toxic to *T. athiasae* than triflumuron 25% and azocyclotin 50%.

El-Banhawy and El-Bagoury (1985) revealed that *Phytoseius finitimus* Ribaga was more tolerable to avermectin than to fenvalerate and residues of fenvalerate showed a longer activity on *P. finitimus*. In addition, fenvalerate was moderately toxic to adult females of *Euseius scutalis* Athias-Henriot while the least toxic was dicofol (Abou-Awad and El-Banhawy, 1985). Fenvalerate have also been shown to be toxic to other predacious mites, e.g., *Amblyseius fallacis* (Garman), *Typhlodromus occidentalis* Nesbitt and *Typhlodromus pyri* Scheuten (Rock, 1979; Wong and Chapman, 1979).

Tedifol was less toxic to the tested predacious mite. Reports have also shown that dicofol was safe to *Phytoseiulus persimilis* A. H. (Smith et al., 1963; Herne and Chant,

Table 2

Total and daily prey consumption of *T. athiasae* sprayed with different concentrations and fed on protonymphs of *T. urticae* formerly kept on untreated substrates

Pesticides	Total No. of prey consumed/female/10 days	Total. No. of prey consumed/Female/day	% Reduction in food consumption
Fenpropathrin	7.8	0.78**	96.04
Fenvalerate	78.5	7.85**	60.17
Propargit	105.05	10.505**	46.70
Tedifol	120.6	12.06**	38.81
Control	197.1	19.71	

L.S.D. at
0.05 = 0.887
0.01 = 1.185
** Highly significant

Table 3

Fecundity, sex ratio and mortality of the predacious mite *T. athiasae* sprayed with different concentrations and fed of protonymphs of *T. urticae* formerly kept on untreated substrates

Pesticides	Total No. of eggs/female/10 days	Total No. of eggs/female/day	% M. female after 10 days	% adverse effect	% Hatching of eggs	Sex ratio male:female
Fenpropathrin	1.45	0.145**	95	99.71	33.33	1 : 0
Fenvalerate	12.9	1.29**	15	55.96	54.55	1 : 1.2
Propargit	19.9	1.99*	10	28.0	90.4	1 : 1.6
Tedifol	23.1	2.31	0	7.0	100	1 : 1.6
Control	24.9	2.49	0	-	100	1 : 1

L.S.D. at
0.05 = 0.375
0.01 = 0.500
* Significant
** Highly significant

1965), *Amblyseius hibisci* Chant (Bartlett, 1964; Jeppson et al., 1975); *Amblyseius brazilii* El-Banhawy (El-Banhawy, 1976); *P. finitimus* (Amer et al., 1993).

When females of *T. athiasae* sprayed with concentration equivalent to LC_{50} of *T. urticae*, the number of prey consumed by females was significantly lower in all pesticides used (Table 2). The percentage reduction in food comsumption of fenpropathrin, fenvalerate and propargit caused a significant reduction in the consumed *T. urticae* for

(more than 40%), the percentage decreased to 38.81% in case of tedifol. The data obtained in Table 3 show that fenpropathrin appeared to be harmful for *T. athiasae*, which females suffered high mortality up to 95%, whereas tedifol proved to be harmless for the predator.

Reproduction decreased, particulary in the case of fenpropathrin and fenvalerate. Jones and Parrella (1983) found that the synthetic pyrethroid permethrin prevented the increase *Euseius stipulatus* (Athias-Henriot) for 83 days, also interrupted oviposition and decreased reproduction in *E. scutalis* were recorded in case of residues of synthetic pyrethroids (Abou-Awad and El-Banhawy, 1985). It is possible that contacting synthetic pyrethroids stopped or decreased reproduction.

The sex-ratio of the progeny of females gave rise to ratio (1) male to (0) female in case of fenpropathrin. This ratio was changed to (1.6) females and (1) male in condition of tedifol and propargit (Table 3).

Further investigations on field tests with sublethal concentrations and selective strategies to fully evaluate the potential of different pesticides as selective pesticide.

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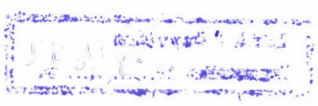

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