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Is taxonomic accuracy important, or can it be given up? How to identify lacewings?

Fontos-e a taxonómiai pontosság vagy fel is adható? Hogyan határozzuk a fátyolkákat?

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Abstract: The common green lacewing, *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) is the best known, most common, most studied, and most widely used lacewing species. It has long been the focus of attention because it is a suitable candidate for IPM programs: it is found worldwide, its larvae are polyphagous predators that are effective natural enemies of pests of essential crops, it is easy and inexpensive to rear, and populations resistant to pesticides have been found. However, its study and use may be hampered by difficulties in its identification. The reason that it has previously been shown to be a complex of sister species, not a single species. These remarkably similar sister species are complicated to distinguish from each other. In the past, taxonomic difficulties have been compounded by naming problems, and the past still haunts us. According to our current knowledge, the following sibling species occur in Hungary: *Chrysoperla carnea*, *Chrysoperla pallida*, *Chrysoperla lucasina*, and *Chrysoperla agilis*. However, due to the difficulties of identification, errors may still occur in publications, and taxonomic accuracy is often sacrificed in favour of referring exclusively to the complex, even though the ecological requirements of the individual species differ, which may determine their use. The author reviews the practice of species complex separation in Hungary and Europe, and its consequences, with particular regard to the methods and knowledge that can help provide accurate information and guidance on the determination, naming, and occurrence of taxa.

Keywords: *taxonomy, Chrysoperla carnea complex, Europe, Hungary, inconsistency*

Összefoglalás: A közönséges fátyolka *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) a legismertebb, a leggyakoribb, a legjobban tanulmányozott, és a leginkább alkalmazott fátyolkafaj. Régóta áll a figyelem középpontjában, mert alkalmas jelöltje az IPM programoknak: Világszerte megtalálható, lárvái polifág ragadozók, amelyek fontos termesztett növényeink károsítóinak hatékony természetes ellenségei, könnyen és olcsón tenyészthető, növényvédő szereknek ellenálló népességeit találták meg. Tanulmányozását és felhasználást azonban keresztezhetik határozásának nehézségei. Az ok az, hogy korábban kiderült, nem egy fajról van szó, hanem testvérfajok komplexéről. Ezek a rendkívül hasonló testvérfajok nehezen különíthetők el. A múltban a taxonómiai nehézségekhez elnevezési gondok is társultak, s a múlt még kísért. Eddigi ismereteink szerint hazánkban a következő testvérfajok fordulnak elő: a *Chrysoperla carnea*, a *Chrysoperla pallida*, a *Chrysoperla lucasina* és a *Chrysoperla agilis*. A határozás nehézségei miatt azonban még a jelenlegi publikációkban is előfordulhatnak tévedések, illetve a szerzők gyakran feladják a taxonómiai pontosságot és csak a komplexet nevezik meg, noha az egyes fajok ökológiai igényei különböznek, ami megszabja a felhasználhatóságukat. A szerző áttekinti a fajkomplex elkülönítésének magyarországi és európai

gyakorlatát, s ennek bizonyos következményeit különös tekintettel arra, hogy milyen módszerek és ismeretek segíthetik a korrekt tájékozódást és tájékoztatást a taxon határozását, elnevezését és előfordulását illetően.

***Kulcsszavak**: taxonómia, Chrysoperla carnea komplex, Európa, Magyarország, ellentmondás*

1 Introduction

Taxonomic accuracy is fundamental to all work involving living organisms. I assume this also applies to plant protection and requires no further explanation. For the sake of formality, I refer to two relevant publications: Van Driesche and Bellows (1996) and Kaplan (2012). This communication concerns lacewings (Neuroptera: Chrysopidae), more specifically the *Chrysoperla carnea* species complex. Its importance as a biological agent has long been extensively researched and recognised in many cases (Principi and Canard, 1984; Ridgway et al., 1980; Hagen and Tassan, 1980; McEwen et al., 1999; Grafton-Cardwell and Hoy, 1985; Tauber et al., 2000). Nevertheless, the above is not always clear. The reason for this, is the separation and naming of the complex as an entity and the individual species that make it up. Recently, this phenomenon has led to confusion, misunderstandings, and difficulties at the user level and among specialists including neuroropterists (Henry et al., 2001). A detailed report on this has also been published in Hungarian (Bozsik, Canard and Thierry, 2014). The taxonomic situation has theoretically stabilised somewhat with the publication of Price et al. (2015), which proved that the original taxon, *Chrysoperla carnea* (Stephens, 1836), is identical to the most common sister species of the same name, which had been the subject of protracted debate. Thus, many problems arose internationally, but the domestic situation regarding the common lacewing was even more complicated (Bozsik, Canard and Thierry, 2014).

The difficulties currently arise from the identification method and the degree of precision required to define the *Ch. carnea* complex at the sibling species level. To a lesser extent, there are also naming anomalies, but I do not wish to discuss these here. The following brief discussion highlights the domestic contradictions that arise from what is presumably an inaccurate identification.

2 The current situation in Europe

Practical species separation of the *Ch. carnea* complex has been remained difficult. There are three primary methods for determining sister species. 1. Recording and analysing the acoustic mating songs of living individuals (Henry et al., 2002). 2. Examining the genetic patterns of living or dead individuals (Price et al., 2015). 3. Traditional morphological separation of living or dead individuals (Mazel et al., 2006). There is also a fourth possibility that expresses each sibling species' tolerance to cholinesterase inhibitors. Still, it has not been developed in detail (Bozsik and Haubruge, 1998, unpublished). According to the literature mentioned above, the first method is the most reliable, followed by the second, and then the third is a distant third. Each has its contradictions and difficulties. Only live lacewings of different sexes can be used for the first method, as well as Charles Henry's laboratory and equipment. Since copulation usually occurs at night or in the early morning hours, continuous video surveillance is essential. According to the author’s data collection, no one in Europe can apply this method as described. Lacewings of some European researchers are determined by Ch. Henry's laboratory in the USA.

The second method requires a biotechnology laboratory and expertise. According to some neuropterists, this is not really suitable for distinguishing between sibling species (P. Duelli, R.A. Pantaleoni, pers. com., 2025). It is certainly not ideal for everyday mass identification. The third, traditional method, based on morphology and genitalia structure, is the most common. However, many are unfamiliar with the subtle species characteristics, which, according to some researchers (Henry et al., 2002; P. Duelli, pers. com., 2025), can only be applied to taxa widespread in a given area, and identification requires extensive practice (Henry et al., 2002; P. Duelli, pers. com., 2025). What makes the situation even more confusing is that not all specialists are aware of the distinctions between sister species and do not use the already somewhat recognised names correctly. One frequently used excuse is that sibling species are not distinguished, even though their ecological requirements differ, but instead the complex name *Ch. carnea* sensu lato is used (Koczor et al., 2010; Berteloot et al., 2024).

3 The situation in Hungary

Hungary's geographical location, topography, climate, and the effects of global warming influence the occurrence of lacewing species. The appearance of alien species that did not previously live here is becoming increasingly common (Bozsik, 2010, 2011, 2012). It is understandable that a nomadic Mediterranean sister species, *Chrysoperla agilis* (Henry et al., 2003), can also be found in Hungary. This species had a permanent population in central Alaska (Henry et al., 2011) and occurred in Switzerland (Henry et al., 2003).

Apart from scarce species, the following sister species occur more frequently in Hungary (Bozsik, 2024, unpublished):

1. *Chrysoperla carnea* (Stephens, 1836).

2. *Chrysoperla pallida* (Henry et al. 2002).

3. *Chrysoperla lucasina* (Lacroix, 1912).

4. *Chrysoperla agilis* (Henry et al., 2003).

The separation of the species mentioned above is based on the descriptions of Henry et al. (2002, 2003), Mazel et al. (2006), comparative specimens (courtesy of D. Thierry and P. Duelli), and communication with neuropterist colleagues (P. Duelli (2014, 2025), D. Thierry (2000-2024), D. Devetak (2025), R. A. Pantaleoni (2025), A. Letardi (2025), and my own decades of experience. The most essential morphological characteristics are formerly described in Hungarian in the report by Bozsik, Canard and Thierry (2014).

These species are very similar. The author is not aware of any new, clear keys for traditional morphological differentiation. So, most experts make their determinations based on scientific articles, comparative specimens, and extensive practice. Some colleagues, such as P. Duelli and R. A. Pantaleoni (pers. com., 2025), verify the correct taxon based on the double, movement-generated, visually perceptible mating song of live specimens. Obviously, this is only possible with living individuals. Based on the author's own experience, even the most clearly identifiable species, *Ch. lucasina*, has been found to exhibit morphological differentiation (Tóth et al. 2009; Bozsik, Canard and Thierry, 2014). It is worth noting again that morphological traits may vary across geographical areas due to the natural variability of lacewing species (P. Duelli, pers. com., 2025; R.A. Pantaleoni, pers. com., 2025). In any case, knowledge of the lacewing species living in different geographical areas can facilitate this work. As the recent literature on chrysopids is not very extensive (Table 1), the author consulted European colleagues (A. Letardi, R.A. Pantaleoni, P. Duelli, A. Gruppe, R. Dobosz, D. Devetak, U. Hiermann and L. Dvořák). Unfortunately, several colleagues could not be reached.

Table 1 Occurrence of some species of *Chrysoperla carnea* complex in Europe

Country	*Ch. carnea*	*Ch. pallida*	*Ch. lucasina*	*Ch. agilis*	References
Spain	x	x	x	x	Bozsik, González Ruiz, 2006
France	x	x	x	x	Mazel et al., 2006
Germany	x	x	x	-	Gruppe et al., 2004
Italy	x	x	x	x	Letardi, 2025
Switzerland	x	x	x	x	Duelli, p.c.[1], 2025, Henry et al., 2002
Poland	x	x	x	-	Dobosz, p.c., 2025
Bohemia and Slovakia	x	?	x	?	Jedlička, et al., 2004
Austria	x	x	x	?	Hiermann et al., 2024
Romania	x	x	x	?	Paulian, 2002
Serbia	x	x	x	x	Devetak et al., 2023
Croatia	x	x	x	x	Devetak et al., 2019ab
Slovenia	x	x	x	x	Deverak, 2025b
Albania	x	x	x	x	Devetak, 2025a
Hungary	x	x	x	x	Bozsik, u.,[2] 2024

[1] p.c. : personal communication, [2] u. : unpublished

Presumably due to the extraordinary similarity or the lack of skills and conditions necessary for correct identification, the species *Ch. agilis* has not yet been confirmed in the Hungarian fauna by anyone other than the author. The relevant domestic and international literature is exceptionally scarce, and the species is not mentioned in the most recent publication (Koczor et al., 2025). However, based on other research, *Ch. agilis* was already present in the domestic fauna in 2018. *Ch. agilis* specimens were already found as early as 2005 (Bozsik, unpublished), and they were verified using specimens sent by P. Duelli and D. Thierry. Funnel traps, used in the article of Koczor et al. (2025), caught 250 *Ch. carnea* complex specimens over a period of about 2.5 months, among which there were only *Ch. carnea*, *Ch lucasina*, and *Ch. pallida* specimens. The authors used the descriptions of Henry et al. (1996, 2002) and Thierry et al. (1998) to define the complex species, but did not mention any comparative specimens, and, to the author's knowledge, no reports on lacewings from other areas have been published. It is striking that the authors identified each individual at the species level, which is interesting because Bozsik has previously identified chrysopids caught in such traps. Still, due to the injuries they sustained (16.8% of all individuals were injured) and the variability in their morphological features, it was not possible to identify all of them. The partial species list of this identification can be found in the article by Tóth et al. (2006). It is possible that among the 197 individuals named as *Ch. carnea* in the article by Koczor et al. (2025), some were *Ch. agilis* individuals, or that several captured individuals were omitted from the publication. It is problematic that, to the author's knowledge, no other identification procedure mentioned above except the morphological method, has yet been implemented in Hungary, as is the case in most European countries. This example, as well as the probable error that occurred in 2009 (Tóth et al., 2009), shows how complex this task is and that much greater care must be taken if you insist on taxonomic accuracy.

4 Recommendations for improving the situation in Hungary

Ch. carnea, *Ch. pallida*, *Ch. lucasina*, and *Ch. agilis* are definitely present in Hungary (Bozsik, 2008, 2024 unpublished), and depending on their natural populations and habitats, they can play a significant role in controlling soft-cuticle pests. However, their usefulness for specific species and their targeted applicability (for use in greenhouses, plantations, or arable crops) requires further study which may need either a new method suitable for mass identification or, traditional identification, highly skilled specialists and a guaranteed comparative series of specimens. The author offers his assistance to the authors of the article by Koczor et al. (2025) or to anyone and is happy to verify their identities.

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Response of field crop species to abiotic stress impacts during early development

Szántóföldi növények fajspecifikus abiotikus stressz-reakciója a kezdeti fejlődés során

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Abstract: Field crops are exposed to various abiotic stresses at all crop sites. The use of adaptable species may reduce the risk of production. In an *in vitro* laboratory trial at the Agronomy Institute of the Hungarian University of Agriculture and Life Sciences, Gödöllő, field bean *Phaseolus vulgaris* L. and maize *Zea mays* L seeds were exposed to various abiotic stress conditions (water supply representing normal and flooded state, temperature with optimal and high exposures, and increasing salinity conditions). Seed samples of both species were tested for viability. The results of the trial suggest that the various abiotic applications had altering effects on the germination performance of seed samples. Bean seeds proved to be more vulnerable in comparison with maize.

***Keywords**: bean, maize, water stress, temperature stress, salinity stress, viability*

Összefoglalás: A szántóföldi növények minden termőhelyen számos abiotikus stressznek vannak kitéve. A termesztés kockázatát az alkalmazkodó fajok használata csökkentheti. A MATE Agronómiai Intézetében Gödöllőn *in vitro* laboratóriumi kísérletben bab *Phaseolus vulgaris* L és kukorica *Zea mays* L magvak csírázását vizsgálták különféle abiotikus stressz körülmények között (vízellátás normál és elárasztott állapot, optimális és magas hőmérséklet, valamint növekvő sótartalom). A kísérletek eredményei arra engednek következtetni, hogy a különböző abiotikus körülmények hatással voltak a magvak csírázására. A bab magvak érzékenyebbnek bizonyultak a kukoricához képest.

***Kulcsszavak:** bab, kukorica, víz-stressz, hőmérséklet-stressz, só-stressz, csírázás*

1 Introduction

Germination of seeds is a physiological process which is influenced by biotic and abiotic factors including water, oxygen, and temperature for a successful germination process. A combination of conducive environmental factors and various cellular processes will allow physiological and morphological changes within the seed which will result in activation of the embryo. Germination starts when the embryo and water (seed imbibition), resulting expansion of the seed and elongation of the embryo, and ends when the radicle has grown out of the seeds coating layers and with the protrusion of the coleoptile (Miransari & Smith, 2014).

Various impacts that may influence the regular physiological processes of live organisms are manifested in stress conditions. Selye (1974; 1983) determined four variations of stress. On one hand he identified good stress (eustress) and bad stress (distress). Regarding the magnitude both may be over-stress (hyperstress) or understress (hypostress). The optimum would be to balance hyperstress and hypostress perfectly and have as much eustress as possible. Viability of plant seeds and the development of germinated plants require certain environmental conditions. The most fundamental factors of these processes are the optimal presence of moisture, temperature, and the availability of oxygen. Any of them whenever missing or the presence of that is out of the optimum range may induce stress impacts on the germination and sprouting processes.

Temperature elevation can cause thermoinhibition on seeds germination. The germination rate decreases for seeds cultivated at a higher temperature. The high temperature also affects the root development of the seedlings (Iloh, et al., 2014).

Soil salinity level may create soil conditions with physiological drought, simultaneously may causes ion toxicity, nutrient imbalance and oxidative stress to the seed (Nayidu, et al., 2012). Kaymakanova (2009) reported that high salinity in soil solutions will result in high osmotic pressure that restricts the seed imbibition by preventing water absorption into the seed. Salt stress caused by NaCl may also decrease the content of essential hormones for germination, such as gibberellins (Atia, et al., 2009).

Water is essential during germination for the seed imbibition process thus, no germination will occur in the absence of water. Nonetheless, excess water will cause waterlogged soil conditions, which will deprive the seeds from oxygen supply. Permanent flood during germination stage resulted by extreme weather conditions, for example torrential rain, or planting in an area with high water table, can threaten crops with low water-logged tolerance by restricting the respiratory activity essential for germination (Zhou, et al., 2020).

It is common for one agricultural land to handle more than one abiotic stress at the same time. Water and temperature interactions often determine yield quantity and quality performance (Jolánkai et al. 2018). A combination of two or more abiotic stress will aggravate the impact of each stress and farmers may need to spend more input to remove or lessen the impacts. High temperature simultaneously with salt stress is a common condition, especially in semiarid and arid regions in the world (Shahid, et al., 2018). The combined stresses may reduce the photosynthetic rate in the same crop due to a decline in pigmentation (Neelambari., et al., 2018).

The aim of the present experiments was to determine the impact of some abiotic factors on seed germination of two crops. Bean *Phaseolus vulgaris* L as a dicotyledonous and maize *Zea mays* L a monocotyledonous plants were studied to determine the impact of water availability, temperature variations and salinity on the viability of seeds.

2 Materials and Methods

The present trial was conducted in the laboratory of MATE Agronomy Institute in Gödöllő, Hungary within an experimental series related to seed viability (Huynh Anh et al, 2024). The objective of the trial was studying the effect of abiotic stresses – among them temperature, salinity and waterlogging – on seed germination. Transparent Petri dishes; micro pipette; filter paper; distilled water; bean and maize seeds; Memmer-type climatic chamber; precision scale were used. Field bean *Phaseolus vulgaris* L. and maize *Zea mays* L seeds were exposed to various abiotic stress conditions (water supply representing normal and flooded state, temperature with optimal and high exposures, and increasing salinity conditions). The methods and the description of the trial were by general laboratory standards. The applications were as follows:

Temperature treatment. We carried out a study on the effect of three levels of temperature (T20; T25; and T30) on the germination of seeds as follows: 20°C, 25°C and 30°C respectively.

Salinity treatment. Two levels of NaCl concentrations on seeds germination (1000 ppm) and (1500 ppm) compared to control seeds (0 ppm) as follows: S0: control seeds. S1: seeds tested to germinate under 1000 ppm of salt. S2: seeds tested to germinate under 1500 ppm of salt.

Water treatment. In this trial, we tried to test three amounts of water in each seed using Petri dishes. The treatments were as follows: W0: seeds soaked in 6 ml of water, W1: seeds soaked in 9 ml of water, W2: seeds soaked in 12 ml of water.

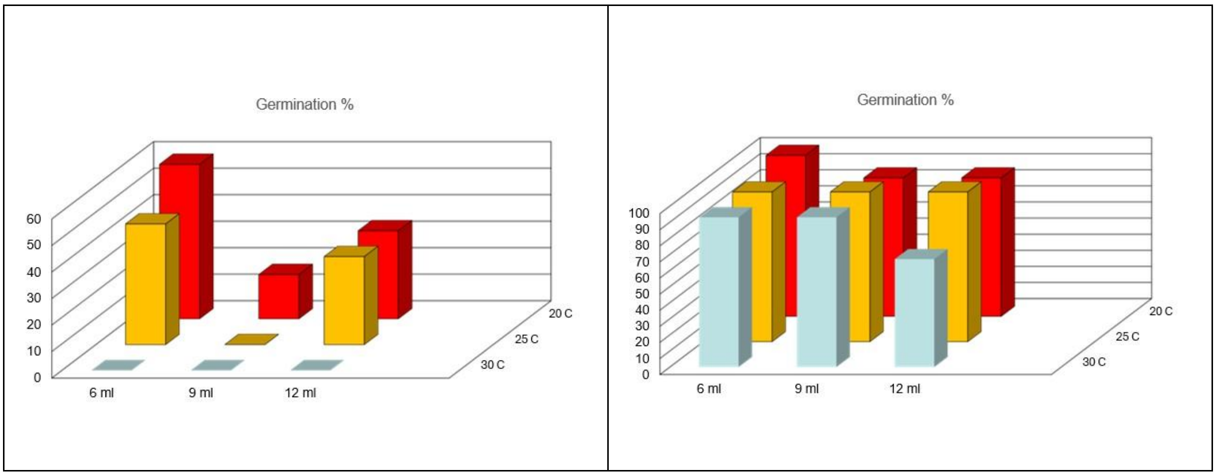

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Figure 1 *Bean (a) and maize (b) germination performance, water supply x temperature assessment*

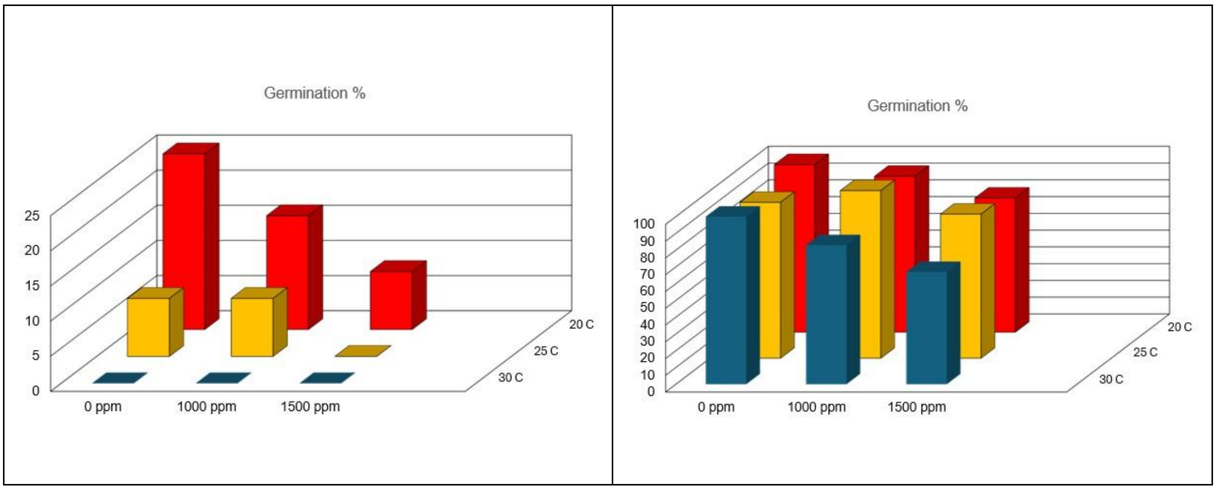

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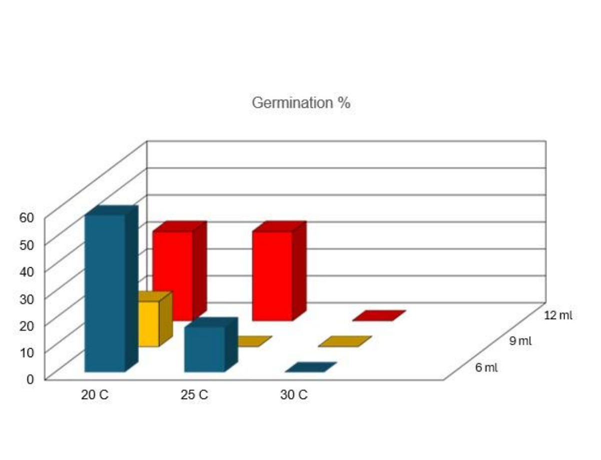
Figure 2 *Bean (a) and maize (b) germination performance, salinity x temperature assessment*



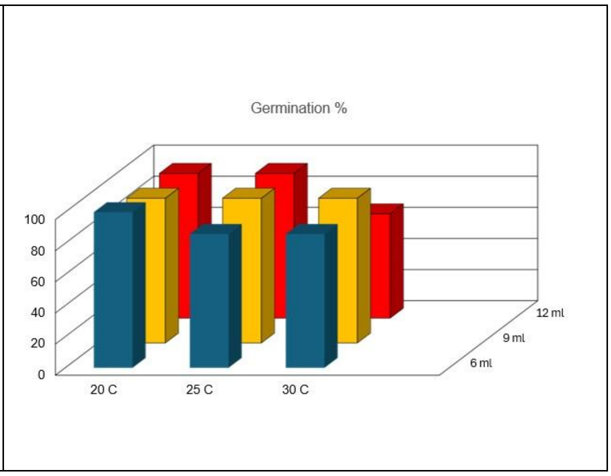


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Figure 3 *Bean (a) and maize (b) germination performance, temperature x water supply assessment*

3 Results and Discussion

3.1 Bean trial

Water supply had a consequent impact on seed germination. The smallest amount (6 ml) resulted in the highest values while bigger doses (9 and 12 mm) flooded seeds and reduced germination (Fig. 1a).

Salinity had strong impact on germination. The higher the salt concentration (1000 and 1500 ppm), the poorer the germination was observed (Fig. 2a).

The most definite impact was detected in the case of temperature. At 30 ºC there was no germination, the best values were obtained in the case of 20 ºC (Fig. 3a).

The ascending values of all treatments resulted in the decrement of the magnitude of all the observed parameters. From among them temperature proved to be significant, while water supply and salinity had detectable but not significant trends.

3.2 Maize trial

Maize germination was affected by the amount of water. The highest germinations were observed in the smaller amount treatments, while flooded seeds produced poorer germination (Fig. 1b).

Salt concentration had definite impact on germination. The 0 ppm control resulted the highest germination figures, while increased salt concentration reduced the viability of maize seeds (Fig. 2b).

Temperature was the least influencing factor affecting maize seed viability. At 30 ºC poorer germination was recorded, while 20 and 25 ºC applications were almost similar in all treatments (Fig. 3b).

The higher levels of all treatments resulted in poorer viability values. However, the strongest significant decrement was detected in the case of salinity.

Table 1 Regression of various stress factors regarding seed viability in case of bean and maize seed germination

Treatment	equation	R^2
Bean		
water	y = - 11x + 46	0,4286
salinity	y = - X + 11,333	0,3324
temperature	y = - 18x + 56,667	0,9982
Maize		
water	y = - 6,5 x + 104,67	0,5841
salinity	y = - 10x + 109,33	0,9494
temperature	y = - 4x + 97	0,5414

4 Conclusions

The aim of the experiments was to determine the impact of some abiotic factors on seed germination of two crops. Bean *Phaseolus vulgaris* L as a dicotyledonous species proved to be more vulnerable than maize *Zea mays* L a monocotyledonous plant regarding seed viability.

The ascending levels of all treatments – water supply, salinity and temperature – were in negative correlation with germination. In case of bean crop temperature proved to be the strongest stress factor resulting no germination at 30 °C. Maize crop was less affected by abiotic stress factors, however higher salinity resulted in the decrement of viability (Table 1).

In the experiment various interactions were observed. The higher levels of all applications resulted in poorer viability values however their interactions suggest that the combination of any high stress treatment with lower applications of other factors could reduce the magnitude of the harmful effects.

Acknowledgements

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Seedborne fungal pathogens associated with *Panicum miliaceum* and *Echinochloa crus-galli* in Hungary

Két egyszikű gyom magfertőző gombabetegségei Magyarországon

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Abstract: The purpose of the study was to investigate the fungal seed diseases affecting two major monocotyledonous weed species, *Panicum miliaceum* L. and *Echinochloa crus-galli* L., in Hungary. Seeds of *Panicum miliaceum* L. were collected from Keszthely in 1985 and 2007, while the seeds of *Echinochloa crus-galli* were gathered from Keszthely and Szombathely regions in 2010. The experiment was set up in two replications with 50 seeds each, resulting in a total of 100 seeds incubated and evaluated per sample. Seeds were arranged in Petri dishes on moistened filter paper, then covered, and incubated in an incubator at 24 ºC for 14 days. Visual scans for early infections were conducted on day 7 and thereafter on day 14. Infected seeds underwent both visual and microscopic examinations to identify fungal pathogens. The microscopic analysis revealed that most isolates were consistent with ascomycetous fungi. The most abundant genera identified were *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Based on the analysis of the results, out of the 400 seeds examined, *Fusarium* was the most dominant with 44.25%, followed by *Alternaria* (21.25%) and *Aspergillus* (16.5%), respectively. *E. crus-galli* showed a higher susceptibility to *Fusarium, Alternaria,* and *Aspergillus* compared to *P. miliaceum*. Given that these monocot weeds are prevalent and serve as reservoirs for plant pathogens, causing considerable yield losses in Hungarian agricultural fields, strengthening the holistic integrated pest management (IPM) strategies is crucial. Furthermore, transitioning towards the use of non-chemical control measures for these fungal groups is recommended to reduce the damage to the environment and overreliance on fungicides.

Keywords: *seed-borne fungi; Panicum miliaceum; Echinochloa crus-galli; Fusarium; Alternaria*

Összefoglalás: A vizsgálat célja két hazai jelentős egyszikű gyomfaj, a termesztett köles gyomosító változata (*Panicum miliaceum* L.) és a kakaslábfű (*Echinochloa crus-galli* L.) magjait fertőző gombabetegségek vizsgálata volt laboratóriumi vizsgálatokban. A *Panicum miliaceum* L. magjait Keszthely térségéből 1985-ben és 2007-ben, míg az *Echinochloa crus-galli* L. magjait Keszthely és Szombathely térségéből 2010-ben gyűjtöttük. A kísérletet két ismétlésben, ismétlésenként 50 mag felhasználásával végeztük, így mintánként összesen 100 mag csíráztatására és magkórtani értékelésére került sor. A magokat nedvesített szűrőpapírt tartalmazó Petri-csészékbe helyeztük, lefedtük, majd 24 °C hőmérsékleten, termosztátban 14 napon keresztül inkubáltuk. A külső kontamináció minimalizálása érdekében az inkubálást megelőzően a magmintákat Neomagnol oldattal felületileg fertőtlenítettük. A korai fertőzések kimutatására a beállítást követő 7. napon értékeltünk, majd ezt követően a 14. napon vizuális megfigyeléseket végeztünk. A fertőzött magokat vizuális és mikroszkópos vizsgálatnak vetettük alá a gombanemzetségek azonosítása céljából. A mikroszkópos vizsgálatok eredményei alapján az azonosított gombák többsége az Ascomycetes osztályba tartozott. A leggyakrabban előforduló gombanemzetségek a *Fusarium* spp., az *Alternaria* spp. és az *Aspergillus* spp. voltak. A 400 vizsgált mag közül a *Fusarium* spp. bizonyult a legdominánsabbnak 44,25%-os előfordulási aránnyal, ezt követte az *Alternaria* spp. (21,25%) és az *Aspergillus* spp. (16,5%). Az *Echinochloa crus-galli* magjai a Fusarium, Alternaria és Aspergillus nemzetségekhez tartozó gombákkal szemben nagyobb mértékű fogékonyságot mutattak, mint a *Panicum miliaceum*. Tekintettel arra, hogy ezek az egyszikű gyomfajok széles körben elterjedtek, és kórokozók rezervoárjaként szolgálhatnak, amelyek jelentős mezőgazdasági károkat okoznak a magyarországi termőterületeken, az integrált növényvédelmi (IPM) stratégiák átfogó megerősítése kiemelt jelentőségű. Emellett a vizsgált gombacsoportok elleni nem kémiai védekezési módszerek alkalmazása is javasolt a környezeti terhelés csökkentése és a fungicidektől való túlzott függőség mérséklése érdekében.

***Kulcsszavak**: magfertőző gombák; Panicum miliaceum; Echinochloa crus-galli; Fusarium; Alternaria*

1 Introduction

Weeds are considered a relevant part of agricultural systems and production, competing with the cultivated crops for essential resources such as light, nutrients, water, and space, resulting in significant decreases in crop yields by up to 32%. In some cases where no weed control measures are implemented, these losses can rise to as high as 72% (Ņečajeva et al., 2023). Weeds can play a significant role as hosts and reservoirs of plant pathogens and vectors, and they can be alternative hosts for some economically important pathogens. Additionally, parasitic weeds may be direct vectors for some plant pathogens, including viruses, bacteria, and fungi, leading to disease development in cultivated crops (Suproniene et al., 2019).

Monocot weeds pose a greater challenge than broad-leaved (dicot) weeds because they occupy comparable ecological niches to cereal vegetation. Cereal crops such as wheat, maize, and rice are among the most widely produced crops worldwide. The presence of monocot weeds in agricultural fields poses a risk to cereal crops directly or indirectly. Direct risk is through their rapid growth and prolific seed production, leading to dense populations and out-competing of the cereal crops, and indirectly mounting disease pressure since the presence of fungal pathogens on weeds can potentially infect the cereal crops as well. Fungal pathogens have been observed to survive in the seeds of many monocots worldwide. Some of the most common phytopathogenic fungal genera, such as *Ustilago* spp., *Fusarium* spp., *Aspergillus* spp., *Alternaria* spp., and *Colletotrichum* spp. (seedborne pathogens) have already been detected in seeds of monocot weeds in several regions of the world (Ņečajeva et al., 2023).

Proso millet (*P. miliaceum*) is a widely distributed weed species that causes economic damage in cereal-growing regions worldwide. Similarly, barnyard grass (*E. crus-galli* L.) is a widespread weed responsible for significant losses in various crops, including rice and maize. Since the European Union (EU), with Hungary being a member, is a significant cereal producer on a global scale, the two weed species are among the most important monocot weeds. *P. miliaceum* L. and *E. crus-galli* L. stand out as major monocotyledonous weeds prevalent in Hungary according to the Fifth National Weed Survey on Arable Fields in Hungary (Novák et al., 2009).

Fungal pathogens that infect the seeds and spread by seeds pose a serious threat to agricultural production, especially in *P. miliaceum* L. and *E. crus-galli* L., which are the most prevalent monocot weeds in Hungary, producing enormous seeds as well (Novák et al., 2009). Despite their importance as weed species common in agricultural fields, as reported by previous scientists, there is a lack of comprehensive knowledge on the fungal pathogens infecting the seeds of the two monocot weeds in Hungary. Addressing this gap is crucial for developing effective disease management and sustainable weed control strategies.

Fungal seed-borne diseases reduce crop yields and quality and increase production costs (Nganje et al., 2004). Fungal pathogens affecting the seeds of *P. miliaceum* L. and *E. crus-galli* L. can potentially spread to cultivated crops. Therefore, understanding the seed-borne diseases in these two monocot weeds will help to reduce the spread of the disease and mitigate the associated economic losses. Additionally, fungal pathogens are becoming a growing concern not only for crop productivity but also for food safety. Some fungal pathogens associated with seeds, in most cases, have been recorded to produce mycotoxins, therefore becoming a major concern to food safety because they pose a risk to humans and livestock if ingested, as reported by Ferrigo *et al*. (2016) and Munkvold (2003). In this experiment, fungal pathogens present in the seeds of *P. miliaceum* L. and *E. crus-galli* L. were investigated. The findings aim to assist Hungarian farmers, government agencies, and other stakeholders in designing a holistic approach to fungal disease management to mitigate economic losses and reduce the use of fungicides to an acceptable level, as recommended in the "Farm to Fork Strategy" EU agricultural policy.

2 Materials and Methods

2.1 Experimental site

The experiment was carried out at the Hungarian University of Agriculture and Life Sciences, Georgikon Campus, at the Festetics Imre Bioinnovation Centre (Seed Pathology Laboratory) between January and March 2024.

2.2 Research materials.

Seeds of *P. miliaceum* L. and *E. crus-galli* L. were collected from agricultural fields in the Keszthely and Szombathely regions of Hungary. Seeds of *P. miliaceum* L. were collected in 1985 and 2007 from Keszthely, while *E. crus-galli* L. seeds were collected in 2010 from both Keszthely and Szombathely. Additional materials included Petri dishes of diameter 12 cm; filter paper; water; Neomagnol tablets, Numigral seed counter, and Thermostat.

2.3 Experiment setup

The experiment was conducted in two repetitions with 50 seeds in each, resulting in 100 seeds germinated per sample. The seeds were thoroughly cleaned to remove foreign impurities and then counted using the Numigral seed counter. Petri dishes were prepared, each layered with a sterile filter paper moistened with approximately 15 ml of sterile distilled water to ensure germination of the weed seeds, holding of the seeds, and providing a suitable environment for fungal microorganisms.

To exclude external contamination and ensure reliable microbiological testing, the seed surfaces were disinfected using Neomagnol. For 1 liter of water, 2 tablets of Neomagnol were used, and then the samples were left in this solution for 10 minutes. After 10 minutes, the samples were rinsed twice with sterilized water and placed on the filter papers with great care, ensuring the seeds did not touch each other to avoid cross-contamination. 50 seeds of each weed species each year were arranged in a grid pattern with 5 rows having 10 seeds each within the Petri dishes, then covered as shown in Figure 1.

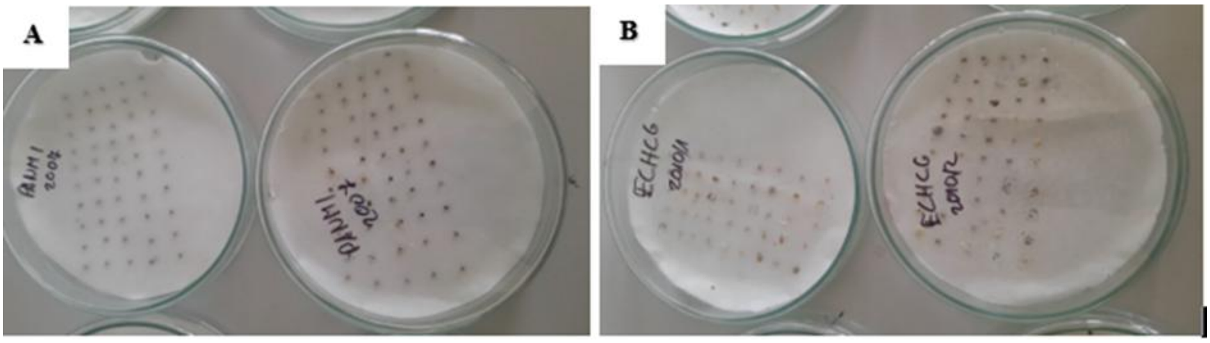

Figure 1 *(A) P. miliaceum L. seed samples on the moistened filter paper. (B) E. crus-galli L. seed samples on the moistened filter paper.*

After preparation, the samples were placed in an incubator set to 24°C (ideal for fungal germination) in sealed containers to minimise contamination. The germination lasted for 14 days. During this time, we made visual scans, and the first one was on day 7 to check for initial fungal infections. The second test was carried out on day 14, when the exact number of seeds infected with fungal microorganisms was determined and recorded. During the study, we sampled infected seed parts and prepared these samples so that we could microscopically examine them to identify the fungi as accurately as possible as shown in Figure 2. The samples in this experiment were destroyed after evaluation.

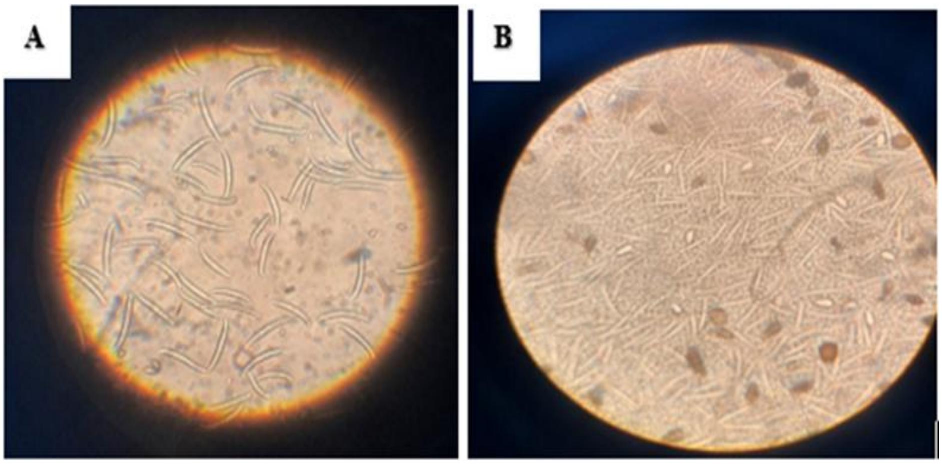

Figure 2 *(A) Sickle-shaped macroconidia of Fusarium spp. from microscopic examination. (B) Round-shaped pale brownish macroconidia of Alternaria spp. from microscopic examination (Photos by György Pásztor).*

After collecting the data from the experiment, the data were organized and analysed using Microsoft Excel 365 to obtain the average infections, trends of disease over time, percentages of infections based on the years of collection, and the locations (regions) where seeds were collected from. T-test and One-way ANOVA using the IBM SPSS Statistics software were used to analyse the comparison of fungal infections between the seeds of the two monocotyledonous weeds.

3 Results

3.1 Overall prevalence of *Panicum miliaceum L.* and *Echinochloa crus-galli L.*

In this study, a total of 400 seeds of *P. miliaceum* L. and *E. crus-galli* L. were examined. The results showed that a total of 177 seeds (44.25%) were found to be infected with *Fusarium* spp., 85 seeds (21.25%) were infected with *Alternaria* spp., 66 seeds (16.5%), were found to be infected with *Aspergillus* spp., and a total of 72 seeds (18%) were found to be healthy (Table 1).

Table 1 Prevalence of fungal infections on *P. miliaceum* L. and *E. crus-galli* L. collected from different locations and years. Each row represents one replicate of 50 seeds. Two replicates (n=100 seeds) were used per species, location, and year

Weed species	Collection year	Collection location	Total number of seeds	Fusarium Infection	Alternaria infection	Aspergillus infection
***P. miliaceum* L.**	1985	Keszthely	50	25	13	5
***P. miliaceum* L.**	1985	Keszthely	50	27	10	5
***P. miliaceum* L.**	2007	Keszthely	50	21	8	3
***P. miliaceum* L.**	2007	Keszthely	50	20	9	3
***E. crus-galli* L.**	2010	Szombathely	50	25	13	12
***E. crus-galli* L.**	2010	Szombathely	50	16	12	11
***E. crus-galli* L.**	2010	Keszthely	50	18	8	17
***E. crus-galli* L.**	2010	Keszthely	50	25	12	10
Total			400	177	85	66

Table 2 below shows the summarized frequency of fungal infections on the seeds of *P. miliaceum* L. and *E. crus-galli* L. per year of collection and overall percentages of infection of each fungal genus.

Table 2 Summarised table showing the total number of seeds and percentages of infection by Fusarium spp., Alternaria spp., and Aspergillus spp. collected from different locations and years

				Frequency of fungal infections			
Weed species	**Year**	**Location**	**Number of seeds**	**Fusarium**	**Alternaria**	**Aspergillus**	**Healthy seeds**
Panicum miliaceum	1985	Keszthely	100	52	23	10	15
Panicum miliaceum	2007	Keszthely	100	41	17	6	36
Echinochloa crus-galli	2010	Szombathely	100	41	25	26	11
Echinochloa crus-galli	2010	Keszthely	100	43	20	24	10
Total number of seeds			**400**	**177**	**85**	**66**	**72**
Percentage of infection				**44.25%**	**21.25%**	**16.50%**	**18%**

3.2 Panicum miliaceum L.

3.2.1 *Panicum miliaceum* L. seeds collected from Keszthely (1985)

After 14 days of incubation, the seeds collected in Keszthely in the year 1985 showed notable fungal infection. Among the observed fungal infections, *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. were the most ubiquitous pathogens identified by physical assessment based on the mycelial colour produced and the confirmation with microscopic analysis. In a total of 100 seeds, 52 seeds (52%) were found to be infected with *Fusarium* spp, 23 (23%) seeds were infected with *Alternaria* spp., and 10 seeds (10%) were infected with *Aspergillus* spp., as shown in Table 1.

3.2.2 *Panicum miliaceum* L. collected from Keszthely (2007)

In this case, *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. appeared to dominate the fungal microflora in these seeds. Out of the 100 seeds incubated for this experiment, 41 seeds (41%) were found to be infected with *Fusarium* spp., 17 seeds (17%) with *Alternaria* spp., and 6 seeds (6%) with *Aspergillus* spp. (Table 1).

3.3 *E. crus-galli* L. collected from Szombathely and Keszthely in the year 2010

After 14 days of incubation, seeds of *E. crus-galli* L. that were collected from Keszthely and Szombathely regions in Hungary in the year 2010 showed high levels of infection based on the visual identification, followed by microscopy revealing a distinct pattern of fungal infestation in seeds collected from these regions.

3.3.1 *Echinochloa crus-galli* L. seeds collected from Szombathely (2010)

According to the results obtained, a total of 41 seeds (41%) were found to be infected with *Fusarium* spp., 25 seeds (25%) were infected with *Alternaria* spp., and 26 with *Aspergillus* spp. Out of the 100 seeds for the year 2010, which were incubated in the two repetitions, 11 seeds (11%) were found to be healthy (Table 1).

3.3.2 *E. crus-galli* L. seeds from Keszthely (2010)

Fungal genera *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. dominated (Table 1). Out of the 100 seeds of *E. crus-galli* L. (2010) incubated, a total of 43 seeds (43%) were found to be infected with *Fusarium* spp., 20 seeds (20%) were infected with *Alternaria* spp., and 24 seeds (24%) with *Aspergillus* spp. while 10 seeds (10%) were healthy, as shown in Table 1 above.

3.4 Comparison between the weed species and locations

Seeds of *E. crus-galli* from Szombathely showed a slightly higher incidence of *Alternaria* and *Aspergillus* than those from Keszthely, while Fusarium infection levels were similar at both locations (Table 2). The seeds of *E. crus-galli* L. showed a higher overall level of fungal infection as compared to that of *P. miliaceum*, across all sampling years, with 179 seeds of *E. crus-galli* and 149 seeds of *P. miliaceum* being infected by at least one of the three fungal species. The statistical results obtained indicated that the p-value for *P. miliaceum* L. is ($p < 0.001$), meaning there were significant differences in the infection by *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Similarly, for *E. crus-galli* L., the p-value ($p < 0.007$) suggests a statistical difference in the infection by *Fusarium* spp., *Alternaria* spp., and *Aspergillus* species (Table 3).

Table 3 Comparison of fungal infections between the seeds of the two monocot weeds

Seed	Fungal Infections	n	Mean ± SE
Panicum miliaceum L.	*Fusarium* spp.	4	23.25 ± 1.65[a]
	Alternaria spp.	4	10.00 ± 1.08[b]
	Aspergillus spp.	4	4.00 ± 0.58[c]
Overall mean		12	12.42
P-Value			**<0.001**
Echinochloa crus-galli L.	*Fusarium* spp.	4	21.00 ± 2.35 [a]
	Alternaria spp.	4	11.25 ± 1.11[b]
	Aspergillus spp.	4	12.50 ± 1.56 [b]
Overall mean		12	14.92
P-Value			**0.007**

3.5 Distribution of fungal genera

The distribution of fungal genera (Fusarium, Alternaria, Aspergillus), the seeds of *P. miliaceum* L. and *E. crus-galli* L. in Keszthely and Szombathely regions in Hungary across the years 1985, 2007, and 2010 showed a consistent dominance of *Fusarium* spp (Figure 3).

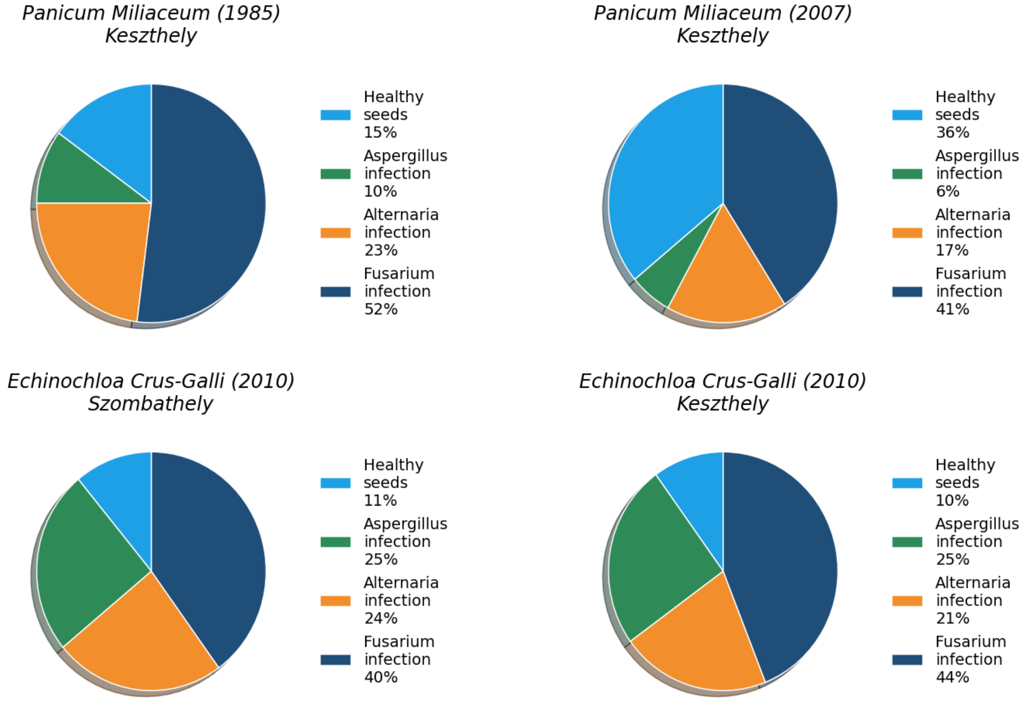

Figure 3 *Distribution of fungal pathogens in seeds of Panicum miliaceum L. and Echinochloa crus-galli L. in Keszthely and Szombathely in the years 1985, 2007, and 2010*

4 Discussion

In this study, a microscopic assessment of fungal pathogens in the seeds of two noxious monocotyledonous weeds, – *P. miliaceum* L. and *E. crus-galli* L. – the results showed that the seeds of these weeds may be heavily infected with seedborne fungal pathogens. Varying degrees of incidence and prevalence of the three phytopathogenic fungal genera were observed. Out of the 400 seeds examined from both weed species, 177 were found to be infected with *Fusarium* spp., 85 with *Alternaria* spp., and 66 with *Aspergillus* spp. (Table 2) and these results confirmed that the weed seeds can act as important pathogen reservoirs.

In this study, we discovered that the seeds of two weed species from the Poaceae family, i.e., *P. miliaceum* L. and *E. crus-galli* L., were infected with *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. These results align with previous studies conducted by Nelson (2018), who found that most of the isolated fungal seed endophytes from Poaceae members were typically filamentous ascomycetes, including *Alternaria, Fusarium, Cladosporium, Phoma, Aerobasidium*, and *Stemphylium.* Similar findings were reported in Latvia by Ņečajeva et al. (2023), using molecular techniques to sequence the ITS region of rDNA, which showed that *Alternaria,* Fusarium*, Sarocladium,* and *Cladosporium* were the most abundant genera colonizing the seeds of *E. crus-galli* L. Additionally, this study supports research conducted by Cavers and Kane (2016) in London and Ontario, who found six genera of fungi – *Alternaria, Aspergillus, Cladosporium, Fusarium, Helminthosporium,* and *Epicoccum* from the seeds of *P. miliaceum* L. that failed to germinate. Although our study was focused on pathogen detection rather than seed viability, fungal infection does not necessarily inhibit germination, and infected seeds may still emerge and contribute to pathogen carryover in the field.

Dominance of *Fusarium* spp. in monocot weed seeds

Fusarium spp. and *Alternaria* spp. showed a higher prevalence than *Aspergillus* spp. In both weed species, with *Fusarium* spp. as the most dominant with 52% and 41% in the *P. miliaceum* L. seeds collected from Keszthely in the years 1985 and 2007, respectively, and in *E. crus-galli* L., the percentage of infection was 43% in Keszthely and 41% in Szombathely (Figure 3). Additionally, in terms of the overall average mean of infection, *Fusarium* spp. showed a significant difference in the degree of infection compared to *Alternaria* spp. and *Aspergillus* spp. In the seeds of both weed species (Table 3). The results from this research align with findings from Simonin et al. (2022), indicating that Fusarium is one of the most widespread fungal genera associated with the infection of seeds, not only weed seeds but also cereal crops. These findings also align with research by Ņečajeva et al (2023), indicating that *Fusarium* and *Alternaria* were the most prevalent genera in the seeds of *E. crus-galli* L. and *Avena fatua* collected in the same location in 2020 and 2021 in Latvia.

Occurrence of *Alternaria* spp. and *Aspergillus* spp.

High frequency of occurrence of *Alternaria* spp. in the seeds of both species, with infection of 23% and 17% from the *P. miliaceum* L. seeds collected in Keszthely in the years 1985 and 2007, respectively. Seeds of *E. crus-galli* L. with 21% from Keszthely and 24% from Szombathely, collected in 2010, showed a relatively higher infection in Szombathely than in Keszthely (Figure 3). Comparing the degree of infection among the fungal infections identified, *Alternaria* spp. showed a higher degree of infection compared to *Aspergillus* spp. in the seeds of both weed species (Table 3) above. The results align with the previous studies of Jasim and Juber (2010), who described *Alternaria* as the most frequent fungal pathogen on the seeds of *E. crus-galli* L.

among the 26 species of fungi tested. *Alternaria* frequently colonizes seeds, exists as a saprophyte, endophyte, and a pathogen in a variety of plants, and is reported in over 400 plant species (Simonin et al., 2022). The most ubiquitous were *Alternaria alternata*, *Alternaria tenuissima,* and *Alternaria porri*, contaminating cereals and other crops, according to Lee *et al*. (2015) and Tralamazza et al. (2018). Additionally, *Aspergillus* spp. showed a lower infection rate in the previous years, that was, 10% and 6% in *P. miliaceum* L. in 1985 and 2007, respectively, and 25% from Szombathely and Keszthely in the year 2010. The identification of *Aspergillus* in research studies in India, where seeds of 5 minor millet species (*Paspalum scrobiculatum*, *Panicum miliaceum*, *Panicum trypheron, Setaria italica*, and *Echinochloa crus-galli* L.) were tested for susceptibility to *Aspergillus flavus,* and the results showed that indeed all these minor millets are susceptible to *Aspergillus flavus,* leading to the production of toxins (Ansari and Shrivastava, 1991).

Differences between weed species

Based on the findings of the experiment, it was discovered that fungal infections occurred differently in *P. miliaceum* L. and *E. crus-galli* L. seeds. While both species were susceptible to *Fusarium* and *Alternaria*, seeds of *E. crus-galli* L. showed a higher likelihood of infection, accompanied by rapid degradation of the seed. A total of 179 seeds of *E. crus-galli* L. were found to be infected by the three fungal genera, compared to a total of 149 seeds of *P. miliaceum* L. infected by the three fungal genera out of the total 400 seeds examined. Therefore, in terms of percentage, *E. crus-galli* L. seeds showed an infection rate of 44.75% and *P. miliaceum* L. with 37.25%. On further analysis based on the overall mean infection, *E. crus-galli* L. recorded 14.92 against 12.42 in *P. miliaceum* L., meaning *E. crus-galli* L. was more susceptible to the three fungal genera compared to *P. miliaceum* L. (Table 3). These observations are similar to what Ņečajeva et al (2023), documented about the high degrading capacity of Alternaria species on the seeds of *E. crus-galli* L., where specifically *Alternaria alternata* and *Alternaria tenuissima* were proven to have a high degrading capacity on the seeds of *E. crus-galli* L. and therefore it has been proposed that this two species can be used as promising biocontrol agents against barnyard grass which is a noxious weed in agricultural fields worldwide. Additionally, the varying susceptibility levels of *E. crus galli* and *P. miliaceum* to *Alternaria* could be attributed to differences in the characteristics of the seed, composition, or ecological preferences of the weed species contributing to overall physical and chemical defense against fungal pathogens (Pollard, 2018).

Co-infection and seed degradation

Some seeds of *P. miliaceum* L. and *E. crus-galli* L. were identified to be co-infected by both Fusarium and Alternaria. In this case, the seeds appeared to be more degraded than those inhabited by one of the genera of phytopathogenic fungi. This correlates with previous studies observed in the research work of Ņečajeva et al (2023) where seeds of *E. crus-galli* L. and *Avena fatua* (both belonging to family Poaceae) were found to be co-infected by the species of the two common fungal genera *Fusarium* and *Alternaria*, and these seeds showed a higher rate of degradation compared to the seeds inhabited by one of the fungal genera. The rapid degradation of the infected seed when co-infected by both *Alternaria* and *Fusarium* is that the species of the two fungal genera may engage in competitive interactions due to their antagonistic mycotoxin production, which can destroy the seed rapidly (Müller et al., 2015). These observations show how fungal pathogen interaction is complex in the natural setting, and this is important for us to understand because it is also the case with cultivated crops and might cause high yield loss and high contamination of the cereal crops.

5 Conclusions

This study identified three fungal genera, demonstrating that the seeds of *Panicum miliaceum* L. and *Echinochloa crus-galli* L. could harbour fungal phytopathogens. The three fungal phytopathogen genera identified in the seeds of both weed species were *Fusarium* spp., *Alternaria* spp., and *Aspergillus* spp. Among the three identified common genera in the seeds of both weed species, *Fusarium* was the most prevalent, with 44.25% of the total seeds examined in the experiment compared to *Alternaria* and *Aspergillus*. This was seen to be consistent in the two locations, Szombathely and Keszthely, for the years 1985,2007 and 2010, where the seeds were collected. *Alternaria* was the second most identified genus in proso millet and barnyard grass, accounting for 21.25% of the total seed examined in this study. Notably, some seeds were co-infected by the two fungal genera *Fusarium* and *Alternaria,* and these seeds appeared to be highly degraded. While the seeds of both *P. miliaceum* L. and *E. crus-galli* L. were susceptible to *Fusarium* spp. and *Alternaria* spp., seeds of *E. crus-galli* L. showed a higher likelihood of infection, accompanied by rapid degradation of seeds, where both *Fusarium* spp. and *Alternaria* spp. co-infected the seeds.

Since the fungal genera identified in this study cause significant economic losses to crops, Integrated Pest Management (IPM) practices are strongly recommended. Strategies such as crop rotation, field sanitation, and seed treatment should be implemented because they focus on a holistic approach to disease control. This reduces reliance on chemical fungicides, which is consistent with the efforts to reduce fungicide dependence in sustainable European agriculture. Further research could involve molecular identification of the fungal pathogens in the seeds of *Panicum miliaceum* L. and *Echinochloa crus-galli* L. at the species level, using advanced molecular techniques such as PCR, DNA sequencing, and metagenomics to identify and characterize these fungal species. Additionally, research on exploring molecular and epigenetic mechanisms underlying fungal infections in the seeds of the two weed species can be done. By investigating the genetic and epigenetic factors influencing susceptibility to fungal diseases, host-pathogen interaction can be understood, leading to specific target strategies for the management of these pathogens and weeds.

Acknowledgements

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Occurrence and Prevalence of Pepper Pathogens in the Vojvodina

A paprika kórokozóinak előfordulása és prevalenciája a Vajdaságban

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Abstract*:* One of the key challenges in pepper production in the Vojvodina Province is the presence of pathogens that cause serious diseases and epidemics, leading to reduced and compromised yields. This study aimed to identify fungal and bacterial pathogens under intensive production conditions. During a two-year period, 2023–2024, a total of 35 pepper samples showing symptoms of diseases, were collected from several locations and examined using classical and molecular methods. The results showed that *Alternara solani* was the most common fungal pathogen, followed by *Colletotrichum* spp., *Botrytis cinerea, Macrophomina phaseolina*, and *Fusarium* spp. (*subglutinans* and *oxysporum*). Among the bacterial pathogens, *Xanthomonas euvesicatoria* was found in all seven examined pepper fields. Additionally, *Pectobacterium* spp. and '*Ca*. Phytoplasma solani' were also detected. These findings provide practical insights that can support the development of effective control measures for pathogens affecting pepper production.

Keywords: *pepper, diseases, detection, identification*

Összefoglalás*:* A vajdasági paprika-termelés egyik legfontosabb kihívása a kórokozók jelenléte, amelyek súlyos betegségeket és járványokat okoznak, ezáltal csökkentve és veszélyeztetve a terméshozamot. A jelen tanulmány célja a gombás és bakteriális kórokozók azonosítása intenzív termelési körülmények között. A 2023–2024-es két év során összesen 35, betegség tüneteket mutató paprika mintát gyűjtöttünk több helyszínről, majd klasszikus és molekuláris módszerekkel vizsgáltunk. Az eredmények azt mutatták, hogy az *Alternaria solani* volt a leggyakoribb gombakórokozó, ezt követték a *Colletotrichum* spp., a *Botrytis cinerea*, a *Macrophomina phaseolina* és a *Fusarium* spp. (*subglutinans* és *oxysporum*). A bakteriális kórokozók közül a *Xanthomonas euvesicatoria* mind a hét vizsgált paprikatáblában jelen volt. Emellett *Pectobacterium* spp. és a '*Ca.* Phytoplasma solani'-t is kimutattuk. Ezek az eredmények gyakorlati betekintést nyújtanak, és hozzájárulhatnak a paprikatermelést érintő kórokozók elleni hatékony védekezési módszerek kidolgozásához.

Kulcsszavak: *paprika, betegségek, kimutatás, azonosítás*

1 Introduction

Pepper (*Capsicum annuum* L.) is widely cultivated worldwide and includes many diverse varieties (Tripodi et al. 2021**;** Živković et al., 2023). In Serbia, most pepper production takes place in the southern part of the country, which accounts for an average of 107,408 tons, or 76.58% of total output. Within this region, the Šumadija and West Serbia region produces about 55,789 tons (39.78%), while the South and East Serbia region contributes around 51,619 tons (36.80%). Northern Serbia produces the remaining 32,842 tons (23.42%), with the Belgrade region averaging 3,664 tons (2.61%) and Vojvodina 29,178 tons (20.81%). Among all regions, Vojvodina achieves the highest average yield at 13.9 tons per hectare (Kljajić et al., 2025).

One of the major challenges affecting pepper production in the Balkan countries is the widespread presence of pathogens that cause severe diseases and epidemics, leading to significant yield losses. Among the most destructive and widely distributed pepper pathogens are *Phytophthora capsici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Fusarium* spp., *Phomopsis capsici*, *Colletotrichum* spp., *Pseudomonas syringae* pv. *syringae*, *Xanthomonas* spp. (*X. euvesicatoria, vesicatoria, performans, gardneri*), *Pectobacterium* spp., *Pepper mild mottle virus* (PMMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Alfalfa mosaic virus* (AMV), and *Potato virus Y* (PVY) (Rodeva et al., 2012; Vučurović et al., 2015; Milošević et al., 2018; Babić et al., 2023; Živković et al., 2023; Popović Milovanović et al., 2025). In Serbia, two phytoplasma diseases, stolbur (‘*Candidatus* Phytoplasma solani’) and aster yellows (‘*Candidatus* Phytoplasma asteris’) have been reported in pepper crops (Duduk and Duduk, 2013).

Disease development in pepper is strongly influenced by weather conditions, including average maximum and minimum temperatures, relative humidity, and rainfall. When control measures are insufficient and conditions favor leaf, fruit–diseases development, infections can intensify and lead to premature defoliation. One of the key strategies for managing and preventing plant pathogens is seed testing and certification, along with producing seed in areas free of pathogens or where conditions limit their development (Živković et al., 2023). Proper detection and identification also play an important role in controlling pathogens in pepper production. The object of this study was to update current knowledge on the most economically significant and emerging *C. annuum* pathogens in the Vojvodina region. Comprehensive knowledge of these pathogens is crucial for the effective disease management in sustainable agriculture production.

2. Materials and Methods

Symptom observation and sample collection. Between 2023 and 2024, the health status of pepper crops was monitored at several locations in the Vojvodina province (Despotovo, Kać, Kovilj, Rimski šančevi, Pivnice, Srbobran, Bački Petrovac). Diseased pepper plants (type babura and kapija) – including 35 samples of leaves, stems, roots and fruits showing various symptoms–were collected during the spring and summer months. The disease incidence was calculated by using the following formula: Disease incidence (%) = Number of infected plants / Total number of plants assessed x 100, collected for all localities and expressed as the average values.

Isolation and pathogen identification. Diseased samples were first rinsed under tap water, dried on sterile filter paper, and surface disinfected with 70% alcohol before being dried again. Samples that showed presence of fungal or bacterial disease were then subjected to isolation on appropriate media: Potato Dextrose Agar (PDA) for fungi and Nutrient Sucrose Agar (NSA) and Yeast Dextrose CaCO3 Agar (YDC) for bacteria. Small fragments taken from the boundary between healthy and diseased tissue were placed on PDA for fungal isolation. For bacterial isolation, tissue fragments were macerated in sterile distilled water (SDW), left for 20 minutes, and then plated on NSA and YDC medium. Petri dishes were incubated at 25 ± 1 °C for 3 to 14 days. Total DNA was extracted from samples or pure cultures grown on NSA, YDC or PDA medium, using a genomic DNA isolation kit (DNeasy Plant Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. The obtained DNA was stored at −20 ºC until use. To identify the pathogens involved in pepper diseases, different methods including classical and molecular tools for fungi, bacteria (including phytoplasma) identification were used (White et al., 1990; Grifoni et al., 1995; Schaad et al., 2001; Agrios, 2005; Koenraadt et al., 2009; EPPO, 2016).

Table 1 Methods used for the identification of pathogens associated with pepper diseases

Pathogen	Method of identification	Reference
Alternaria	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Colletotrichum	Colony, conidia and acervuli morphology	*Stojšin et al. (2008)*
Fusarium	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Botrytis	Colony and conidia morphology; PCR confirmation	*White et al. (1990); Agrios (2005)*
Macrophomina	Colony morphology; microsclerotia	*Stojšin et al. (2008)*
Xanthomonas	Colony morphology; PCR confirmation	*Schaad et al. (2001); Koenraadt et al. (2009)*
Pectobacterium	Colony morphology; PCR confirmation	*Grifoni et al. (1995); Schaad et al. (2001);*
Ca. Phytoplasma	Real-Time PCR confirmation	*EPPO (2016)*

3 Results and Discussion

This study describes the main fungi, bacteria and phytoplasma affecting pepper production in Vojvodina, with the aim of identifying which pathogens are most frequent and potentially harmful under local agro–ecological conditions. Using several pathogen–identification methods (Table 1), our analysis indicates that the pathogen structure in pepper is highly complex. The pathogens listed below appear each year, though their intensity varies with agro–ecological conditions and the cultivated pepper assortment. The following section summarizes the findings from the period 2023–2024.

Diseases caused by fungi. Among the fungal pathogens found in diseased pepper samples, *Alternaria solani* was the most common, causing both leaf spots and fruit rot (Figure 1a). This pathogen can infect pepper plants at almost any stage of growth and spreads gradually. It enters through leaves, roots, or wounds on plant tissues (Attia et al., 2025), allowing it to cause damage throughout the entire season. In addition, several *Colletotrichum* species and *Botrytis cinerea* were identified as major causes of pepper fruit rot in the field (Figure 1b, 1c). Their ability to spread and overwinter has contributed to notable reductions in pepper yield. Isolates of *Fusarium* (including *F. subglutinans* and *F. oxysporum*) and *Macrophomina phaseolina* were isolated from the roots of infected pepper plants, after the flowering stage. *F. subglutinans* was also found in both internal and external fruit rot. In some cases, these pathogens appeared together as mixed infections. All identified pathogens are generally polyphagous, infecting a wide range of plant species worldwide (Van Poucke et al., 2012; Anila Younas et al., 2016; Frans et al., 2017; Cerkauskas, 2017; Engalycheva et al., 2024), including pepper, which adds to the challenges of effective disease management. Fusarium wilt typically begins with slight vein clearing on young leaves, followed by drooping of older leaves. Affected plants become stunted, lower leaves turn yellow, wilting and defoliation occur, and eventually the whole plant dies. Average disease incidence of pepper pathogens across all surveyed locations is presented in Figure 2.

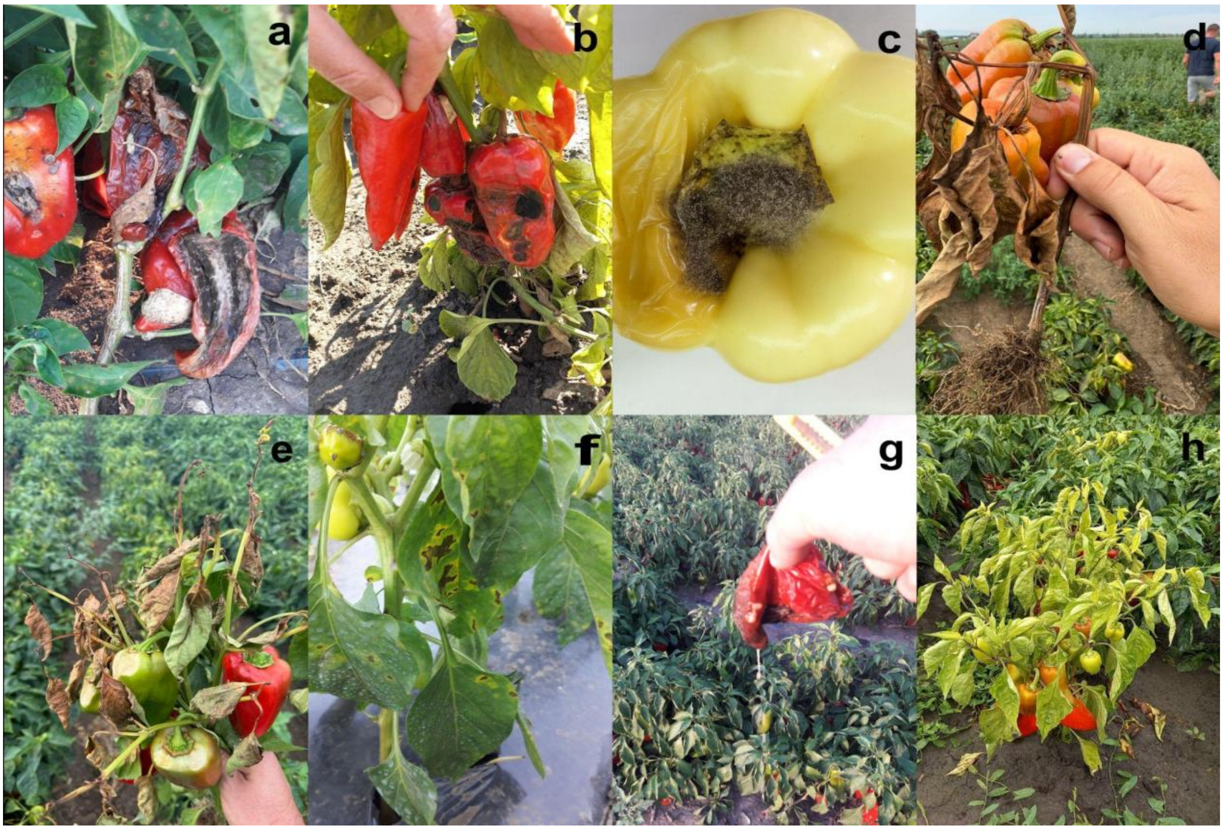

Figure 1 *Symptoms on pepper plants: a) A. solani; b) Colletotrichum spp.; c) B. cinerea; d) M. phaseolina; e) Fusarium spp.; f) X. euvesicatoria; g) Pectobacterium spp.; h) 'Ca. P. solani'.*

Diseases caused by bacteria. The predominant bacterial pathogen affecting pepper production was *Xanthomonas euvesicatoria*, causing bacterial spots on both leaves and fruits (Figure 1f). This pathogen was detected at all surveyed locations, during the 2-4 leaf stage of development, with disease incidence varying mainly according to agroecological conditions and pepper cultivars. The results obtained were expected given that *X. euvesicatoria* is one of the most common pepper diseases in Serbia (Vlajić et al., 2017; Popović Milovanović et al., 2025). Bacterial spot occurs widely in both open-field and greenhouse pepper production, causing extensive necrotic lesions on leaves, stems, and fruits, along with defoliation and notable reductions in yield and fruit quality. Under favorable conditions, *X. euvesicatoria* often becomes the dominant species, driving epidemic development and increasing disease severity in susceptible pepper cultivars (Živković et al., 2023). Seedborne transmission further supports its spread, especially in production systems that rely on undeclared or non-certified seed. Furthermore, based on the occurrence of bacterial diseases, presence of *Pectobacterium* spp. was determined as the causative agent of fruit soft rot in the pepper samples. *Pectobacterium* species cause soft rot in plant tissues and were detected in ripe pepper fruits, leading to pronounced parenchyma softening. Typical symptoms include water-soaked lesions and a characteristic foul odor resulting from cell wall degradation (Van Gijsegem et al., 2021; Ma et al., 2007; Popović Milovanović et al., 2023). Under high humidity and in the presence of mechanical injuries, these bacteria can trigger significant losses, especially during storage and postharvest handling. In our survey, the pathogen was observed mainly on red-ripening pepper fruits harvested in the autumn.

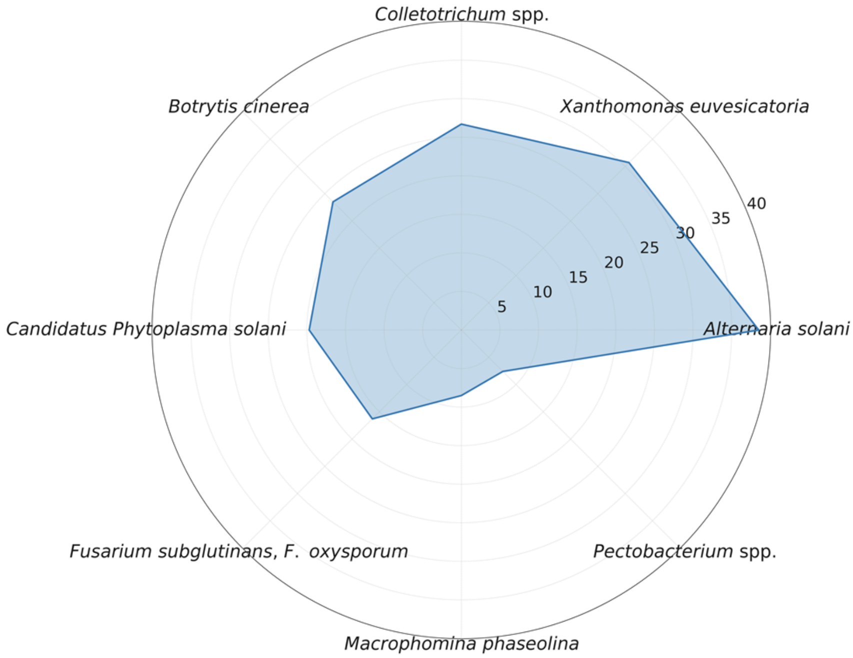

***Figure 2** Average disease incidence of pepper pathogens across surveyed locations in the Vojvodina Province.*

Additionally, based on the results, the presence of '*Candidatus* Phytoplasma solani' (Stolbur phytoplasma) was confirmed in the pepper samples after flowering stage resembling the symptoms of yellowing, chlorosis and stunting of the leaves with sporadic necrosis (Figure 1h). With the development of the disease regardless of soil moisture and intensity of insolation diseased plants continue rapidly to lose turgor. In recent years, phytoplasmas have increasingly affected not only pepper production but other vegetables as well in Serbia (Mitrović et al., 2013, 2016; Popović et al., 2021; Iličić et al., 2022). As the use of insecticides for controling leafhoppers in vegetables is very limited and generally ineffective, protection should focused on removing the dual host-plants, simultaneously hosting '*Ca*. P. solani' and vector, is a precondition for the reduction of the disease incidence (Popović et al., 2021). Although no detection of virus diseases in pepper was carried out during this research, they are common under field conditions and of considerable economic importance. Nevertheless, their presence in the surveyed production areas cannot be ruled out, as certain symptoms may visually resemble those of abiotic disorders or infections caused by other pathogens.

Managing diseases in pepper production is challenging because the detected pathogens are highly variable, survive well in complex environments, persist in plants as latent infections, and remain in the soil for extended periods. Although chemical pesticides have traditionally been used, their effectiveness often declines over time, they can be costly, and they may pose risks to human health and the environment. These limitations highlight the need for continuous monitoring of pathogen presence in pepper fields, as well as the integration of preventive measures such as the use of healthy planting material, crop rotation and sanitation, and growing resistant cultivars. Strengthening monitoring and implementing integrated disease management strategies is therefore essential for reducing disease pressure and maintaining sustainable pepper production.

5 Conclusions

In conclusion, our results showed that the pathogens affecting pepper production present complex, involving various fungi, bacteria, phytoplasmas, and visually observed—but not laboratory-confirmed—viruses that threaten both production and yield. As many of these pathogens are seed-borne, the findings highlight the importance of using pathogen-free seeds as a primary control measure, supported by precise pathogen detection and effective control management.

Acknowledgements

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Testing a field methodology for assessing the vegetational impacts of access pathways traversing the reed beds of Lake Balaton at a study site in Balatonfenyves

A Balaton nádasait átszelő bejárók növényzetre gyakorolt hatásainak vizsgálatához szükséges terepi módszertan tesztelése balatonfenyvesi mintaterületen

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Abstract: Our study examined the effects of four public access pathways on the surrounding vegetation within the reed beds of Lake Balaton at a study site in Balatonfenyves. The vegetation was analyzed using 1×1 meter quadrats to determine species composition and abundance. The research aimed to assess the extent of the pathways' influence on the vegetation, the spatial scale of this impact, and how the disturbance zone changes with increasing distance from the shore zone. The primary goal of this work was to develop a simple field method to support future, more comprehensive investigations. Our findings suggest that the pathways' impact on the vegetation is most significant in a wider zone near the land-water interface, decreases towards the center of the reed bed, but intensifies again in close proximity to the open water.

Keywords: *anthropogenic effect, Lake Balaton, reed bed, tourism, shore zone vegetation*

Összefoglalás: Kutatásunk során Balatonfenyvesen négy, a nádast átszelő közösségi bejáró mentén vizsgáltuk ezeknek a vonalas létesítményeknek a hatását a környező növényzetre. A vegetáció vizsgálatához 1×1 méteres kvadrátokat használtunk, amelyeken belül meg tudtuk határozni az egyes növényfajok arányát. Azokra a kérdésekre kerestük a választ, hogy a bejárók mekkora mértékben hatnak a körülöttük lévő vegetáció összetételére, ez a hatás milyen távolságban érvényesül a bejáróktól számítva, valamint ez a hatásterület változik-e a nyílt víz felé haladva. Ezeknek a problémáknak a kutatására egy egyszerűen használható terepi módszer kifejlesztésére van szükség. Ezzel az volt a célunk, hogy további részletesebb kutatásokat készítsünk elő. Eredményeink azt mutatják, hogy a szárazföld közvetlen közelében nagyobb szélességben van hatása a növényzetre a bejáróknak, amely a nyílt víz felé haladva csökken, azonban a nyílt víz közelében ismét felerősödik az építmények zavaró tulajdonsága.

Kulcsszavak: *antropogén hatás, Balaton, nádas, turizmus, partmenti növényzet*

1 Introduction

Despite its nearly 600 km² surface area, Lake Balaton is an exceptionally shallow standing water body, with an average depth of only 3.3 m (http1), and therefore responds rapidly even to minor changes. Owing to its shallowness, an extensive littoral vegetation zone has developed, which functions as a buffer area. Reed beds filter pollutants arriving from the terrestrial environment and protect the shore zone from wave-induced erosion (Jakucs 1981). This well-functioning ecosystem—often comprising zonation from open water through marsh meadows to woody terminal communities—is subject to anthropogenic mechanical disturbances. Several types of disturbance affect the littoral vegetation, including land reclamation and the construction of boat passages. According to a survey performed in 2012, along the entire shore zone of Lake Balaton an interruption of some type in the vegetation of littoral zone could be observed on average every 59 metres. The study indicates that the reed beds of Balatonmáriafürdő, Balatonfenyves, and Balatonkenese are the most affected by human disturbance (Vidovenyecz 2012).

However, the most common disturbances are access paths, predominantly originating from private properties in recreational areas (Gyurina 2025), which cut through reed beds and open the formerly closed vegetation both toward open water and toward land. Under these conditions, species indicative of human disturbance and invasive alien plants, such as *Echinocytis lobata* are able to penetrate the reed beds from the terrestrial side (Pomogyi 2011). In the areas of Balatonfenyves and Balatonmáriafürdő, these pathways also indirectly promote shrub spread within reed beds (Gyurina & Boromisza 2025). In addition, wave action can enter the reed beds from the open water side along these access paths, initiating internal erosion of the vegetation (Pomogyi 2011).

Numerous studies have addressed disturbances affecting littoral vegetation in lakes; however, relatively few have focused specifically on the issue of access paths, which cause substantial fragmentation of shore zone vegetation along Lake Balaton (Vidovenyecz 2012). In 2011, natural and developed shore zones of three lakes in northern Germany were compared. In the three studied lakes, shore zone development affected 9%, 24%, and 65% of the total shore zone length, respectively, resulting in reductions of macroinvertebrate biomass of 5%, 20%, and 36%.

An investigation of beaches established within the littoral vegetation of tourist-frequented lakes in a nature conservation area in Germany revealed that plant cover in disturbed reed belts was considerably lower than in control sites. Furthermore, the proportion of ruderal species was slightly higher in areas close to the beaches, although the difference was modest. The study ultimately concluded that, among the examined habitat types, reed beds exhibited the greatest contrast between control and disturbed sites, although even in this case the differences were not pronounced (Meyer et al. 2023).

Along lake shores with well-developed infrastructure, it is common for owners of waterfront properties to consider littoral vegetation as aesthetically undesirable and therefore to cut or remove it. This practice reduces habitat diversity and reduces the capacity of vegetation to fulfill its water-quality protection functions. A study of lake shore zones in The USA, Maine found that, along access paths leading to docks, macrophyte species in the littoral zone presented significantly lower shoot densities (Ness 2006). Similar phenomena can also be observed along certain shoreline sections of Lake Balaton.

In 2009, potential damage caused by anglers was examined along the shores of Canadian lakes and rivers. On shoreline sections that were rarely used by anglers, the area devoid of vegetation was almost four times greater than on frequently used sections; however, this effect was moderated to the immediate shoreline and reduced toward open water (O'Toole et al. 2009). Comparable effects can be expected in relation to access paths occurring within the reed beds of Lake Balaton.

The protection of the reed beds of Lake Balaton is regulated by two legal instruments: the provisions of Act CXXXIX of 2018 concerning the Lake Balaton Priority Resort Area, and

Government Decree No. 22/1998 (II.13.). The first regulation stipulates that, with limited exceptions, all mechanical interventions are prohibited in class I–III reed beds and within a buffer zone of at least 2 meters around them, as well as in reed beds located in protected natural areas and their buffer zones. Prohibited activities include dredging, infilling, construction, and the establishment of piers or access structures, where such actions may damage reed stands, degrade their quality, or lead to their destruction. Exceptions are limited to environmental damage prevention, interventions required for life and accident safety, specific maintenance of the lakebed, the removal of unauthorized structures or infill, and management measures defined in nature conservation or Natura 2000 management plans.

The latter regulation further stipulates that the conservation of class IV and V reed beds must be ensured and, where possible, their restoration should be undertaken. In addition, Government Decree No. 283/2002 (XII. 21.) also addresses the protection of reed beds by requiring that, during the preparation of shoreline regulation plan documentation, the areas of classified reed beds be taken into account. This provision therefore offers a form of protection against the designation of inappropriate land-use categories. Although these legal instruments could provide suitable protection for the reed beds of Lake Balaton, the vegetation of the lake's littoral region remains highly fragmented (Vidovenyecz 2012).

This review of the relevant literature also reveals that the effects of access paths crossing the reed beds of Lake Balaton on the composition of riparian vegetation have not been investigated in sufficient depth. The aim of the present study is to develop a simple, easily applicable, and spatially replicable field methodology suitable for assessing the impact of access paths on vegetation.

2 Materials and Methods

The access paths selected as sampling sites for this study are located in Balatonfenyves, at the boundary between the Nagyberek and Lake Balaton microregions (Csorba 2021). The entire study area can be classified as the General Habitat Classification System (ÁNÉR) habitat type B1a, Eu- and mesotrophic reed and Typha beds (Bölöni et al. 2011). In the vicinity of the investigated access paths, in addition to the dominant *Phragmites australis*, *Carex riparia*, *Salix cinerea*, and *Typha angustifolia* are also present as frequent species.

As shown in Figure 1, four access paths were selected for investigation. Vegetation was surveyed at four sampling points along each access path, with the exception of the access path located at the end of Daru Street, where the structure did not extend across the entire reed bed. In this case, but the access path was included in the study because conducting the survey within a single, ecologically coherent vegetation patch was considered essential in order to minimise variability associated with differing shore zone characteristics along Lake Balaton. This criterion was met during the selection of the access paths; however, the access path located on Fogoly Street is situated farther from the other investigated structures. At the time of the field surveys, this was the nearest access path that was not closed and could be accessed from the terrestrial side.

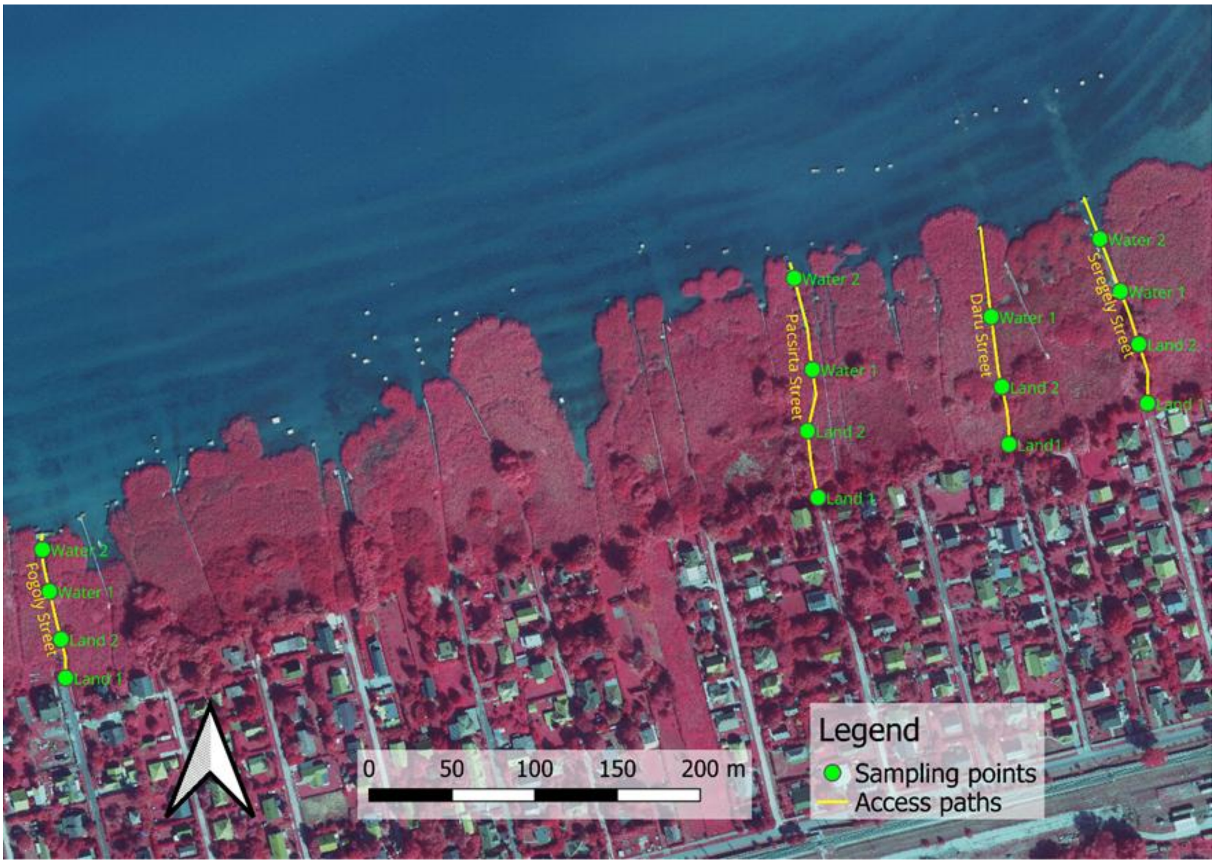

Figure 1 *The location of the examined pathways and the sampling points*

The primary criterion for selecting the study area was the presence of a reed bed along the shoreline that is heavily fragmented by access paths. This allowed the research to be performed in an area where the problem addressed by the study is particularly relevant.

The development of the field methodology was informed by a study published in 2023 that examined the effects of beaches established within riparian vegetation on lakeshores in Germany (Meyer et al. 2023). In that study, 2 × 8 m quadrats were designated in each vegetation zone. Adapting this approach, sampling points were established within the vegetation from the terrestrial side towards open water, such that botanical surveys were conducted at the terrestrial end of the access paths (land 1), at one third of their length (land 2), at two thirds of their length (water 1), and at the border at the open water (water 2).

Field surveys were carried out in early June 2025. At each sampling point, vegetation was recorded using the Braun–Blanquet method; however, the cover of each species was expressed as a percentage (Braun-Blanquet 1964). In contrast to the German study (Meyer et al. 2023), 1 × 1 m quadrats were used, as these are easier to establish in the field than those applied in the referenced study, in line with the objective of developing a simple and easily applicable methodology. Furthermore, the use of larger field quadrats would not have provided a sufficiently detailed representation of the vegetation at varying distances from the access pathways.

Unlike the German research, which also considered bathing areas arranged in continuous strips, the linear extent of the access paths themselves was not included in the survey. This decision was motivated by the aim of determining the distance from the structures at which their effects on vegetation remain detectable. Quadrats were placed progressively farther away from the access paths; at each sampling point, a minimum distance of two quadrats (i.e. 2 m) from the structures was applied. Where the proportion of disturbance-tolerant species exceeded 20%, the distance was increased to 4 m from the structures.

This approach subsequently allowed the distance over which disturbance-indicator plant species can spread from the structures to be identified during data analysis. Based on these results, an acceptable proportion of degradation-indicator species within reed bed habitats could also be determined.

For the categorisation of the recorded plant species, Borhidi's system of social behaviour types was applied (Borhidi 1995). The species identified in the survey were assigned to seven types, which were subsequently grouped into two main categories. The first category comprised habitat stability indicator species, including specialists (S), generalists (G), and natural competitors (C). The second category consisted of disturbance-indicator species, including weeds (W), disturbance-tolerants (DT), adventive aliens (A), and alien competitors (AC). Open water and bare mud surfaces observed during the field survey were also assigned to the latter category, as these features generally indicate some form of disturbance within reed bed habitats.

For each quadrat, the proportional representation of the two species groups was calculated, and the results were compared across distances from the access paths as well as among sampling points. Field data were compiled in tabular form. Within the scope of the present study, priority was given to testing the applicability of the methodology; therefore, the analysis focused primarily on descriptive statistics, including mean species proportions and trends related to band width.

3 Results/ Results and Discussion

A total of 26 species were recorded across 34 quadrats established along the surveyed transects. Among the disturbance-tolerant species, *Calystegia sepium* exhibited the highest frequency of occurrence, being recorded in 14 quadrats. Notably, *Solanum dulcamara* and *Urtica dioica* were each present in 12 quadrats. Table 1 presents the mean proportions of habitat stability indicator species and disturbance-indicator species at varying distances from the surveyed transects.

Table 1 Average proportions of two groups of species present at measured distances from the examined pathways

	Land 1				Land 2		Water 1			Water 2	
	1. meter	2. meter	3. meter	4. meter	1. meter	2. meter	1. meter	2. meter	3. meter	1. meter	2. meter
Habitat stability indicator species	67.5%	73.75%	85%	100%	87.5%	95%	65%	75.5%	65%	43.3%	86.7%
Disturbance-indicator species	32.5%	26.25%	15%	0%	12.5%	5%	35%	24.5%	35%	56.7%	13.3%

It is clearly evident that, at sampling points closest to the terrestrial zone, the access structures exert an influence on the surrounding vegetation across a wider spatial band. This effect reduces at sampling points located one meter farther from the terrestrial zone along the access routes; however, in those sections of the riparian vegetation where permanent water cover is present, the influence of the access structures intensifies again. Table 1 presents only mean values; therefore, these patterns are not equally pronounced at all access routes.

For example, at the Seregély Street access route, at the first sampling point the proportion of disturbance-indicator species in the quadrat located within the first meter laterally from the structure was slightly lower (20%) than in the second meter (25%). In contrast, at the Fogoly Street access path, a more pronounced difference was detected: the proportion of disturbance-indicator species was 15% within the first meter from the structure, increased to 50% at the second meter, and subsequently decreased again to 15% at the third meter.

At the remaining sampling points of the access routes, the proportion of disturbance-indicator species in the quadrats generally decreased or remained stable with increasing distance from the structures.

4 Discussion

The access paths fragmenting the reed beds of Lake Balaton, together with the associated vegetation degradation, have posed a threat to the maintenance of the lake's favourable ecological status for decades. In order to determine the extent to which these structures negatively affect vegetation, a simple and easily applicable field methodology is required for vegetation assessment.

At the sampling points which are closest to the terrestrial zone of the access paths, within the first meter from the structures, disturbance-indicator species may occur in higher proportions as a result of anthropogenic impacts originating from the shoreline. At the open-water end of the access paths, directly adjacent to the structures, the proportion of habitat stability indicator species is low despite the lower intensity of other forms of human disturbance. This can be attributed to the penetration of wave action along the access paths into the vegetation of the littoral zone, where it is able to cause erosion. At the boundary of open water, wave action is less capable to degrade the reed bed at a lateral distance of two meters from the access path.

Based on field observations and the description of the ÁNÉR B1a habitat type, which states that in reed beds classified within naturalness category 3 the proportion of alien species does not exceed 20% (Bölöni et al. 2011), we propose defining the acceptable proportion of disturbance-indicator species at the same threshold, although the concepts of alien and disturbance-indicator species do not fully overlap. Accordingly, we consider it optimal to apply this proportion as a definition of vegetation of littoral zone in favourable condition in future studies.

Based on our experience to date, in order to reliably achieve this threshold during vegetation surveys along access paths, it is necessary to move at least four meters away from the structures. Only in a single case was it necessary to conduct sampling at this distance, as the proportion of disturbance-indicator species exceeded 20% in quadrats located at distances of 1–3 meters from the structures. Consequently, within individual quadrats the proportion of habitat stability indicator species reaches 80%. Figure 2 illustrates, together with this threshold value, the proportions of plant species belonging to the two examined groups within the quadrats.

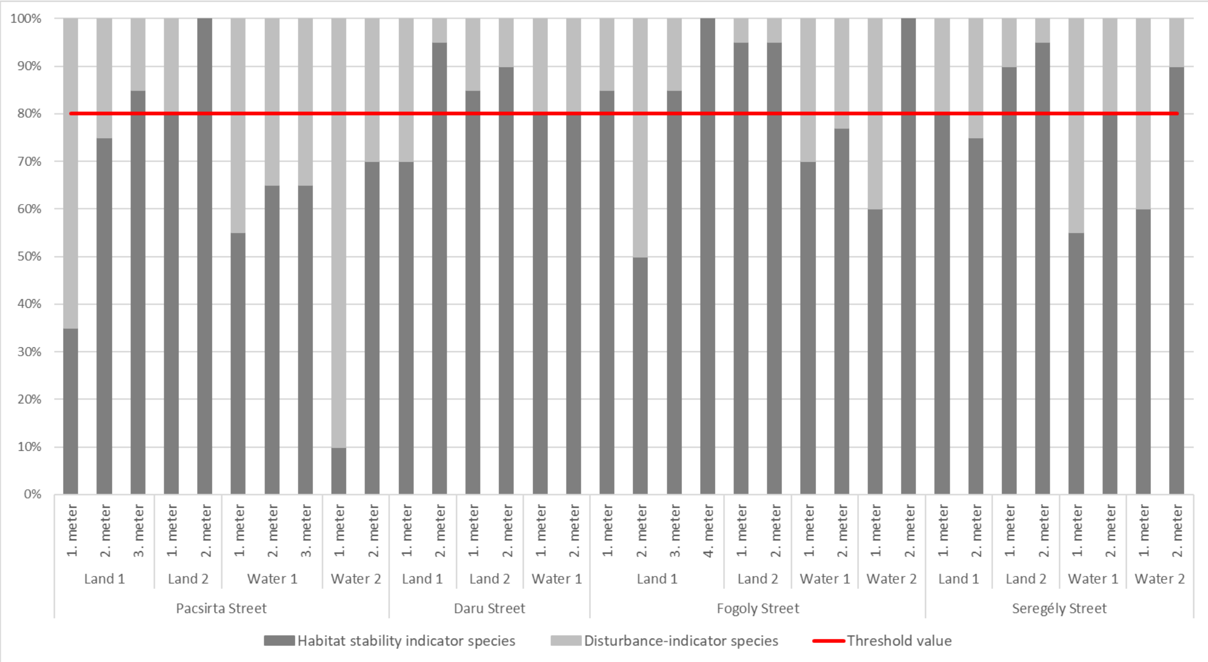

Figure 2 *The ratios of the two target species groups per quadrat and the associated threshold for a desired ecological state*

In addition, further indicators may be required for the field assessment of the impacts of access structures on reed beds in order to obtain more accurate results in future studies. Such indicators may include, for example, measurements of reed shoot height or estimates of the proportion of dead reed stems, which are also applied within the National Biodiversity Monitoring System (NBmR) to assess the condition of reed beds (Bata et al. 2010).

Long-term research may subsequently determine the density of access paths that can cause reed dieback along a 100 m shoreline section. The removal of these structures would promote more natural vegetation dynamics. This could also benefit species inhabiting the margins of reed beds, as anthropogenic disturbance is more pronounced along artificially created edges associated with built structures. Furthermore, the results of this research may support the rehabilitation of vegetation the littoral zone at sites where access paths have been removed.

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