LICHEN-BAGS AS A BIOMONITORING TECHNIQUE IN AN URBAN AREA

PETROVA, S.P.[1*] – YURUKOVA, L.D.[2] – VELCHEVA, I.G.[1]

[1] *University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and Environmental Conservation, Plovdiv 4000, Tzar Assen Str. 24, Bulgaria*
(phone: +359-32-261519)

[2] *Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, Sofia 1113, Acad. G. Bonchev Str., Bl. 23, Bulgaria*

**Corresponding author:*
e-mail: slaveya_petrova@uni-plovdiv.bg

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Abstract. The suggested work presents the first application of the lichen-bags technique in the city of Plovdiv (Bulgaria). Plovdiv was pointed as one of the most polluted cities in Europe for 2008 by National Institute of Statistics of Italy (ISTAT, 2011). The study was carried out to estimate the air quality in the areas with high traffic activity, industrial and suburban zones by means of lichens. For this purpose, the concentrations of 21 chemical elements (Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, U, V and Zn) were investigated using *Pseudevernia furfuracea* (L.) Zopf. samples in bags, which were exposed at 4 different sites in the city of Plovdiv. The elements Na, Mn, Cu, Zn and Sr increased significantly their concentrations in the lichen-bags, whereas K, S and Cd decreased theirs in comparison with the unexposed lichenized fungi. Highest values of RAF (relative accumulation factor) were found for Na (3.49), Zn (2.92) and Cu (3.33).

Keywords: *atmospheric pollution, urbanization, active biomonitoring, lichen-bags, Pseudevernia furfuracea*

Introduction

Since the beginning of the twentieth century, air pollution has worsened as a consequence of urbanization and industrialization, together with the rapid growth of the motorized transportation and population. Of the main components of air pollution, an important group to be considered is that of heavy metals, mostly associated with airborne particulate matter. This is particularly true in city centers, where vehicle engines, as well as the heating systems inside buildings, represent the most important sources of air pollution (Sawidis et al., 1995; Monaci et al., 2000).

Lichens are widely used in biomonitoring studies of air pollution, either as bioindicators of air quality or as bioaccumulators of atmospheric deposition (Conti and Geccheti, 2001; Sczepaniak and Bizuik, 2003; Cansaran-Duman et al., 2012). These organisms can be easily sampled, are low in cost, and allow wide areas to be monitored. Moreover, lichens do not have root systems or waxy cuticles, and thus they are strongly dependent on wet and dry deposition for their mineral nutrients (Bermudez et al., 2009). Unlike many other plants, they lack variability in morphology throughout the growing season, thus they are used for biomonitoring purposes in many ways (Giordano et al., 2005).

Some lichen species were successfully applied from many years for assessing the atmospheric deposition of heavy metals and toxic elements in background and polluted areas. Lichens naturally occur in many terrestrial ecosystems, but the anthropogenic

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factors can be responsible for their lack in urban areas. In such cases, the passive biomonitoring is not useful and the active biomonitoring comes to replace it. One of the techniques is exposing bags containing lichen in the polluted area to measure concentrations of contaminants affecting the samples (Adamo et al., 2003; Giordano et al., 2005; Yildiz et al., 2008).

This technique has a lot of advantages: well-defined exposure time, known original concentration of elements in the biomonitor, flexibility both in site selection and in number of stations that can be chosen. The major limitations of the method are in the unknown collection efficiency for different contaminants in different biomonitors and, therefore, the impossibility to standardize the exposure time for the exact species in bags and/or for urban, industrial or other source of pollution.

The suggested work shows the first application of the lichen-bags technique in the city of Plovdiv (Bulgaria). Furthermore, Plovdiv was pointed as one of the most polluted cities in Europe for 2008 by National Institute of Statistics of Italy (ISTAT, 2011). The study was carried out to estimate the air quality in the areas with high traffic activity, industrial and suburban zones by means of lichens. For this purpose, the concentrations of 21 chemical elements (Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, U, V and Zn) were investigated using *Pseudevernia furfuracea* (L.) Zopf. samples in bags, which were exposed at 4 different sites in the city of Plovdiv.

Materials and methods

Study area and sampling sites

Plovdiv (42°9' N, 24°45' E) is the second-largest city after the capital Sofia with a population of over 338 000 inhabitants (NSI, 2011). It is situated in Southern Bulgaria on the two banks of the Maritsa River. Air quality in Plovdiv is quite worsened due to the motor vehicle emissions and residential heating, than the industrial sector, which has a minor impact (Atanassov et al., 2006; RIOSV, 2010).

For the main purpose of our study, four sites (Northen, Eastern, Western and Central) were selected on the basis of the type of anthropogenic impact (*Fig. 1*).

The Northern site (N 42º10′32.3″, E 24º45′53.4″) is located in the city outskirts and was chosen because it is close to an industrial nonresidential area (Northern industrial zone in the city of Plovdiv) where there are a fossil fuel power plant, cosmetic and glass factories, as well as other small-point sources of contaminants.

The Eastern site (N 42º08′24.3″, E 24º47′06.2″) is located in the “Trakiya” suburb, which is characterized by a high traffic and moderate household pressure.

The Western site (N 42º07′54.4″, E 24º42′18.0″) was chosen in the “Smirnenski” suburb, which is subjected to a moderate anthropogenic impact.

The Central site (N 42º08′22.8″, E 24º44′24.1″) is located in the main center of the city of Plovdiv, along one of the major streets (Ruski Boulevard) with a very intensive daily traffic.

Experimental set up

Pseudevernia furfuracea (L.) Zopf. was applied as a suitable biomonitor of air pollution. Lichen material was collected from the Vitosha Natural Park, Bulgaria (42°37′ N, 23°19′ E, altitude 1710 m) in April 2010. Samples were cleaned from bark particles and air-dried in the laboratory. The unwashed material (3 g) was placed in a nylon mesh (10 × 10 cm) and 12 lichen-bags from each site were prepared. A novel hanging system (*Fig. 2*) at the same height from the ground (approximately 6-7 m) was used in the four studied sites in the city of Plovdiv (north, east, central and west part). Lichen-bags were exposed to the ambient air from May to October 2010.

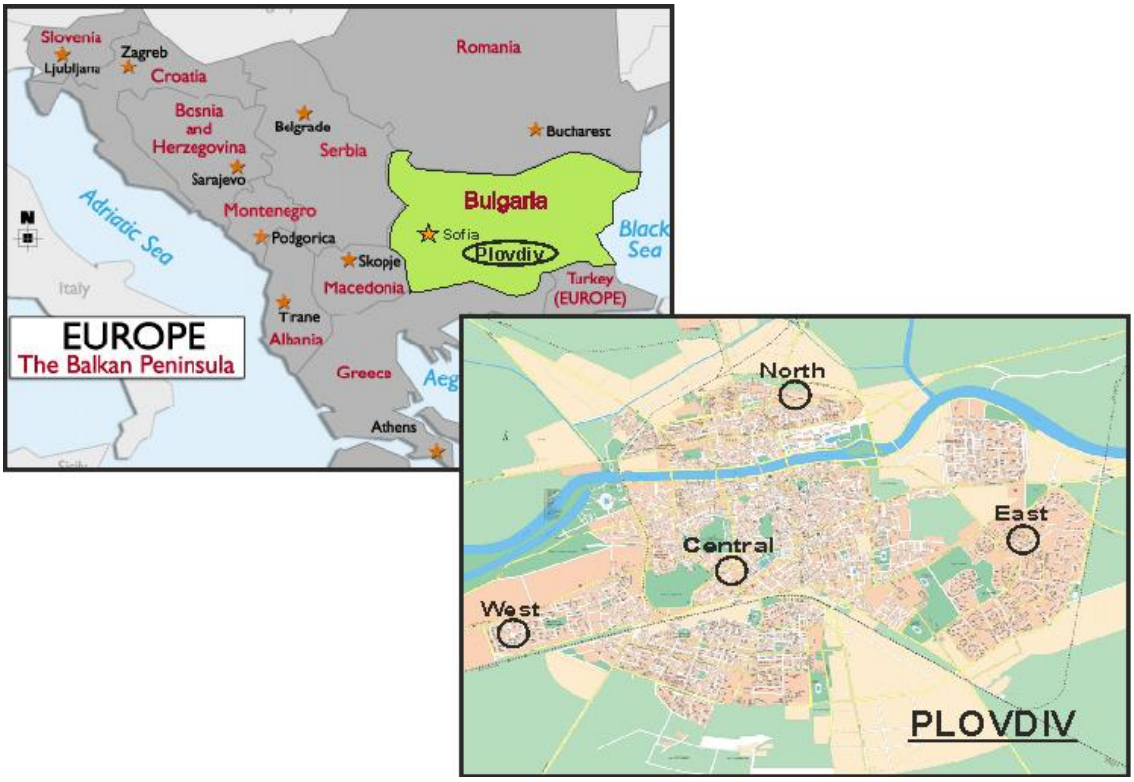

Figure 1. *Plovdiv map, which shows the studied sites' location*

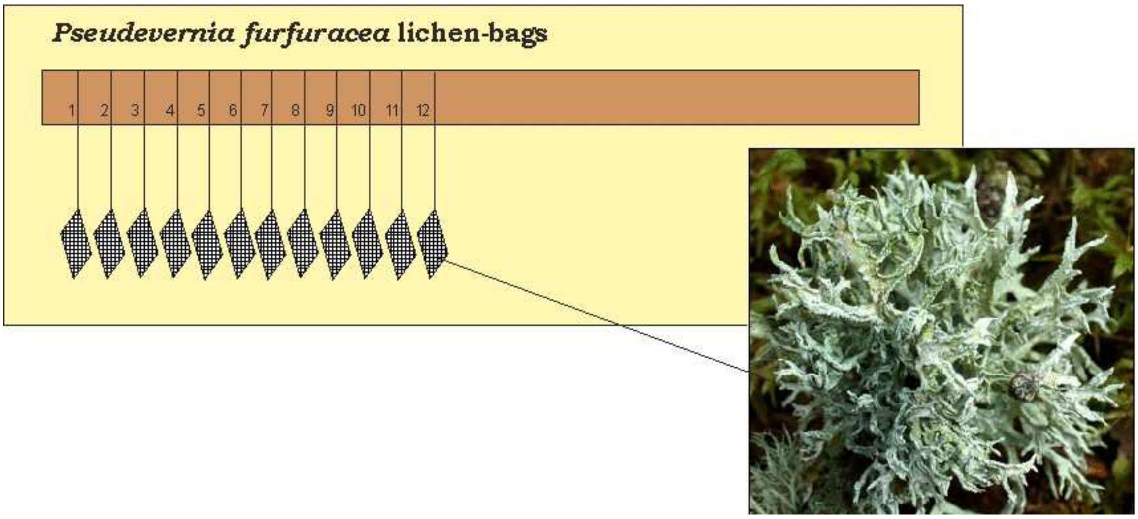

Figure 2. *The novel hanging system with lichen-bags*

At the beginning of the experiment and every week during the study, respectively each lichen-bag was carefully moistened with drops of distilled water. Lichens, which were exposed for one, three and five months, respectively were collected at the end of each period and stored in clean, labeled polyethylene bags until chemical analysis.

Analytical procedures

Approximately 1 g ground plant material was treated with 5 ml 65% HNO_3 (Merck) for 24 h at room temperature. The wet-ashed procedure was carried out with a Microwave Digestion System CEM MDS 81D. Samples were treated for 5 min at maximum power (600W) in closed vessels. After cooling, the vessels were opened and 2 ml HNO_3 and 3 ml 30% H_2O_2 were added and left to react for 1 hour. The vessels of the Microwave Digestion System were closed and treated for 10 min at 600W once again for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

All samples, blanks and standards were spiked with internal standards – Ge 50 ppb and Rh 5 ppb final concentration in the solutions. Calibration standards Multy VI (Merck) were freshly prepared from 1 to 1000 ppb in 0.05 volume % HNO_3 (p.a.). Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotops for the tested elements were measured twice in both modes – without and with helium gas collision cell.

Concentrations of 22 macro- and microelements were analyzed by FAAS (Zn, Fe, K, Mg, Mn, Na, Cu, Ca) using Atomic Absorption Spectrometer PERKIN-ELMER 4000 and ICP-MS method (Al, S, P, Cr, V, Co, Ni, As, Sr, Mo, Cd, Hg, Pb, U) with instrument Agilent 7700 (2009).

Statistical analysis

Data analyses using STATISTICA 7.0. for Windows (StatSoft Inc., 2004) were conducted. One-way ANOVA was applied in order to determine the significant differences ($p < 0.05$) occurring between the element concentrations in the lichen bags from the four studied sites and the unexposed sample.

Results and discussion

Element concentrations in the transplant material of the lichen biomonitor before and after exposure could be compared to track the anthropogenic load in time (*Fig. 3*). Macroelements K (up to 4 times), P and S decreased their concentrations by extending the exposure period ($p<0.05$) in comparison with the unexposed *Pseudevernia furfuracea*. Loss of K in moss-bags after exposure has been observed in Sofia by Culicov and Yurukova (2006) and in many other studies (Yurukova and Ganeva, 1997; Adamo et al., 2003; Culicov et al., 2005), but no additional data in respect to loss of macroelements in lichen-bags were found in the literature.

Elements As, Cd, Hg and U had insignificantly higher concentrations in the unexposed *Pseudevernia furfuracea* in comparison with the exposed samples. A possible reason for this result could be the higher content of these elements in the background area for the material in Vitosha Mountain as similar results were reported for the moss *Sphagnum girgensohnii,* collected in the same region (Yurukova et al., 2013). Furthermore, an interesting result for this group of elements (As, Cd, Hg, U) was the dynamics during the three exposure periods: an insignificant decrease of the concentrations between the control (unexposed sample) and exposed lichen after 30 days period (for Hg – till 90 day), then an increase to 150 day of exposure. Theseelements are connected with burning processes of the fuels, which during the summer were not so heavy as in the winter. Thus a possible explanation for this result could be the emissions emanated from the non-ferrous metallurgy plant, located nearby the city of Plovdiv (to the South direction), as well as the predominant wind from south to north during the period of exposure. This suggestion is supported by the highest concentrations of As, Cd, Hg and U, found in the lichen-bags from the Southern site.

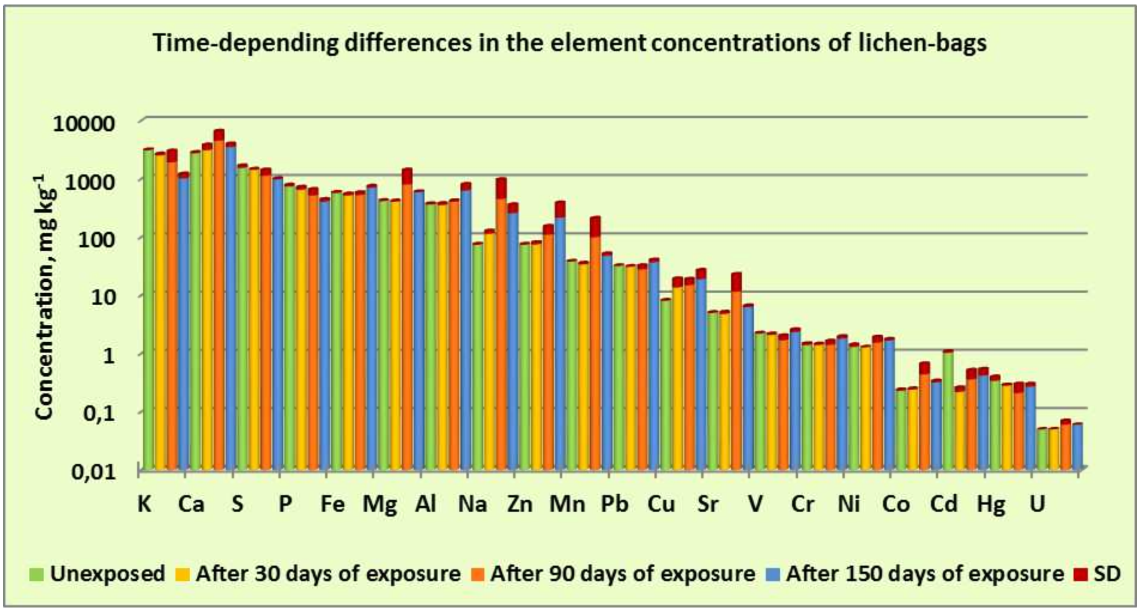

Figure 3. *Element concentrations in Pseudevernia furfuracea bags after 30, 90 and 150 days of exposure in the city of Plovdiv*

Overall, the reasons for these dynamics in the elements concentrations could be explained by the hypotheses of Sanita di Toppi and Gabrielli (1999). According to them, the Cd ions are transported into the cell vacuole of phytochelatins and they are accumulated until the ionic binding to the cell wall is less effective. Metal ions are able to bind to the negative charges on the pectin compounds (e.g., galacturonic acid) from the wall by the dynamic conversion process (passive ion exchange). Moreover, the authors state that the dead plant tissue showed a higher cation exchange capacity compared with the live cells, probably due to the destruction of the membrane, allowing the cations to bind more protein regions than in the normal state. On the other hand, some abiotic factors, such as rainfall, can dissolve and wash out the associated cell-wall elements more easily than in the vacuole.

Elements Al, Cu, Ni and Zn significantly increased their content in lichen-bags with extending the exposure period ($p<0.05$). These data correspond with the finding of many authors that used *Pseudevernia furfuracea* (Basile et al., 2008) and other lichens (Sujetoviene and Sliumpaite, 2013) in urban areas. Element concentrations, which we measured in the lichen-bags from Plovdiv in the present study were higher compared to the results of Basile et al. (2008) from Italy (Cd 1.5 times, Cu 4 times, Mn 1.5 times, Pb, 2 times and Zn 9 times). was quite higher when compared to the data from Italy.

Relative accumulation factors (RAF) were calculated for all elements as follows: the lichen-bags mean content of each element after 150 days of exposure ($C_{exposed}$) in all studied sites was divided by the content of each element before exposure ($C_{unexposed}$).

$$RAF = C_{exposed}/C_{unexposed}$$

The values of RAF are given in *Table 1*. Highest values were found for Na (3.49), Zn (2.92) and Cu (3.33). Culicov and Yurukova (2006) reported similar RAF for Na in lichen-bags from Sofia.

Table 1. *Relative accumulation factors (RAF) of the analyzed elements in Pseudevernia furfuracea bags after 150 days of exposure in Plovdiv*

Element	Mean content in lichen-bags after 150 days of exposure, mg kg^{-1}	Content in unexposed sample *Pseudevernia furfuracea*, mg kg^{-1}	RAF
Al	633	364	1.74
As	0.55	2.0	-0.275
Ca	3535.5	2761	1.28
Cd	0.425	1.03	-0.41
Cr	1.8	1.4	1.29
Co	0.32	0.23	1.40
Cu	18.9	8.1	2.33
Fe	686.75	573	1.20
Hg	0.27	0.34	-0.79
K	1020	3114	-0.33
Mg	595.25	417	1.43
Mn	47.75	38	1.26
Na	258	74	3.49
Ni	1.675	1.3	1.29
P	407.5	740	-0.55
Pb	37	32	1.16
S	984	1547	-0.64
Sr	6.3	5	1.26
U	0.06	0.05	1.2
V	2.35	2.2	1.07
Zn	215.75	74	2.92

Bioaccumulation capability of lichens was determined by their significant cation exchange capacity, which is due to the negatively charged cell wall bases (mostly acidic carboxyl groups), which may also be connected via ionic bonds with the dissolved cations. Elements can be detained and particles can be suspended in the intercellular spaces or on uneven surfaces (Freitas, 1994). Many factors determine the volume and speed of the processes of uptake and release of various elements in lichens, such as chemical characteristics of the element, chemical composition and size of the particles associated with the element, and impact of toxic substances contained in the organism.

In the present study 40 positive and 21 negative correlations were proved for the analyzed elements ($p<0.05$). Strongest positive one was found for P, S and K; followed by the correlation between Na, Ca, Mg, Mn, Ni, Sr Cd and U; as well as Al, V, Pb, Cr and Fe. Negative relationships existed between the macroelements and the group of heavy metals.

Site-depending differences are presented in *Fig. 4*. Mean concentrations of K, Ca, S, P, Fe and Mg proved insignificant differences in the lichen-bags exposed in the studied sites.

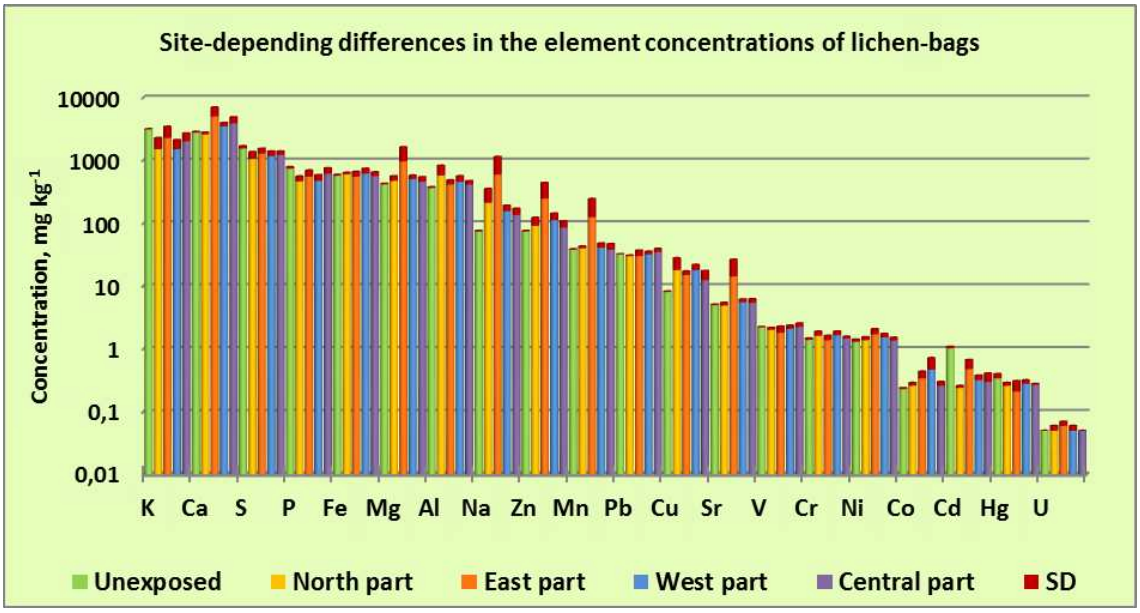

Figure 4. *Element concentrations in Pseudevernia furfuracea bags after 150 days of exposure in four selected sites in the city of Plovdiv*

A tendency to enlarge the number of maximal values of the elements (P, Pb, V, Cr, Hg, Fe) by approaching the central part of Plovdiv was found. The Eastern zone was characterized with highest concentrations of K, Ca, S, Mg, Na, Zn, Mn, Sr, Ni, Cd and U. However, none of the elements had highest concentrations in the Western site. In addition, the applied cluster analysis showed a close relationship between the Northern and Western sites, connected then with the Central zone but quite separated from the Eastern site (*Fig. 5*).

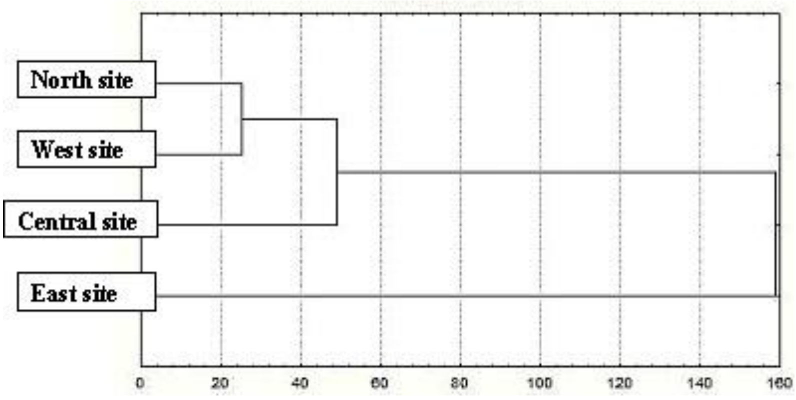

Figure5. *Cluster analysis of the studied sites in the city of Plovdiv based on element content in Pseudevernia furfuracea bags after 150 days of exposure*

Conclusions

In general, in the present study it was attempted to describe and assess the atmospheric pollution levels in the city of Plovdiv using lichen-bags technique for three periods. Na, Mn, Cu, Zn and Sr increased significantly their concentrations in the lichen-bags, whereas K, S and Cd decreased theirs in comparison with the unexposed lichenized fungi. Highest concentrations of 11 elements were measured in the collectors, which were exposed in the Eastern site of the urban area, probably due to non-ferrous complex nearby.

Pearson`s analysis showed a strong positive correlation between the macronutrients P, S and K, followed by the correlation between Na, Ca, Mg, Mn, Ni, Sr, Cd and U, and lastly between Al, V, Pb, Cr and Fe, which mainly indicates the vehicle emissions impact.

We can conclude that this study confirms that the species *Pseudevernia furfuracea* is an efficient element accumulator in lichen-bags exposed in urban areas. Moreover, the results from this preliminary study can be successfully used in future comparative analyses for the atmospheric pollution and risk assessment in similar urban areas.

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DO WEATHER AND MOON HAVE ANY INFLUENCE ON SPOTLIGHTING MAMMALS? THE CASE OF HARE IN UPLAND ECOSYSTEM

SOKOS, C.[1,2,3*] – PAPASPYROPOULOS, K. G.[3] – BIRTSAS, P.[3,4] – GIANNAKOPOULOS, A.[1] – BILLINIS, C.[1,2]

[1]*Laboratory of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Trikalon 224, 43100 Karditsa, Hellas (Greece)*

[2]*Department of Biomedicine, Institute for Research and Technology of Thessaly, Centre for Research and Technology Hellas (CERTH), Larissa, Hellas*

[3]*Research Division, Hunting Federation of Macedonia and Thrace, Ethnikis Antistasis 173-175, 55134 Thessaloniki, Hellas*

[4]*Laboratory of Wildlife, Department of Forestry and Management of Natural Environment, Technological Institute of Thessaly, End of Mavromichali Str., 43100 Karditsa, Hellas*

**Corresponding author*
e-mail: sokos@vet.uth.gr

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Abstract. Nocturnal spotlight survey is a common census technique for many mammal species. The influence of abiotic factors may be essential for the appropriate design of censuses, however this method has not been examined sufficiently in relation to abiotic factors such as weather conditions and the moon. We tested their influence for a commonly counted species, the brown hare (*Lepus europaeus*). Spotlight surveys were conducted at certain night time and transects in three upland Mediterranean areas, at different times of the year, different moon phases and under a variety of weather conditions. Wind speed and direction had an influence on hare counts with stronger winds resulted in fewer juvenile hares being observed, and more hares were counted when wind was blowing from the hare towards the vehicle. No significant effects were found from the other factors probably due to the intense relief and thick vegetation of the studied areas.

Keywords: *activity, abundance, census method, lagomorphs, mammals.*

Introduction

Spotlighting (spotlight count or night lighting) is widely used to survey mammals during night (Scott et al., 2005; Sunde and Jessen, 2013). This is a favored method of estimating abundance due to its efficiency, repeatability and no necessity for animals capture (Langbein et al., 1999). However, numerous studies have shown that several factors can affect the spotlight counts of mammals. These include the animal's activity patterns and behaviour (Brown and Peinke, 2007), visibility (Parkes, 2001; Barnes and Tapper, 1985), relief and amount of vegetation cover (Ralls and Eberhardt 1997; Langbein et al., 1999; Tizzani et al., 2013), observer experience (Sunde and Jessen, 2013), time of night and moonlight (Langbein et al., 1999; Scott et al., 2005) and weather conditions (Langbein et al., 1999).

Spotlighting is a very common survey method for lagomorphs (Strauß et al., 2008; Tizzani et al., 2013) and studies reveal sources of variation in counts due to weather conditions and moon phase. Newman (1959) found that the number of cottontails (*Sylvilagus floridanus*) observed during early morning or late afternoon roadside counts was higher with increasing

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snow cover, frost, rain preceding the count, and decreasing temperature and wind speed. In contrast significantly fewer brown hares were spotlighted when the temperature fell below 15°C in Britain (Barnes and Tapper, 1985). Heydon et al. (2000) found no influence of rain or visibility on brown hares counted in Britain. Fafarman and Whyte (1979) refer that cottontail spotlight counts tended to be higher when rain was falling during the count and lower when moon illumination was increasing. Wind direction had also an influence, but no other weather factor affected their counts. In an arid region of Jordan, moonlight had a varied effect on spotlight counts of cape hare (*Lepus capensis*), but with similar densities during full and no moon (Scott et al., 2005).

The above results seem to be contradictory for some factors and inconclusive for some others; one explanation may be the differences in abiotic and biotic conditions between geographical areas and differences between lagomorph species behaviour. Therefore, there is currently a difficulty in designing a study that takes weather and moonlight effects into account; consequently, their influence is generally overlooked in hare spotlight counts (Strauß et al., 2008; Sunde and Jessen, 2013). In upland and shrubland ecosystems in particular we are unaware of any studies concerning the influence of abiotic factors on spotlighting brown hares. Thus, the objective of this study was to determine the influence of moonlight and weather conditions on the number of brown hares counted along spotlight transects.

Materials and Methods

Study area

The study was part of a larger research project on the influence of wildfires on brown hare that was carried out in the Kassandra Peninsula of Chalkidiki Prefecture, Macedonia, northern Hellas (*Fig. 1*).

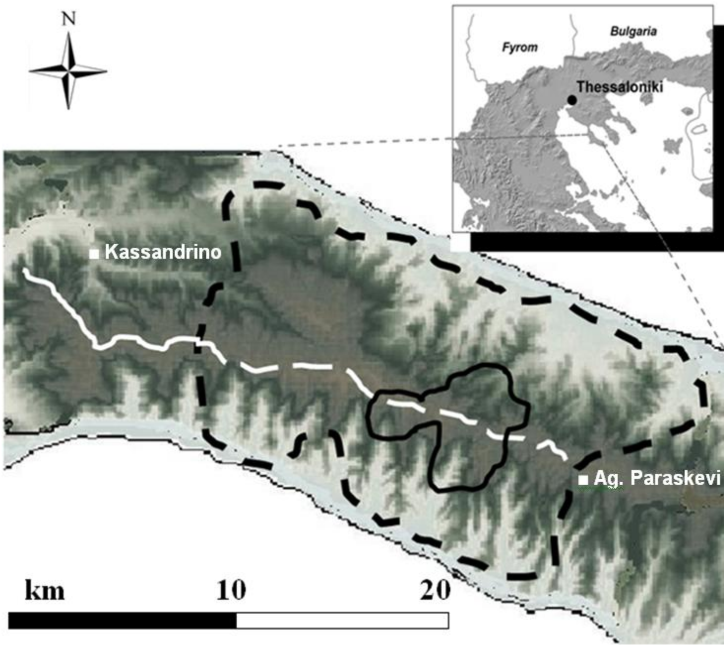

Figure 1. *Location of the study area. The dashed black line indicates the moderately burned area and the solid black line denotes the intensely burned area. The solid white line shows the transect within the unburned area, and the dashed white line shows the two transects within the burned areas*

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The Kassandra Peninsula has altitudes ranging 150-280 m above sea level, and the climate is Mediterranean with hot and dry summers, mild winters, and moderately rainy autumns and springs. Mean annual precipitation is 581 mm and the mean annual air temperature 16.3°C. The landscape has intense relief with steep slopes in some positions.

The study area is covered mainly by forest vegetation of Aleppo pine (*Pinus halepensis*) and broadleaved shrubs (*Quercus coccifera*, *Pistacia lentiscus*, *Phillyrea media*, *Cistus incanus*), while there are also scattered agricultural fields, mainly comprising olive groves, cereal and few vetch crops. Wildfire occurred in Kassandra on 21/8/2006 and burnt an area of 68.7 km². The woody plants regenerated quickly after wildfire and shrubs resprouts reached the height of about half a meter in the first year after the wildfire.

The spotlight transects stretch along the ridge of the peninsula and the unsurfaced forest roads. The transects had a total length of 22 km: 8.5 km passing through unburned areas, 9.5 km through moderately burned areas, and 4 km passing through intensely burned areas (*Fig. 1*).

Spotlight counts

Hares were spotlighted in the study area at different times of the year, under different weather conditions and moon phases, but along the same transects and at the same time of night and all transects were surveyed each night. To avoid observer bias, almost the same crew undertook surveys in each visit. Hares were counted from a 4-wheel truck traveling at about 15-20 km/h on forest roads. The crew consisted of the driver and two observers, each one holding two million candle spotlights which were used to light up both sides of the road. Surveys began two hours after sunset (Verheyden, 1991; Langbein et al., 1999). Visits were postponed during intensive rains and in thick mist. About one visit was carried out each month in spring and summer and two visits in autumn and winter. The survey lasted for two years during the first and second year after wildfire, and the total number of visits was 30.

Additionally, as most of the hares were seen initially or were approached later at a distance of less than 30 meters, it could be distinguished which hares weighed more than 3 kg (developed hares) and which less than 2.5 kg (juvenile hares), that is younger than three months old (Bray et al., 2002). Those which could not be seen well or appeared intermediate in weight were not categorized.

Weather conditions and moon phase

According to the literature concerning influences on spotlight counts, we selected nine explanatory variables to describe the variable “hare counts” (*Table 1*). During each visit, wind level and cloud cover were recorded. Available climatic data for the study area on each visit day were obtained from the nearest climatic station of the National Observatory of Athens (http://penteli.meteo.gr/meteosearch/). Precipitation values of the visit day and two days before, the mean air temperature, wind speed and direction were taken into consideration in all analyses (*Table 1*). Moonlight illumination values for each day were obtained from the US Naval Observatory (http://aa.usno.navy.mil/data/docs/MoonFraction.html). The level of moonlight was adjusted by multiplying illumination of the moon and cloud cover according to Brown and Peinke (2007). Cloud cover was categorized as 1=no cloud cover, 0.5=intermediate, 0.1=complete cloud cover. We used 0.1 instead of zero for complete cloud cover, because the darkest of the nights with a full-moon was brighter than any new moon nights, as the moonlight penetrated the clouds (Griffin et al., 2005).

Table 1. *Weather and moon variables and their values during the counts*

Variable	Meaning	Type	Mean ± SE	Min.	Max.
Moon	Degree of moon appearance during the visit	Scale	0.42±0.07	0	1
Clouds	Degree of cloud cover during the visit	Ordinal	1^a	0.1	1
Moonlight	Interaction of moon and clouds during the visit	Scale	0.28±0.06	0	1
Temperature	Mean temperature on the day of the visit (°C)	Scale	13.4±1.55	0	33.8
Rain1	Precipitation during the day of visit (mm)	Scale	0.3±0.16	0	4
Rain2	Precipitation during the day of visit and one day before (mm)	Scale	2.11±1.03	0	23.6
Rain3	Precipitation during the day of visit and two days before (mm)	Scale	3.79±1.25	0	23.6
Windspeed	Mean wind speed on the day of the visit (km/h)	Scale	8.1±0.91	1	22.7
Windir	Direction of wind on the day of the visit	Circular	east^b	-	-

^{a}median
^{b}the mode value of wind direction

Statistical analysis

The hares observed during each visit were divided into two categories, each one consisting of three dependent variables. The first category was relevant to the hares' age. It included the variable "juv" with the juvenile hares, the variable "dev" with the developed hares, and the variable "total" with the sum of counted hares. The second category was relevant to the study area. It included the variable "intBurned" with the observed hares/visit in the intensely burned areas, the variable "modBurned" with the observed hares/visit in the moderately burned areas, and the variable "unburned" with the observed hares/visit in the unburned areas. We also used an additional variable, "totBurned", which was the sum of "intBurned" and "modBurned" variables.

As shown in *Table 1*, our aim was to examine the influence of seven scales, one ordinal and one circular variable to the dependent variables. Circular variables have a special statistical analysis not suitable for the usual types of variables, and for this reason we transformed Windir into two variables sinWindir and cosWindir. That is, we followed the transformation suggested by Jammalamadaka and Lund (2006) by computing the sine and cosine of the wind angular measurement in order to use it in the typical statistical analysis of weather and moon data.

Firstly, descriptive statistical methods were used to analyze the data. We computed mean values, standard errors and median values of the variables. We then used the non-parametric Sign test. This test is robust to normality and homogeneity of variance and makes no assumptions about the original distributions of the variables (Gray and Kinnear, 2012). The test was used to examine if there was a statistical significant trend in hare counts due to hare abundance changes and other factors.

When a statistical significant difference was confirmed, we removed the trend from the series of observations. This is a common procedure in time series analysis (Box et al., 2008). To determine the real influence of the weather and moon variables on the hare population, one has to remove any existing population trend. In the time series $\{y_t\}_{t=1}^{n}$, the trend can be removed if we create a new time series $\{x_t\}_{t=1}^{n-1}$ using the

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equation (Chatfield, 2004): $x_t = y_t - y_{t-1}$. We used the Kendall tau-b correlation coefficient to relate the new series with the weather and moon variables. The analysis was performed using the statistical software IBM SPSS 20.0 and the significance level was set to P<0.05 and tending towards significance at P<0.1.

Results

A total of 30 visits to the study area identified 104 hares in a total distance of 660 km. During the counts, the moon was usually below crescent, but in four visits it was around full moon and cloud cover was absent (*Table 1*). Mean daily temperature varied from zero in winter to 33.8°C in summer. There was no snowfall and rainfall was low on the day of the count, although for the period of two days before the visit, rainfall >10mm was recorded in five visits. A light breeze blew during most counts with a maximum speed of 22.7 km/h (tree branches move).

The Sign test confirms that there is an increasing trend of observed hares in the second year after the wildfire in the intensely burned and the moderately burned areas of the study area (P = 0.016 and P = 0.004 respectively). The unburned areas show no trend (P = 1.0). Therefore, the procedure of differencing was applied in order to eliminate the trends. An example in *Fig. 2* shows the result of differencing the trended series. The left Figure shows the initial total count of hares, where a trend is confirmed, and the right Figure shows the detrended series.

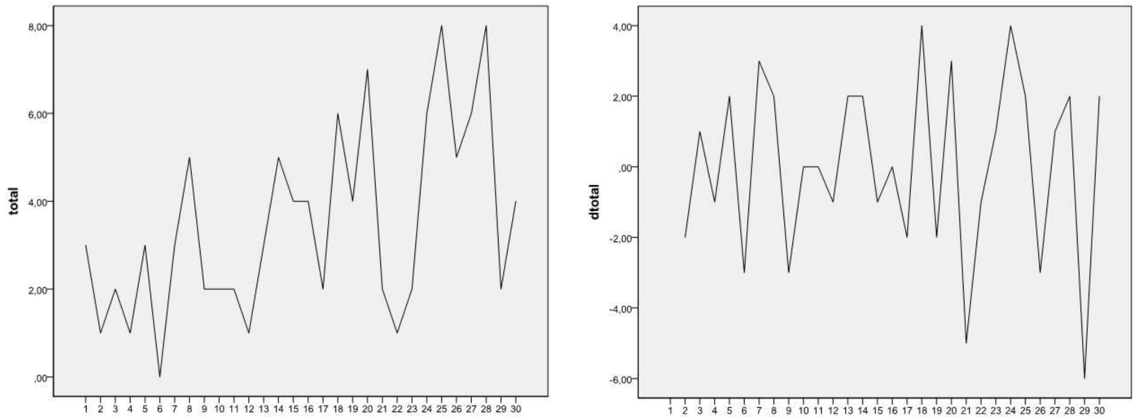

Figure 2. *Initial (left) and detrended series (right) of hare counts during the 30 visits (total of counts from the three transects)*

The detrended series were checked for their bivariate correlations with the weather and moon variables. *Table 2* shows that significance at the 10% level was found between wind speed and the counts of juvenile hares, showing a negative linear correlation. This means that stronger winds resulted in fewer juvenile hares being observed (*Fig. 3*). This relationship is marked in bold in *Table 2*. Another marked relationship, the one between intensively burned areas and the sine of Wind Direction (sinWD), indicates that east winds are coincided with an increased number of recorded hares.

Table 2. *Kendal's tau-b correlation coefficient of counted hares and weather and moon variables. The bold numbers mark significant relationships at the P<0.1 significance level*

Variable	moon	clouds	illumin	temp	rain1	rain2	rain3	windspeed	sinWD	cosWD
juv	0.163	0.087	0.106	0.124	-0.136	-0.046	-0.044	**-0.267**	0.057	0.109
P	0.252	0.597	0.453	0.378	0.404	0.772	0.775	**0.058**	0.705	0.474
dev	-0.048	-0.024	0.005	-0.010	-0.151	0.092	0.045	0.205	-0.071	0.009
P	0.727	0.880	0.971	0.942	0.344	0.555	0.765	0.138	0.636	0.951
total	-0.115	-0.063	-0.144	0.026	-0.161	-0.158	-0.006	0.026	0.202	0.032
P	0.403	0.690	0.295	0.849	0.306	0.310	0.967	0.849	0.172	0.831
intBurned	-0.194	-0.012	-0.215	-0.041	0.102	-0.158	0.127	0.163	**0.300**	0.047
P	0.184	0.943	0.141	0.775	0.544	0.340	0.420	0.261	**0.057**	0.765
modBurned	-0.115	-0.080	-0.085	0.057	-0.110	-0.214	-0.023	-0.076	-0.093	-0.205
P	0.415	0.618	0.547	0.684	0.492	0.178	0.881	0.587	0.539	0.174
totBurned	-0.153	-0.050	-0.166	0.035	-0.048	-0.206	0.067	0.013	-0.019	-0.188
P	0.274	0.754	0.234	0.803	0.765	0.191	0.658	0.924	0.897	0.210
unburn	0.097	0.119	0.074	-0.019	-0.186	0.148	0.052	-0.036	0.131	0.197
P	0.504	0.474	0.607	0.894	0.263	0.364	0.740	0.804	0.395	0.202

juv: juvenile hares, dev: developed hares, total: sum of juvenile and developed hares, intBurned: total hares in the intensely burned areas, modBurned : total hares in the moderately burned areas, unburned: total hares in the unburned areas, totBurned: sum of intBurned and modBurned

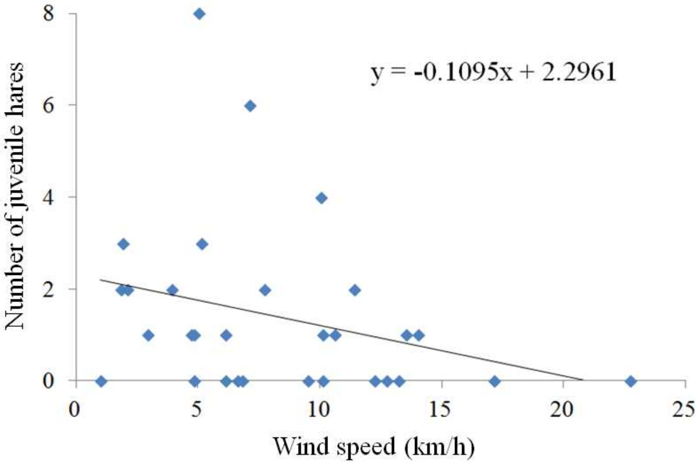

Figure 3. *Total number of juvenile hares which were counted from the three transects in each visit in relation with wind speed*

Discussion

Analyses on spotlight counts showed that wind speed and wind direction influenced counted hares in some cases. Similarly, Newman (1959) found that the number of cottontails observed during roadside counts was higher with decreasing wind speed. Wind causes the moving of vegetation and thus the ability of observer to detect the hares and especially the smaller juveniles appears to be decreased. Moreover the movable of vegetation may cause fear to juveniles and thus the decreasing of their movements. High speed winds have been associated with decreased activity in lagomorphs (Lechleitner, 1958; Smith, 1990; Villafuerte et al., 1993).

From the other side, the movement and the noise by the wind-blown vegetation apparently make the detection of the approaching vehicle more difficult for hares and thus hares may stay nearer to the road and thereby the probability of be counted is increased. Perhaps this is why a positive relationship was found, although not significant, between the intensity of the wind and the recorded developed hares, which as more experienced than juveniles may know that should go away when a car is approaching and this was observed during this study. Other studies have also found that the distance at which a mammal move away from an approaching human is negatively correlated with wind speed (Karlsson et al., 2007). Moreover, wind direction was also significantly associated with hares counted in intensely burned area, which tended to increase when the wind was blowing from the east direction, this means from the hare to vehicle (*Fig. 1*). The east wind appears to take away the noise under the circumstances of intensely burned area with a straighter west-east road than in other areas.

Analyses on spotlight counts indicated that no other weather or moon variable had an impact on the counted hares. Precipitation variation was relatively small and rainfall in the day of visit was below 2.5mm, under these conditions none important influence of rains was detected. These conditions provided us the opportunity to better examine the influences of temperature and moonlight. However, none appeared to have any effect on hare counts.

In our study area the steeply upland area and the thick vegetation do not permit the observation of hares at long distances as in open areas, thus in upland landscapes, such as the Kassandra Peninsula, the counting of hares seems to depend more on randomness: that is if a hare is close to the road at the time the car passes. Nevertheless, in flat landscapes hares can be observed at greater distances. In this case the observer's ability and hare's behaviour may have a higher importance for the detection, and may be more influenced by weather and moon. This may explain why in Britain it was proved that temperature reduced brown hare activity, with significantly fewer hares spotlighted when the temperature fell under 15°C (Barnes and Tapper, 1985). For other lagomorph species, Newman (1959) found that the number of cottontails counted increased with the decrease of temperature, and Rogowitz (1997) found that temperature had no significant effect on movements of white-tailed jackrabbits (*Lepus townsendii*).

Many nocturnal mammals react to increasing moonlight by restricting their movements and the use of open areas to avoid detection by predators (Kolb, 1992; Beier, 2006). In Ireland, moonlight negatively affected counts of Irish hare (*Lepus timidus hibernicus*) during winter in areas where the vegetation is usually short (Reid et al., 2007). The same was found for white-tailed jackrabbit that reduces its activity during periods of greater moonlight in grasslands (Rogowitz, 1997).

In our forested study area, the vegetation, even in burned areas a few months following the wildfire, provided adequate cover for hare, a fact that may decrease the detection probability by predators and the moonlight influence. A similar case may be that of snowshoe hares (*Lepus americanus*) which are less active in open areas during nights with increased moonlight in winter (Gilbert and Boutin, 1991; Griffin et al., 2005), but this was not found in the snow-free season when the vegetation cover and hiding opportunities were higher (Griffin et al., 2005). In contrast, black-tailed jackrabbits in northern Utah were more active when moonlight was more intensive during fall, winter and spring. During summer moonlight did not appear to influence jackrabbit activity (Smith, 1990).

In conclusion, only wind speed and direction had an influence on hare counts tending towards significance. Thus, the researcher should expect to see fewer juvenile hares in windy days, something that would not apply to developed hares. Yet if the wind is blowing from the hares towards vehicle the researcher is likely to count more hares. Temperature and moon did not influence spotlight counts. Precipitation also had no effect possibly due to its low variability in this study. In a future study, will be a need to test the method in relation to weather and moon in different ecotopes, especially concerning relief and vegetation structure.

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ANALYSIS OF LAND COVER CHANGES IN THE BONSA CATCHMENT, ANKOBRA BASIN, GHANA

ADUAH, M. S.* – WARBURTON, M. L. – JEWITT, G.

Centre for Water Resources Research, University of KwaZulu-Natal, PBAG x01, 3209,
Scottsville, Pietermaritzburg, South Africa.
(phone:+27-033-260-5678; fax: +27-033-260-5818)

**Corresponding author*
e-mail: msaduah@umat.edu.gh

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Abstract. Multi-temporal land cover changes in the Bonsa catchment of the Ankobra Basin in Ghana were determined using four satellite images from 1986, 1991, 2002 and 2011. The results indicate that evergreen forest is the largest class occupying an area of 68% in 1986, 62% in 1991, 50% in 2002 and 51% in 2011 and during the past 26 years, the largest land cover change has been the conversion of evergreen and secondary forests to shrubs/farms, mining areas and settlements. During this period, mining areas increased over two-fold, while settlements and shrubs/farms increased more than three and four-fold, respectively. This resulted in increasing annual deforestation rate of 0.33% between 1986 and 1991, 0.70% between 1991 and 2002 and 2% between 2002 and 2011. The results suggests that the drivers of the land cover changes in the Bonsa catchment, are both local and global and include, international trade, local population growth, agriculture extensification and urbanization. The identified land cover changes have the potential to impact negatively on the hydrological regimes of the Bonsa River and the local communities by leading to flooding, soil erosion and the siltation and pollution of the river during peak seasons and the scarcity of water during dry seasons. Therefore, the maps and statistics generated can be applied to assess the impacts of the land use changes on the local hydrology and provide a better basis for future land use planning. Through these findings the importance of multi-temporal analysis of satellite imagery for planning in data poor regions is highlighted.

Keywords: *Bonsa catchment, Ghana, hydrological regimes, land cover change, multi-temporal analysis*

Introduction

Land cover/land use changes occur by the alteration of the natural landscape through both anthropogenic activities and natural processes (Coppin et al., 2004; Hu et al., 2005). Natural causes include volcanoes, storm surges, floods, droughts, earthquakes and wildfires, while anthropogenic land cover/land use changes are influenced mainly by socio-economic drivers such as population growth, rural-urban migration, immigration, urbanization, government policy and economic development (Lambin et al., 2003; Cohen, 2004; D'Orgeval and Polcher, 2008). These changes in land use, in turn impact the hydrological responses of a catchment by altering the partitioning of precipitation into different components of the hydrological cycle, such as interception, infiltration, runoff, evapotranspiration and groundwater recharge rate (Costa et al., 2003; D'Orgeval and Polcher, 2008), as well as influencing water quality (Rogers, 1994; Randolph, 2012). To predict and manage the impacts of land cover/land use changes on hydrology, it is imperative to understand the land cover/land use change processes, the rates and possible causes of the changes. Satellite sensors, with their wide spatial and synoptic coverage over large areas, can provide spatially continuous and consistent data, which can be analysed to derive land cover maps and change statistics

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(Foody, 2002), required for effective land use planning and water resources management in a catchment. Such land cover maps and change statistics can be derived through the process of change detection and land cover mapping (Song et al., 2001).

Land cover/land use change detection is the process of categorizing differences in the state of the land cover by mapping it at different times over a period (Lambin and Ehrlich, 1997). The analysis of satellite images to detect land cover/land use changes is, based on the assumption that the recorded electromagnetic radiation, which is the basis of categorizing land covers, is altered as the land cover/land use of the same geographic area changes (Lambin and Ehrlich, 1997; Abuelgasim et al., 1999). Change detection techniques can be categorized as algebraic, transformation, classification and visual analysis techniques (Coppin et al., 2004). Algebraic based methods include image differencing, image regression, image ratioing, normalised difference vegetation index (NDVI) differencing and change vector analysis (CVA) (Braimoh and Vlek, 2004; Coppin et al., 2004), while those in the transformation category include multi-date Principal Component Analysis (PCA), Kauth-Thomas (KT) and Chi-square transformations (Coppin et al., 2004). The classification methods consist of post-classification comparison, multi-date classification, spectral–temporal combined analysis and unsupervised change detection (Coppin et al., 2004; Braimoh and Vlek, 2005). Algebraic and transformation methods are suitable for detecting continuous changes, while classification methods are effective for categorical changes (Abuelgasim et al., 1999), but depend on the accurate geometric registration and classification of individual images (Coppin et al., 2004). Continuous changes refer to changes in the concentration or amount of an attribute (e.g. biomass and the leaf area index of a forest), while categorical changes are the conversion of one land cover type to another (e.g. evergreen forest to a mining area). Visual analysis techniques (Lu et al., 2004) are primarily based on the visual interpretation of aerial photographs and high resolution images.

Image classification techniques, using either unsupervised or supervised classification (Campbell, 2002; Foody, 2002; Richards and Jia, 2006) can be used to implement the post-classification comparison methods. However, supervised classification systems have found a wider usage, because it is possible to create specific thematic classes (Campbell, 2002) with respect to the objectives of a study. Supervised classification systems can be grouped as either parametric or non-parametric methods. The parametric methods include maximum likelihood classification (MLC) (Campbell, 2002; Richards and Jia, 2006), fuzzy-set classifiers (Suresh Babu and Viswanath, 2009; Stavrakoudis et al., 2011), sub-pixel classifiers (Verhoeye and De Wulf, 2002; Frazier and Wang, 2011), spectral mixture analysis (Nichol et al., 2010; Youngentob et al., 2011) and object-oriented classifiers (Geneletti and Gorte, 2003; Platt and Rapoza, 2008). The non-parametric methods include artificial neural networks (ANN) (Atkinson and Tatnall, 1997; Abuelgasim et al., 1999; Laurin et al., 2013), decision tree and support vector machines (Huang et al., 2002). In terms of image classification accuracies, literature shows that the ANN and the MLC algorithms perform better than the other methods (Atkinson and Tatnall, 1997; Abuelgasim et al., 1999; Laurin et al., 2013). Nevertheless, the application of the statistically-based MLC algorithm is more common, as it is widely accessible in much remote sensing software and it is considered easier to implement, compared to ANN (Richards and Jia, 2006; Lu and Weng, 2007).

When applying methods of land cover change detection, the advantages vary, depending on the techniques employed and the nature of the change under investigation.

According to Coppin et al. (2004) among others, complementary use of more than one technique has the potential to generate the best results. Nonetheless, available literature indicates that post-classification comparison techniques are preferred by many researchers (Braimoh and Vlek, 2004; Aduah and Aabeyir, 2012; Kumi-Boateng et al., 2012; Wasige et al., 2013). It is advantageous to use post-classification comparison methods because individual images are classified separately, which minimizes the requirement to conduct absolute radiometric corrections and the techniques also produce change matrices (Coppin et al., 2004), which are needed to identify change trajectories. The methods are also simple and accessible in a variety of software and can be used to detect categorical land cover changes.

Previuos studies (Kusimi, 2008; Schueler et al., 2011; Kumi-Boateng et al., 2012) within the Ankobra River basin of the Western region of Ghana indicates that there is extensive deforestation, as a result of increasing urbanisation, mining and farming. Even though these studies analysed land cover changes for the Wassa West District within the Ankobra River basin (Kusimi, 2008; Schueler et al., 2011; Kumi-Boateng et al., 2012), none covered the entire Bonsa sub-catchment nor applied the results of the studies to project potential future land use changes, as well as assessing the impacts of the land use changes on catchment hydrology. As a first stage in projecting potential future land use changes and assesing their impacts on Bonsa catchment hydrology, a more recent and multi-temporal land cover information for the entire catchment is needed. In this study, the post-classification comparison and the MLC methods were selected to map and to detect the multi-temporal land cover changes in the Bonsa sub-catchment of the Ankobra River basin in Ghana, West Africa, over a period of 26 years (i.e 1986 to 2011). The study seeks to determine the rates of change in the land cover types, the transitions between different land cover types and to elicit an understanding of the possible drivers of the land cover changes and their impacts on the environment.

Methodology

Description of Study Area

The Bonsa catchment, a sub-catchment of the Ankobra River basin in Ghana, West Africa (*Figure 1*), is located between longitudes 1° 41′ and 2° 13′ West and latitudes 5° 4′and 5° 43′ North and it straddles the intersection of four administrative districts, namely: Twifo Heman Lower Denkyira to the north, Tarkwa Nsuaem and the Prestea-Huni Valley to the west and Mpohor Wassa East to the east. The catchment has a generally low relief, with the elevations ranging between 30 and 340 m above mean sea level and it drains an area of 1482 km^2. The rainfall regime is bimodal, with the peak season between February and July and the minor season between August and November. The rainfall ranges between 1578 mm and 1982 mm per annum and the annual average minimum and maximum temperatures are 22ºC and 32ºC, respectively. Predominant land cover consists of thick evergreen and secondary forests, with scattered shrubs and farms. The basin's geology is characterized by Birimian and Tarkwaian rock systems (Akabzaa et al., 2009), while the soil is composed mostly of Ferric Acrisols, according the Food and Agricultural Organisations'(FAO) soil classification system and forest oxysols (Dwomo and Dedzoe, 2010), according to the Ghana soil classification system. Major economic activities in the catchment include open-pit gold mining, rubber cultivation and small-scale cocoa and food crop production.

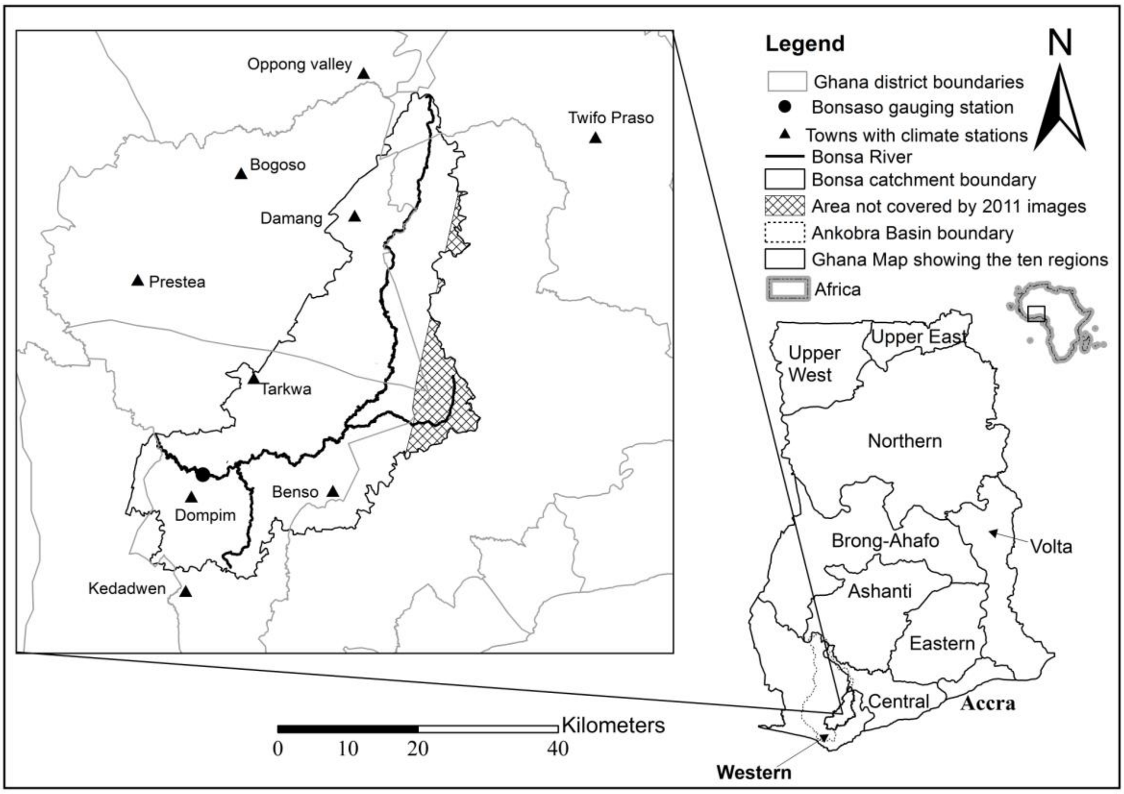

Figure 1. Map of Bonsa catchment, a sub-catchment of the Ankobra River basin in Ghana, West Africa

Data Acquisition

Data used for the study included two digital topographic maps obtained from the Survey of Ghana (SOG). One of the maps is part of the SOG's 1:5 0000 series topographic maps produced in 1974 for the whole country, while the other is a 1:5 000 topographic and detail map (produced in 2003) covering only the urban area of Tarkwa. Four satellite images covering the period 1986 to 2011 were also acquired from the United States Geological Survey (USGS) and the European Space Agency (ESA) (*Table 1*).

Table 1. *Satellite images used for the study*

Sensor	No. bands	Path/row orbit/frame	No. scenes	Spatial resolution (m)	Acquisition date	Source
Landsat TM/ETM+	7, 8	194/056	3	30, 60	12/1986, 01/1991, 01/2002	USGS
ALOS AVNIR-2	4	26495/3490, 26495/3500	2	10	01/2011	ESA category 1 proposal (Third party mission data)

In selecting the satellite images, near-anniversary image acquisition dates were selected to minimize seasonal vegetation differences. Fieldwork was conducted between July and August 2013 to collect ground truth data for image classification. The field data collection was based on an unsupervised classified map of the 2011 satellite images. In order to obtain information on the land cover for the historical images, the local knowledge of the author and that of some inhabitants of the sites visited, was applied, as well as the SOG topographic map for 2003.

Image Pre-processing

Errors in mapping land cover/land use changes can be reduced by performing image pre-processing before classification and change detection methods are applied. There are two main types of image pre-processing: geometric and radiometric corrections (Campbell, 2002; Narumalani et al., 2002; Richards and Jia, 2006). The geometric distortions resulting from sensor and platform errors, as well as the rotation of the Earth in relation to the sensor, are corrected by image registration. Geometric correction converts the satellite image geometry to real world coordinate and projection systems (Narumalani et al., 2002; Lu and Weng, 2007), to enable the comparison of two or more images, the extraction of information for use in a Geographic Information System (GIS) and to overlay images with maps from different sources. Radiometric correction, on the other hand, corrects for the effects of the atmosphere on satellite images (Campbell, 2002). The objective of radiometric correction in change detection is to ensure that available images are compared on the basis of similar radiometric properties (Coppin et al., 2004). Image pre-processing in this study consisted of only geometric correction. All the images had been geo-referenced to the UTM WGS84 Zone 30 North projection by the data suppliers, but they did not match with the geo-referenced topographic map of the study area. Hence each of the images was geometrically corrected, using the study area topographic map as a reference. The high resolution Advanced Visible and Near Infrared Radiometer Type 2 images of the Advanced Land Observation Satellite system (ALOS AVNIR-2) were first geometrically corrected after which the rest of the images were co-registered to them. Haze removal was not executed, because available algorithms distorted the images and reduced their quality. However, haze was not considered problematic since the change detection technique adopted was post-classification comparison, which relies on classified individual images. As the Bonsa catchment was the area of interest for the study, images were clipped, based on the catchment boundary (*Figure 1*).

Image Classification and Accuracy Assessment

Image classification was conducted by generating spectral signatures, using training samples created for each satellite image. The training samples were created by randomly selecting 70% of the sample class data for each satellite image. Six thematic classes were selected to represent the land cover of the Bonsa catchment, using the USGS's land cover classification scheme for Landsat data (Jensen, 2000). The classes used are secondary forests, water, evergreen forests, settlements, shrubs/farms and mining areas (*Table 2*). After creating the spectral signatures, the separability of the thematic classes was checked using the Jefferies-Matusita's matrices (Richards and Jia, 2006), and finally, the MLC algorithm was used to classify all the images using the generated spectral signatures. Since the ALOS AVNIR-2 images covered only 92% of the Bonsa Catchment, a strip of a 2009 SLC-off Landsat image was used to map the remaining (*Figure 1*) and it was merged with the land cover data from ALOS AVNIR-2 images to produce land cover data for 2011. It was observed from a field visit that the study area not covered by the 2011 images was part of a protected forest reserve (evergreen forest area as at January 2014). Hence in the absence of a current satellite image for the area, it was reasonable to map its land cover using the 2009 image, which depicts the area as evergreen forest.

Table 2. *Land cover classification Nomenclature*

Land cover	Description	Photo
Water	water courses, ponds/flooded mine pits and rivers	
Shrubs/farms	short tree species and non-tree vegetation such as herbs, grasses and farms (cocoa, palm, plantain, cassava, maize), recently cleared forests	
Evergreen forest	tall trees including indigenous species and mature rubber located mostly in forest reserves and plantation farms	
Secondary forest	degraded/re-growth forest and tree crops (cocoa, palm) and rubber with open canopy	
Settlement	urban areas, villages, paved/unpaved roads, car/lorry parks, bare lands, playing fields	
Mining areas	areas where open cast/surface mining has taken place and mining infrastructure (roads, factories, workshops, houses)	

To evaluate the performance of the classification algorithm, accuracies of the land cover/land use maps were assessed by using 30% of the sample class data generated for each satellite image. The accuracy assessment was conducted by creating confusion matrices between the land cover maps and the test data and calculating the overall accuracy, user accuracy, producer accuracy and the kappa statistic (Congalton, 1991). The kappa statistic was based on the formula provided by Congalton (1991), as shown in Equation (1).

$$K=\frac{N\sum_{i=1}^{r} x_{ii}-\sum_{i=1}^{r}(x_{i+}*x_{+i})}{N^2-\sum_{i=1}^{r}(x_{i+}*x_{+i})} \qquad \text{(Eq.1)}$$

Where K is the kappa statistic, N is the total number of observations in the matrix, r is the number of rows, x is the number of observations in row i and column i, x_{+i} and x_{i+} are the marginal totals of row i and column i, respectively.

Change Detection

Land cover/land use change was detected using the post-classification comparison method. The land cover maps for 1986, 1991, 2002 and 2011, generated after image classification were reclassified and combined to form multiple bi-temporal land cover change maps for the periods 1986-1991, 1991-2002 and 2002-2011, using GIS spatial analysis. The areas and proportions of change from one thematic class to another (change matrix) between the periods and the annual rates of the changes, were computed to explain the magnitudes and directions of the land cover transitions within the three periods. Finally, the overall deforestation rates were determined, by merging evergreen and secondary forest classes in each period and calculating the proportion of changes per year, between the three periods, using the Food and Agriculural Organisation's (FAO) definition of forests (FAO, 2010). The change detection in this study is based on the assumption that land cover classes between 1986 and 2011 remained the same.

Results

The land cover/land use maps generated from image classification for 1986, 1991, 2002 and 2011 are presented in *Figure 2*, while the land cover proportions are shown in *Table 3*. The classification accuracies of the maps ranged between an overall accuracy of 90%, 80%, 88% and 78%, while the Kappa statistic was 87%, 76%, 86% and 73% for the 1986, 1991, 2002 and the 2011 images, respectively. The Kappa statistics achieved in the land cover mapping are acceptable, according to the ranking by Landis and Koch (1977) and they are comparable to results of previous studies in the same study region (Kusimi, 2008; Schueler et al., 2011; Kumi-Boateng et al., 2012) and similar fragmented landscapes in West Africa (Braimoh and Vlek, 2005; Laurin et al., 2013). In Appendix A, the confusion matrices, showing how the accuracies were calculated, are presented.

Figure 2 and *Table 3* show that the Bonsa catchment is covered by five broad land cover classes: evergreen forest, secondary forest, shrubs/farms, mining areas and settlements. The percentage of the water class, which is mainly water contained in tailings dams, is less than 0.2% of the study area. Overall, the areal coverage of evergreen forest and secondary forest have reduced between 1986 and 2011, while mining areas and settlements have increased substantially. Evergreen forest, the dominant land cover, occupied 68% of the catchment area in 1986, reduced to 62% in 1991, decreased substantially again between 1991 and 2002 (50%), after which it remained relatively constant (51% for 2011). The results for the secondary forest class, however, were not consistent. The area under secondary forest increased from 27% in 1986 to 31% in 1991, 35% in 2002 and decreased again to 19% in 2011 (*Table 3*). For the shrubs/farms class, the area increased consistently between 1986 and 2011. In 1986 the area under shrubs/farms was 5% and it increased to 6% in 1991, doubled in 2002 and more than quadrupled in 2011(*Table 3*). For the non-vegetative classes, the areal coverage increased substantially between 1986 and 2011. Settlement areas increased from 0.32% (4.8 km^2) in 1986 to 0.84% (12.4 km^2) in 1991 and remained almost the same for 2002, but increased again in 2011 by 1.5% (22.4 km^2), while mining areas increased from 0.49% (7.2 km^2) in 1986 to 1.63% (24.2 km^2) in 2011 (*Table 3*).

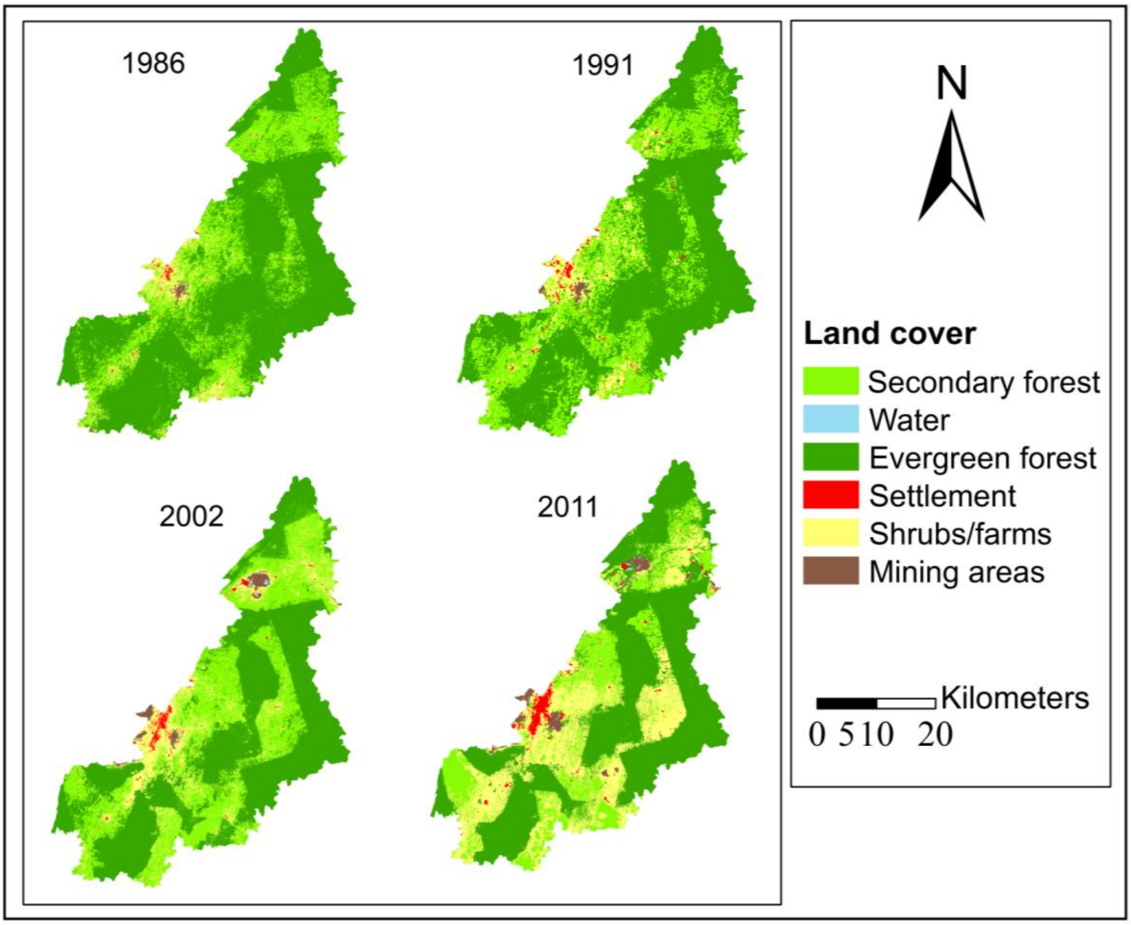

Figure 2. *Land cover maps of 1986, 1991, 2002 and 2011*

Table 3. *Proportion of land cover between 1986 and 2011*

	1986		1991		2002		2011	
Land cover	area (Km^2)	%	area (Km^2)	%	area (Km^2)	%	area (Km^2)	%
Secondary forest	393.8	26.6	457.3	30.8	523.7	35.3	280.3	18.9
Water	0.9	0.1	0.2	0.0	1.9	0.1	1.4	0.1
Evergreen forest	1003.2	67.7	916.5	61.8	744.0	50.2	754.6	50.9
Settlement	4.8	0.3	12.4	0.8	12.6	0.8	22.4	1.5
Shrubs/farms	72.4	4.9	90.0	6.1	180.1	12.1	399.4	26.9
Mining areas	7.2	0.5	5.9	0.4	20.1	1.4	24.2	1.6
Total	1482.3	100.0	1482.3	100.0	1482.3	100.0	1482.3	100.0

The change matrices and the annualized rates of changes for 1986, 1991, 2002 and 2011 land cover/land use are presented in *Table 4* and *Figure 3*, respectively. The change matrix illustrates the change in proportions of land cover from the initial year to the final year. The diagonal elements in the change matrix indicate areas of no change, while the off-diagonals show changes from one class to the other. Therefore, the sum of the diagonal elements represents the total area of land cover that did not change. The annualized rate of change shows the percentage of change in land cover area in a period divided by the number of years between the initial and the final year. *Table 4* illustrates that between 1986 and 1991, 75% (1110 km^2) of the land cover did not change, compared to 67% (998 km^2) between 1991 and 2002 and 63% (933 km^2) between 2002 and 2011.

Table 4. *Land cover/land use change matrices (%)*

(a) 1986-1991

		From 1986					
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining areas
To 1991	Secondary forest	16.9	0.0	11.7	0.0	2.1	0.1
	Water	0.0	0.0	0.0	0.0	0.0	0.0
	Evergreen forest	6.1	0.0	55.4	0.0	0.3	0.0
	Settlement	0.3	0.0	0.1	0.2	0.2	0.0
	Shrubs/farms	3.2	0.0	0.5	0.1	2.2	0.1
	Mining areas	0.1	0.0	0.0	0.0	0.1	0.2

(b) 1991-2002

		From 1991					
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining areas
To 2002	Secondary forest	19.1	0.0	13.5	0.2	2.5	0.0
	Water	0.1	0.0	0.0	0.0	0.0	0.0
	Evergreen forest	4.9	0.0	45.1	0.0	0.2	0.0
	Settlement	0.2	0.0	0.0	0.3	0.4	0.0
	Shrubs/farms	6.1	0.0	2.9	0.3	2.6	0.2
	Mining areas	0.5	0.0	0.2	0.1	0.4	0.2

(c) 2002-2011

		From 2002					
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining areas
To 2011	Secondary forest	12.9	0.0	3.5	0.0	2.4	0.1
	Water	0.0	0.0	0.0	0.0	0.0	0.1
	Evergreen forest	6.5	0.0	42.2	0.0	2.0	0.2
	Settlement	0.1	0.0	0.0	0.6	0.8	0.0
	Shrubs/farms	15.7	0.0	4.5	0.1	6.4	0.2
	Mining areas	0.2	0.1	0.1	0.0	0.5	0.8

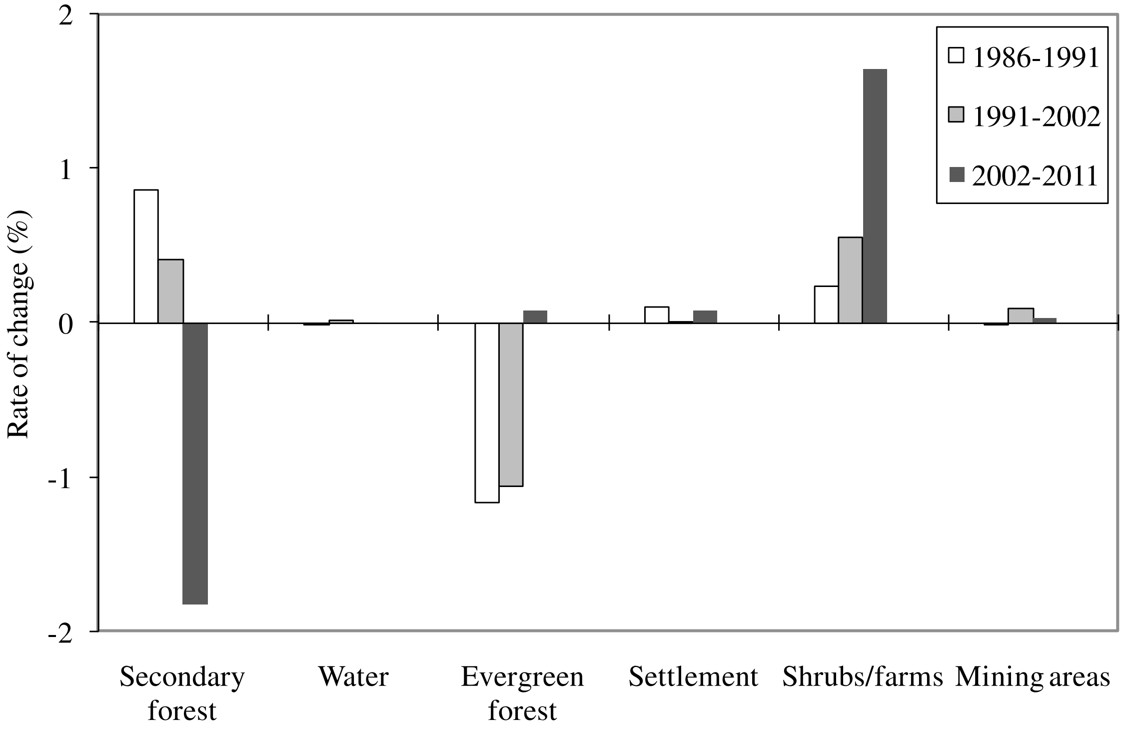

Figure 3. *Annual rate of land cover changes*

Table 4 further shows that transition from evergreen forest to secondary forest was higher between 1986 to 1991 (11.7%) and between 1991 and 2002 (13.5%) than between 2002 and 2011 (3.5%), while transition from secondary forests to shrubs/farms was higher (16%) between 2002 and 2011 than the other periods: i.e 3% between 1986 and 1991 and 6% between 1991 and 2002. The transition from evergreen to secondary forest occurred with an annual rate of reduction of 1.2% between 1986 and 1991 and 1.1% between 1991 and 2002, but during the period 2002-2011, the transition to secondary forest stopped (*Figure 3*). During the period 2002-2011, transition from secondary forest to shrubs/farms occurred, with the highest annual rate (1.8%) of reduction in secondary forest, confirming an overall reduction in secondary forest (*Figure 3*). Using FAO's definition of forests (FAO, 2010), the evergreen and secondary forest areas (*Table 3*) were merged into one class. Therefore, annual deforestation in the Bonsa catchment, defined as the overall conversion of forest areas to other land cover types per year, ranged from 0.3% between 1986 and 1991, 0.7% between 1991 and 2002 and 2% between 2002 and 2011(*Table 5*).

Table 5. *Annual deforestation rates for Bonsa catchment*

Period	change in area	% change (total change)	% change (annual)
1986-1991	-23.3	-1.7	-0.3
1991-2002	-106.0	-7.7	-0.7
2002-2011	-232.8	-18.4	-2.0

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Table 4 also demonstrates that forest recovery in the Bonsa catchment occurred from the re-growth of shrubs/farms and secondary forests. The re-growth from secondary forest to evergreen forest was similar for all the periods: i.e 6% between 1986 and 1991, 5% between 1991 and 2002 and 7% between 2002 and 2011. Re-growth from shrubs/farms to secondary forest was also similar for the three periods: i.e 2% between 1986 and 1991, 3% between 1991 and 2002 and 2% between 2002 and 2011. However, the re-growth between 2002 and 2011 is marginally higher than the other two periods.

Discussion

Land Cover and the Drivers of Changes

The results of the study indicate that deforestation in the Bonsa catchment is widespread, confirming the previous land cover study by Kusimi (2008). The present study shows that between 1986 and 2011, evergreen forest and secondary forest reduced by 25% and 28%, respectively, while mining areas increased over two-fold and settlements and shrubs/farms increased more than three and four-fold, respectively (*Table 3*). The increasing deforestation rate (0.3% per year between 1986 and 1991, 0.7% per year between 1991 and 2002 and 2% per year between 2002 and 2011), recorded in this study (*Table 5*), is consistent with the 2% per year deforestation rate for Ghana, estimated by the FAO (2010), which is also consistent with trends in deforestation in Africa between 1990 and 2000 (1.1 million ha) and between 2000 and 2005 (2.7 million ha) (FAO and JRC, 2012). The results further show that, although the deforestation rate has been increasing with time, more than 50% of the land cover in the Bonsa catchment remained unchanged between 1986 and 2011. Evergreen forest represented the largest stable class (55% between 1986 and 1991, 45% between 1991 and 2002 and 42% between 2002 and 2011), while secondary forest and shrubs/farm were the most fragmented classes (*Table 4*). The recorded land cover changes indicate that some areas which were originally covered by either evergreen forests or secondary forests have been converted to other cover types, and the recovery of the original vegetation (reforestation) during the three periods is small, compared to the overall deforestation.

The land cover/use changes in the Bonsa catchment can be attributed to anthropogenic drivers, which can be grouped into two broad categories, namely global and local factors. The global factors include globalization of agriculture, urbanization, international trade regimes and global politics, while the local factors include population growth, urbanization, immigration, economic development and government policy (Sage, 1994; Barbier, 2000; Meyfroidt et al., 2013). The recorded deforestation in the Bonsa catchment was influenced by local population growth (Ghana Statistical Service, 2005; Kusimi, 2008; Ghana Statistical Service, 2013), agricultural extensification (Kusimi, 2008; Sutton and Kpentey, 2012), timber logging (Asante, 2005; Kusimi, 2008) and increased surface mining activities (Akabzaa and Darimani, 2001) since 1986, when the implementation of the structural adjustment programme of the World Bank, liberalized Ghana’s economy (Barbier, 2000) and increased foreign direct investment in the mining, timber and agricultural industries. Kusimi (2008) provided a discussion on how these driving forces have influenced land use changes within and around the Bonsa catchment.

In addition, population growth and per capita income are the two main drivers of settlement expansion (Sage, 1994). As the number of people in a community increases,

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there is the need to provide social infrastructure in transportation, housing, sanitation, health, education and recreation, which are mostly built on agricultural or forested lands. Hence, increases in a country's Gross Domestic Product (GDP), which result in higher per capita incomes, coupled with higher population growth rates, can lead to higher urbanization rates. In the Western region of Ghana, where the Bonsa catchment is located, the total urban population increased from 22.6% in 1984 to 42.4% in 2010 (Ghana Statistical Service, 2013) and correlates well with the expansion of settlements, recorded in this study, and is not surprising, considering that Ghana's GDP has increased from $8 billion in 1984 to $32 billion in 2010 (Kwakye, 2012). Therefore, for settlements, especially in the Tarkwa urban area, three main drivers namely (i) population growth (both natural growth and immigration), (ii) increase in per capita income and (iii) increases in mining activities, jointly influenced its expansion. According to Kusimi (2008) mining influenced population growth and urbanization by attracting people from other towns and cities in search of mining-based jobs The expansion of settlements, puts further pressure on the surrounding rural areas to produce more food to feed urban dwellers and more cash crops to pay for the importation of foreign products, which are associated with urban populations (Sage, 1994). The increased demand from urban settlements further causes the conversion of more forested lands into food and cash crop farms, thereby increasing the rates of deforestation. International trade and rising per capita incomes of the developed world and the ermerging markets, also reinforces deforestation in the developing world through higher demands for natural resources and commodities (Sage, 1994; Meyfroidt et al., 2013). This phenomenon, which results in volatility in prices of commodities such as, cocoa, rubber and gold, has also influenced deforestation in the Bonsa catchment.

Despite the increasing deforestation rate, the results of the study also indicate that concurrent vegetation regeneration is taking place in the Bonsa catchment. Vegetation regeneration occurred mainly because of programmes implemented by the Forestry Commission of Ghana, as well as some mining companies, maturing of rubber trees and the restrictions placed on the timber industry; banning exportation of round logs (Sutton and Kpentey, 2012) and the criminalization of chainsaw operations. The Forestry Commission of Ghana embarked on reforestation programmes in 2000 to reforest about 4000 km^2 of degraded forests, at a rate of 200 km^2/year (FAO, 2010), but had only replaced 25 km^2/year by 2012 (Sutton and Kpentey, 2012). Mining companies in the Bonsa catchment have also carried out limited reforestation programmes in mined out areas (Tetteh, 2010). However, the results of this study show that, compared to deforestation, vegetation regeneration is less significant in the Bonsa catchment.

Anthropogenic land cover changes are caused by both proximate and global drivers, while the impacts are largely felt at the local scale. Therefore, in addition to mapping and identifying the drivers of the changes, there is also the need to identify the potential impacts of any changes in land use, in order to plan and manage land use effectively. Thus, the remainder of the discussion is focused on identifying the potential impacts of land cover/land use changes and discussing the need for further research.

Potential Impacts of the Land Cover Changes

The impacts of land cover changes can have substantial socio-economic, environmental and health impacts on communities, especially in poor countries. The impacts include change in hydrological regimes, reduced biodiversity, reduced soil organic carbon leading to poor fertility, pollution of surface and groundwater and changes in the albedo and the microclimate (Meyer and Turner II, 1994) and the invasion by alien species. Other impacts include emission of atmospheric trace gases (Penner, 1994), which contribute to climate changes. Impacts on hydrology and water resources are one of the most significant impacts of land cover changes. Land cover changes have impacts on water flows through changes in the partitioning of rainfall into the components of the hydrological cycle (Costa et al., 2003; D'Orgeval and Polcher, 2008). Land cover changes that increases imperviousness, such as surface mining and urbanization for example, reduce infiltration and increase the velocity and volumes of runoff, which can lead to floods, soil erosion and sedimentation, and pollution of rivers. Reduced infiltration can also lead to less groundwater recharge and less baseflows and drying of rivers, during low flow periods (Rogers, 1994; Randolph, 2012). Therefore, in the long-term, as population size and water use in the Bonsa catchment increases, if land cover changes are not controlled, deforestation and urbanization, could further lead to scarcity of water (Rogers, 1994).

Additionally, conversion of more forested lands to agriculture can increase soil erosion and loss of soil fertility, thereby making it more difficult to produce enough food and cash crops to meet the demands of the growing population in Bonsa catchment. Land degradation and the loss of soil fertility has the potential to reinforce more land cover changes through the extensification of agriculture (Barbier, 2000). Since agriculture in the Bonsa catchment is of the low-input type, the only means to increase production will be to increase the area under cultivation, which can further cause changes in land cover. Soil erosion, as well as the use of fertilizers and other chemicals, can also cause pollution of water bodies, destroy aquatic habitats and reduce aquatic biodiversity, if proper agricultural practices are not adopted. The removal of forests, also leads to loss of indigenous plant species, the destruction of animal habitats and reduction in biodiversity of the forest ecosystem. Planting rubber trees and other non-native tree species for example, can reduce groundwater recharge and baseflows significantly, because exotic plants consume more water than the native species.

Apart from deforestation and soil erosion, mining activities in the Bonsa catchment have the potential to cause heavy metal pollution of the water sources and increases in respiratory diseases, caused by dust pollution, resulting from blasting of rocks and movement of heavy duty mining equipment. Akabzaa et al. (2009) concluded that heavy metals, such as mercury, copper and nickel of concentrations above World Health Organisation (WHO) maximum allowable limits for drinking water, have been released into streams and groundwater systems by mines within the Ankobra basin. Similarly, Armah et al. (2012), concluded that the pH, COD and turbidity levels of groundwater in the Tarkwa minng area are above WHO standards. Further, between 1998 and 2006, eigth (8) spillages of cynanide were reported in the Tarkwa area (Tsuma, 2009). Impacts of both urbanization and surface mining also include changes in land surface albedo and the microclimate, which can create urban heat islands (UHI). UHI can cause skin cancer, as well as result in increased energy consumption of buildings. UHI is a phenomenon that results in higher temperatures in urban areas than the surrounding rural areas (Jensen, 2000), which is due to the presence of less vegetation/heat absorbing surfaces in urban/bare land areas.

According to Klocking and Haberlandt (2002), it is difficult to draw generalised, quantitative conclusions about impacts of land cover/use changes, without a specific local study. For example, Mahe et al. (2005), Boulain et al. (2009) and Warburton et al. (2012) among others, showed that the hydrological responses to land cover changes, depend on the scale of the changes, the land cover type, the climate and the location of the changes within a catchment. It is therefore imperative that locally relevant studies, consistent with the objectives of planning and development, within a specific basin, are undertaken.

Further Research

The population of people living in urban areas around the world is expected to grow from 3.15 billion in 2008 to 7.2 billion by 2050 (Randolph, 2012). The population pressure will make it difficult to provide services to people living in urban areas and many people in the developing world will not have access to portable water, sanitation and safe living environments, as urbanization increases. It is estimated that half of the people living in the developing world lack access to these services already (Randolph, 2012). The increasing rate of deforestation in Africa (FAO and JRC, 2012) for example, will have negative impacts on the livelihoods of people, who primarily depend on the natural resources for their development, while the goods and services provided by the natural environment will be reduced. The need to therefore plan and manage land use and water resources effectively in Africa and the rest of the developing world cannot be overemphasized.

In addition to the current land cover/land use changes, in the near future, the population of the Bonsa catchment in Ghana, is expected to grow at an average of 2% per annum (Ghana Statistical Service, 2005), mining activities will be intensified, as more mining leases are approved (*Figure 4*). Urbanization will also increase, as a result of both natural population increase and immigration to mining towns, and deforestation will increase. Therefore, in order to carryout effective land use planning, the impacts of both the current and the potential future land cover changes need to be quantified. Unfortunately, few studies of this type have been carried out and of these studies, all focus on impacts of surface mining on either groundwater pollution or surface water pollution (Kortatsi, 2003; Akabzaa et al., 2009; Armah et al., 2012), and airborne particulate matter pollution (Bansah and Amegbey, 2012). Although previous studies have mapped land cover changes between 1986 and 2002 of different sections of the catchment (Kusimi, 2008; Schueler et al., 2011; Kumi-Boateng et al., 2012), none assessed the impacts on the environment. The land cover change impacts on the hydrological flows, biodiversity and soil fertility are not known. Quantifying these impacts will provide the necessary information to protect life and property, protect the environment and ensure sustainable utilization of the natural resources of the catchment. In evaluating the impacts, the derived land cover information, as well as projected changes in the land cover, will be key variables.The potential drivers, identified in this study, will be vital in projecting future changes in the land cover. Therefore, further research will need to focus on modelling future changes in the land cover and quantification of the impacts of both historical and potential future changes in land cover on hydrology of Bonsa catchment. Additionally, future research will need to assess the impacts of climate change and land cover changes jointly.

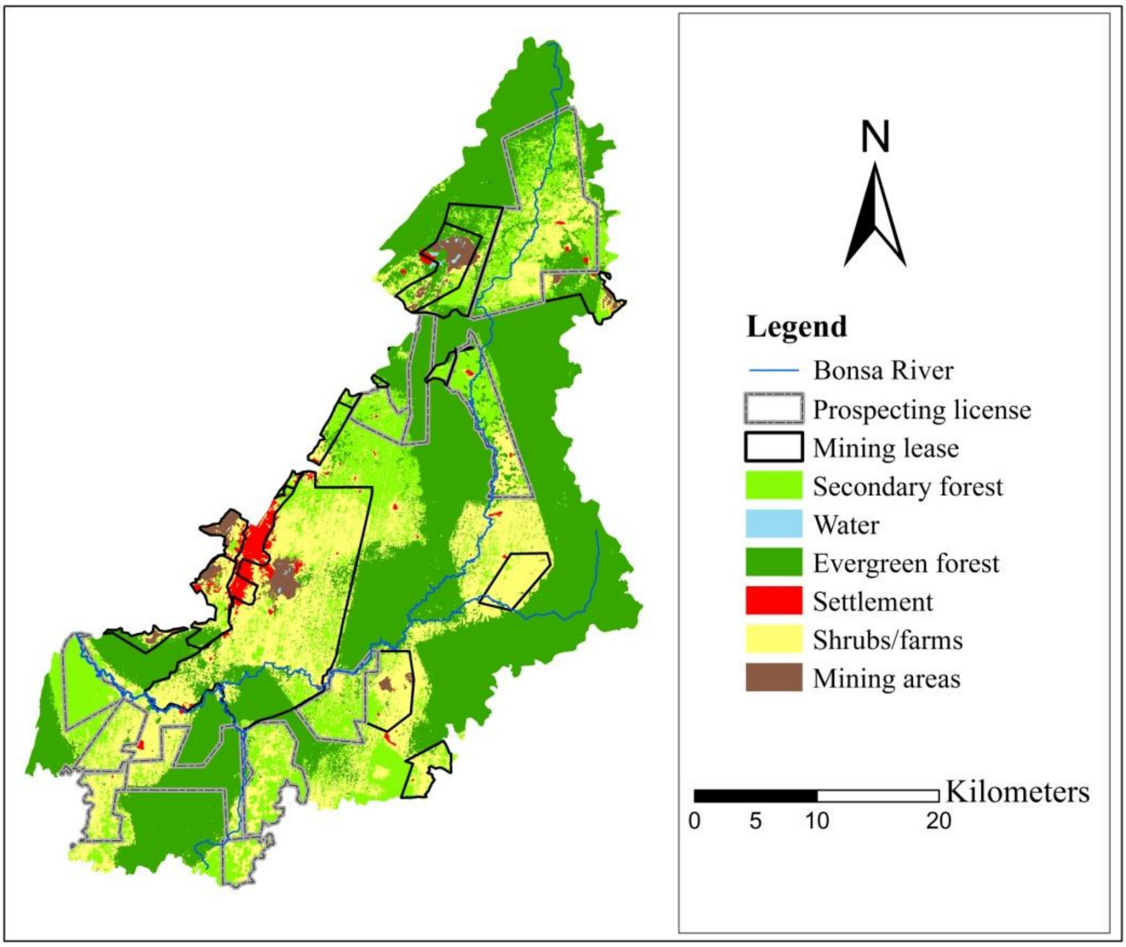

Figure 4. *Land cover (2011) and approved mining leases and prospecting lincenses in the Bonsa catchment. Source : documents from mining companies (Bourke* et al.*, 2007; Gold Fields Limited, 2012b, a; Castle Peak Mining Ltd, 2013).*

Conclusion

Land cover mapping and change detection for the Bonsa catchment was executed successfully with Kappa statistics ranging between 72 and 87%, using the maximum likelihood classification algorithm. The study has generated multi-temporal land cover data from 1986 to 2011, which shows that the Bonsa catchment is predominantly covered by forests, which are currently reducing at the rate of 2% per year, implying that the ongoing re-afforestation programmes undertaken by stakeholders in the catchment is occurring at a lower rate, compared to the deforestation. The study further indicates that the potential drivers of the land cover changes are both local and global, which include international trade, local population growth, agriculture extensification and urbanization. The study highlights the value of a multi-temporal land cover mapping approach to provide data to guide effective land use planning and water resources management, especially in data poor regions.

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APPENDIX

Appendix A. Confusion Matrices

Appendix A1(a). Confusion Matrix for 1986 land cover map

		Reference Data (No. of points)						
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining area	Total
1986 Land cover map	Secondary forest	32	0	1	0	0	0	**33**
	Water	0	29	0	0	0	0	**29**
	Evergreen forest	2	0	52	0	0	0	**54**
	Settlement	0	0	0	35	2	8	**45**
	Shrubs/farms	1	0	0	1	33	2	**37**
	Mining area	2	0	0	4	1	27	**34**
	Total	**37**	**29**	**53**	**40**	**36**	**37**	**232**

Overall accuracy =
208/232 *100% = 89.66%

kappa= 87.49%

Land cover	Producer's accuracy (%)		User's accuracy (%)	
Secondary forest	32/37=	86.49	32/33=	96.97
Water	29/29=	100.00	29/29=	100.00
Evergreen forest	52/53=	98.11	52/54=	96.30
Settlement	35/40=	87.50	35/45=	77.78
Shrubs/farms	33/36=	91.67	33/37=	89.19
Mining area	27/37=	72.97	27/37=	79.41

Appendix A 1(b). Confusion Matrix for 1991 land cover map

	Reference Data (No. of points)							
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining area	Total
1991 Land cover map	Secondary forest	26	0	2	0	0	0	**28**
	Water	0	27	0	0	0	1	**28**
	Evergreen forest	3	3	62	0	0	0	**68**
	Settlement	0	0	0	57	4	2	**63**
	Shrubs/farms	17	0	0	0	26	0	**43**
	Mining area	0	4	0	24	0	48	**76**
	Total	**46**	**34**	**64**	**81**	**30**	**51**	**306**

Overall accuracy =
246/306*100%= 80.39%

kappa =76.09%

Land cover	Producer's accuracy (%)		User's accuracy (%)	
Secondary forest	26/46=	56.52	26/28=	92.86
Water	27/34=	79.41	27/28=	96.43
Evergreen forest	62/64=	96.88	62/68=	91.18
Settlement	57/81=	70.37	57/63=	90.48
Shrubs/farms	26/30=	86.67	26/43=	60.47
Mining area	48/51=	94.12	48/76=	63.16

Appendix A 1(c). Confusion Matrix for 2002 land cover map

	Reference Data (No. of points)							
	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining area	Total
2002 Land cover map	Secondary forest	64	0	1	0	0	0	**65**
	Water	0	51	0	0	0	0	**51**
	Evergreen forest	11	1	61	0	0	0	**73**
	Settlement	0	0	0	70	1	6	**77**
	Shrubs/farms	19	0	0	0	46	0	**65**
	Mining area	0	3	0	2	5	73	**83**
	Total	**94**	**55**	**62**	**72**	**52**	**79**	414

Overall accuracy =
365/414*100% = 88.16%

kappa = 85.76%

Land cover	Producer's accuracy (%)		User's accuracy (%)	
Secondary forest	64/94=	68.09	64/65=	98.46
Water	51/55=	92.73	51/51=	100.00
Evergreen forest	61/62=	98.39	61/73=	83.56
Settlement	70/72=	97.22	70/77=	90.91
Shrubs/farms	46/52=	88.46	46/65=	70.77
Mining area	73/79=	92.41	73/83=	87.95

Appendix A 1(d). *Confusion Matrix for 2011 land cover map*

Reference Data (No. of points)

	Land cover	Secondary forest	Water	Evergreen forest	Settlement	Shrubs/ farms	Mining area	Total
2011 Land cover map	Secondary forest	44	0	0	0	2	0	**46**
	Water	0	36	0	4	0	0	**40**
	Evergreen forest	6	0	34	0	12	0	**52**
	Settlement	0	1	2	36	1	6	**46**
	Shrubs/farms	26	0	1	0	67	0	**94**
	Mining area	0	9	0	7	0	50	**66**
	Total	**76**	**46**	**37**	**47**	**82**	**56**	**344**

Overall accuracy =
267/344 *100% = 77.62 %

kappa = 72.84%

Land cover	Producer's accuracy (%)		User's accuracy (%)	
Secondary forest	44/77=	57.89	44/46=	95.65
Water	36/46=	78.26	36/40=	90.00
Evergreen forest	34/37=	91.89	34/52=	65.38
Settlement	36/47=	76.60	36/46=	78.26
Shrubs/farms	67/82=	81.71	67/94=	71.28
Mining area	50/56=	89.29	50/66=	75.76

ARTHROPOD PHOTOTAXIS AND ITS POSSIBLE EFFECT ON BIRD STRIKE RISK AT TWO NAMIBIAN AIRPORTS

HAUPTFLEISCH, M.L.[1*] – DALTON, C.[1]

[1]*Department of Agriculture and Natural Resources Sciences, Polytechnic of Namibia*
Private Bag 13388, Windhoek, Namibia
(phone: +264-81-124-1365; fax: +264-61-207-2141)

**Corresponding author*
e-mail: mhauptfleisch@polytechnic.edu.na

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Abstract. Aircraft wildlife collisions are a global safety and financial problem for the aviation industry, with birds being the main concern. In Namibia, 97% of collisions at Namibia's two main airports are reported to be with insectivorous birds. Phototaxis was identified as a major attractant to insectivorous birds, which feed on the arthropods attracted to airport apron and terminal lights. This study considered the effect of light as an attraction at the rurally situated Hosea Kutako International and urban Eros airports. It further investigated the attractiveness of light colour (or wavelength) on arthropod abundance, biomass and diversity. The study found that phototaxis was a significant factor at Hosea Kutako only, and that white light was the main attractant for arthropods, specifically for large moths (Order Lepidoptera), while yellow and orange light attracted significantly less arthropods. The study indicates a high likelihood that the Hosea Kutako apron lights (white) are an important attractant for arthropods, and therefore indirectly insectivorous birds, which can be reduced by replacing them with orange or yellow filters.

Keywords: *airport, arthropods, bird strike, phototaxis*

Introduction

Aircraft-wildlife collisions (AWCs) are a global concern. Thorpe (2003) reported 42 fatal accidents, 231 human deaths and 80 destroyed aircraft in the world's aviation sector as a result of AWCs between 1912 and 2002, while Dolbeer et al. (2012) cited 229 human deaths and 210 destroyed aircraft since 1988 alone. At Namibia's two major airports, Hosea Kutako International and Eros (domestic), 128 AWC incidents were recorded between 2006 and 2010 (Hauptfleisch et al., 2013). Only two serious incidents were recorded in Namibia in the last five years. Damage from these incidents amounted to damages of over US$ 2.5 million (Hauptfleisch and Avanant, 2012). The number of bird collisions in Namibia seems to fluctuate from between one or two collisions to 25 collisions per year per airport (Hauptfleisch et al., 2013). This is probably not a true version of the number of collisions, as reports on these incidents are not always filed, especially when there is little or no damage to the aircraft (Hauptfleisch et al., 2013).

Airports are described as complex habitats (Soldatini et al., 2010) and provide niches and ecosystem services such as primary food supply (grass / vegetation, insects, small mammals and carrion), water, shelter and nesting sites. Studying ecosystem components that provide these services should consequently be useful to airport wildlife management. At airports arthropods provide ecosystem services and food for birds and other wildlife (Bernhardt et al., 2010), were found to be attracted to the heat of runways and taxiways (Pennell, 2007), and use airport grasslands as refugia (Kutchbach-Brohl et al., 2010). Reductions in arthropod numbers at airports is expected to reduce risk of AWCs (Buckley and McCarthy, 1994; Bernhardt et al., 2010; Steele and Renner, 2010;

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Washburn et al., 2011) The possible influence of arthropods on bird strikes can therefore not be ignored.

Different methods to discourage birds from using airports, including killing them, have proved to have little effect on the numbers of birds (Froneman, 2000). It was however found that the management and manipulation of foraging opportunities to birds could be an effective strategy (Blackwell et al., 2013). Habitat management efforts, such as alteration of plant communities are an integral part of wildlife hazard management programs to reduce the risk of wildlife strikes at major airports globally (Washburn, 2011). Thus a more holistic approach which includes research into the ecosystem services provided to birds at the airports is useful.

During the period 2006 to 2010, fifteen bird species were responsible for bird strike incidents at Hosea Kutako and Eros Airports Namibia. Of those, 14 (92%) all except White-backed Vulture (*Gyps africanus)*, are described to rely on arthropods as a food source. The attractiveness of light to arthropods is widely known (Weiss et al., 1941; Van Tets et al., 1969; Ashfaq et al., 2005; Blanco and Hernández, 2006; Fox et al., 2006) and the effect of this phenomenon on attracting birds to the airports formed the motivation for this study. For safety and security reasons the International Civil Aviation Organisation (ICAO, 2004) requires that lights at airports be positioned in such a way that all aircraft on the apron are clearly visible at all times (ICAO 2004). Only white, yellow or orange light will suffice, as other colours like red and blue are too dark and are used for signalling (ICAO, 2004). According to Ashfaq et al. (2005) white light attracts more arthropods than yellow light, while red light, few or no arthropods. Arthropods are photosensitive and are thus either attracted to light (photo-positive) or repelled by it (photo-negative) (Shockley Cruz and Lindner, 2011), while natural environmental factors like humidity and temperature and surrounding land management may play a role in the behaviour of arthropods and thus influence their numbers at any given time (Jonas et al., 2002; Blanco and Hernandez, 2006). The attraction to light, however, can cause arthropods to become easy meals to birds as they become disorientated, collide into the light or congregate around it. Birds have been found to readily make use of these “artificial feeding stations” (Van Langevelde et al., 2011). Variations in intensity and spectral composition of artificial lighting have also been found to affect its attractiveness to arthropods (Van Langevelde et al. 2011). For example certain beetles (Order Coleoptera), crickets (Order Orthoptera), bees and desert ants (Order Hymenoptera) have been found to use the light polarisation patterns during dispersal and migration, while many nocturnal arthropods are attracted to artificial sources of polarised light (Horváth et al., 2009).

This study used field experiments to determine the extent to which light attracts insects, and also investigated whether light colour (wavelength) and surrounding land use plays a role in attractiveness to light to arthropods. It futher attempts to relate these results to the risk of aircraft-bird collision risk at the study airports.

Materials and methods

The study area is in the central highlands of Namibia’s Khomas region. Three study sites were established: i) at Hosea Kutako International airport (Longitude: 17° 28' 0" E and latitude 22° 28' 59" S), 40 km east of Windhoek, ii) a neighbouring game and cattle farm (Longitude: 17° 29' 09" E and latitude 22° 28' 13" S Oupembamewa, and iii) Eros

Airport (longitude 17° 4' 59" E and latitude 22° 37' 0" S) within the suburbs of Windhoek, Namibia's capital city.

Light traps were located at the three study sites for 6 consecutive nights (during April 2013). Each light trap consisted of a clear plastic tray as well as a light source. Controls consisted of the same without a light source. Mono-ethylene glycol was used to kill and preserve arthropods that fell into the light traps.

The light traps were set up in a grid pattern with random treatments (white, yellow, orange or no light), 50 metres apart. Light Emitting Diodes (LED lights) were used as it does not produce heat, a factor which can influence the attraction of arthropods (Fox et al., 2006). The lights were switched on at dusk and switched off at dawn the following morning.

Arthropods collected from each trap were identified, dried for 48 hours at 50°C and weighed. Individuals were identified to Order level to determine order richness and diversity using the Shannon Information Index (Manly, 2001) As the Order Lepidoptera were most abundant and a major prey component of insectivorous birds (Van Langevelde et al., 2011;Washburn et al., 2011), the assistance and expertise of the National Museum was called upon to identify Order Lepidoptera to Family level. A species of the order Hymenoptera (*Anaplolepis custodiens* – Pugnacious ant) which is diurnal (Picker, et al., 2004), were trapped in abundance in the control (especially at Eros airport). It was clear that this species was not attracted to light (being diurnal) and was probably attracted to the moisture in the light traps (personal observation, 13 April 2013). Hymenoptera was subsequently removed from further analysis. Trap success was calculated as the mean number of individuals trapped per night over 6 nights. Biomass was calculated as dry mass per order per light trap.

Normality of data was determined with the Shapiro-Wilk's W test. As the data was not normally distributed, a standard nonparametric test (Kruskal - Wallis for multiple analysis of variance), was used to compare land use and light colour. All data were statistically analysed by using Statistica ® for Windows version 10 (StatSoft Inc., 2011). A statistically significant level of 95% ($p<0.05$), was observed for all tests.

Results

A total of 3 878 arthropods were trapped. 2 760 (71.17%) were collected at the farm (Oupembamewa), 283 (7.29%) were collected at Eros Airport and 835 (21.53%) were collected at Hosea Kutako (*Table 1*). Significantly higher trap success and order richness ($H = 36.44$, $p < 0.01$) were found at the farm compared to Eros and Hosea Kutako Airports.

When combining the three study sites a total of 2 066 (53.27%) arthropods were trapped in light traps with white light, 737 (19%) were trapped in light traps with orange light, 957 (24.67%) were trapped in light traps with yellow light and 118 (3.04%) were trapped in the control. Significantly higher arthropod numbers were found in traps bearing white light ($H=30.39$, $p < 0.01$) when compared to the control traps and traps fitted with yellow and orange lights.

Overall, white light showed a significantly higher trap success ($H=51.63$, $p < 0.05$) than orange light ($H=46.79$, $p < 0.05$), yellow light ($H=44.15$, $p < 0 .05$) and the control ($H=5.24$, $p < 0.05$)(*Fig. 1a*) (*Table 1*). Although less arthropods were caught at orange light traps than yellow, this difference was not significant. Significantly less arthropods were captured at the control compared to all light colours.

***Table 1**. Abundance (number of individuals trapped), biomass, order richness and diversity of arthropods at light traps in three land use areas (W = white light, O = orange light, Y = yellow light, C = control / no light).*

	Hosea Kutako Airport				**Eros Airport**				**Oupembamewa Farm**			
Orders	W	O	Y	C	W	O	Y	C	W	O	Y	C
Class-Arachnida												
Araneae	40	22	30	3	14	5	14	2	96	109	92	1
Acari	3	5	4	1	1	0	0	0	1	0	1	0
Amblypygi	0	1	2	0	0	0	0	0	0	0	0	0
Solpugida	1	0	0	0	0	0	0	0	0	0	1	0
Class-Entognatha												
Collembola	30	40	13	1	9	3	8	0	16	16	25	4
Class-Insecta												
Lepidoptera	156	22	18	1	30	6	7	1	856	140	268	2
Hemiptera	124	41	29	9	59	2	14	10	177	115	123	6
Orthoptera	6	4	5	3	6	2	2	5	10	8	10	5
Coleoptera	32	17	16	2	4	4	3	8	34	30	29	1
Diptera	77	11	10	8	27	5	10	4	178	87	72	9
Phasmatodea	0	0	0	0	0	0	0	0	0	1	0	0
Blattodea	4	7	4	2	7	1	2	2	0	1	0	0
Isoptera	0	0	0	0	0	1	1	0	0	1	1	0
Mantodea	2	0	0	0	3	0	1	0	53	22	43	0
Plecoptera	0	0	0	0	0	0	0	0	0	0	1	0
Thysanoptera	1	2	6	16	0	0	0	0	0	4	4	5
Psocoptera	3	0	0	0	0	0	0	0	5	2	3	1
Neuroptera	1	0	0	0	0	0	0	0	0	0	0	0
Total n	480	172	127	46	160	29	62	32	1426	536	673	34
Order richness	14	11	10	10	10	9	10	7	10	13	14	9
Shannon diversity (Orders)	1.77	1.93	1.86	1.92	1.74	1.19	1.94	1.39	1.2	1.73	1.74	1.92
Biomass (g)	21.95	32.34	35.18	4.55	17.45	11.1	8.54	4.1	6.92	13.02	9.03	4.13

Although the difference between white light and other light traps was significant at all three study sites, Hosea Kutako (*Fig. 1b*) and the farm (*Fig. 1d*) site showed a greater difference than at Eros (*Fig. 1c*) (*Table 1*).

Arthropod biomass was highest at Hosea Kutako and lowest at the farm. Total arthropod biomass at Hosea Kutako was 94.02 g (49.09%), at Eros 56.27 g (29.38%) and Farm Oupembamewa 41.23 g (21.52%). Overall order richness was highest at the farm Oupembamewa, followed by Hosea Kutako and Eros Airports. Per light colour order richness was highest at the rural study sites, Hosea Kutako (14) and Oupembamewa (14), order richness was highest at the farm for yellow light and at Hosea Kutako for white light. Overall order diversity was highest at Eros for yellow light (Shannon 1.94) as well as lowest at Eros for orange light (Shannon 1.19). Highest order diversity at Hosea Kutako was found at orange light (Shannon 1.93) and lowest at white light (Shannon 1.77)traps, while at Oupembamewa order diversity was highest at the control (Shannon 1.92) and lowest at white light (Shannon 1.2) (*Table 1*).

Individuals of the order Lepidoptera were most abundant at 1507 (38,86%) individuals (*Fig. 2*), of the families Lymantriidae, Limacodidae, Noctuidae and Geometridae. Hemiptera were second most abundant at 709 (18.28%) individuals, followed by orders Diptera, Araneae, Coleoptera, Collembola, Mantodea and

Orthoptera. Significantly greater abundance of Lepidoptera was attracted to white light than to any either orange or yellow light. (H=85.32, p < 0.01)(*Fig. 2*).

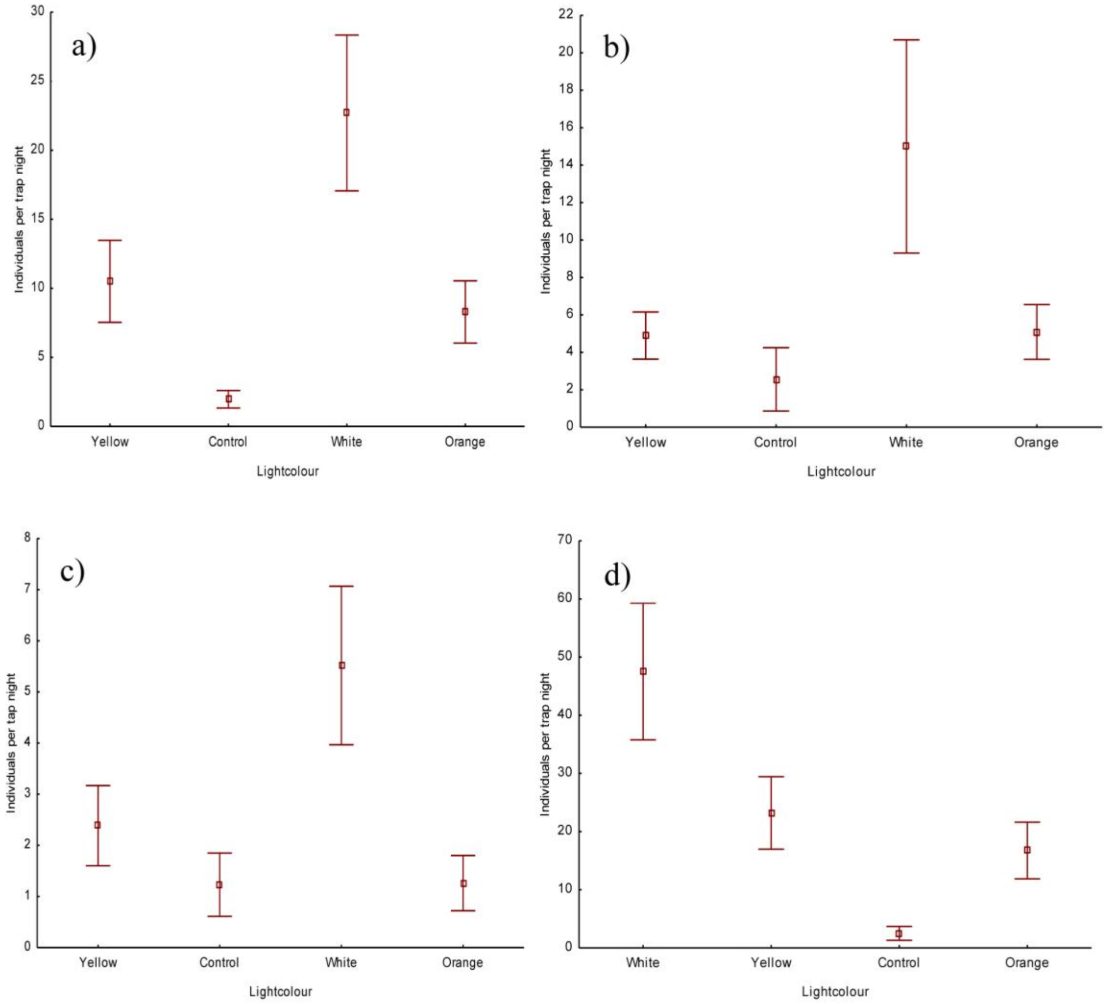

Figure 1. *Trap success per light colour at a) All light traps, b) Hosea Kutako Airport, c) Eros Airport, and d) Farm site (mean, whiskers = 95% confidence interval).*

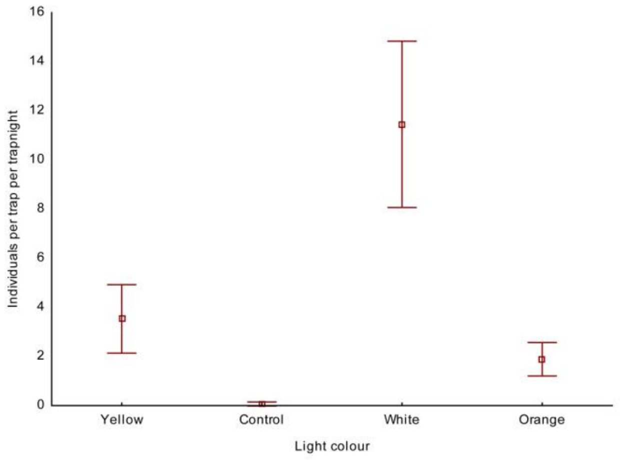

Figure 2. *Trap success of Order Lepidoptera per light colour.*

Discussion

This study confirmed other findings (Weiss, 1946; Prokopy and Owens, 1983; Frank, 1988; Stavenga, 2002; Ashfaq et al., 2005; Cloyd et al., 2007; Fox et al., 2007; Buschke and Seaman, 2011; Shockley Cruz and Lindner, 2011; van Langevelde, 2011) that white light is a major attractant to arthropods. When considering land use, the effect of light as attractant was reduced by increased ambient light sources. Being in an urban setting, Eros Airport is surrounded by artificial light sources, while Hosea Kutako Airport is surrounded by farming and conservation areas with minimal artificial light. The results indicate a clear increase in attractiveness of all (particularly white) light sources to arthropods with decreased surrounding light sources, making it a significant factor at airports situated rurally (e.g. many parts of Africa) (Hauptfleisch, 2014). This factor is already in use to reduce the nuisance of nocturnal insects in the tourism (Roqoe Albello et al., 2007) and other industries (Chartered Institute of Environmental Health, 2009), while only one other study (Frank, 1988) of this phenomenon at an airport could be found.

Birds most commonly involved in collisions with aircraft at Hosea Kutako Airport are Crowned Lapwing (*Vanellus coronatus*) (16%) and Helmeted Guineafowl (*Numida meleagris)* (9%) (Hauptfleisch et al., 2013), both insectivorous (Hockey et al., 2005). Crowned Lapwing (*Vanellus coronatus*) were responsible for two thirds of collisions at Eros Airport (Hauptfleisch et al., 2013). Stomach and crop content analyses at the two study airports during 2010-2012 revealed arthropods as a major food source (Hauptfleisch, 2011).

Of the family Geometridae, moths with larger body mass, larger wing dimensions and larger eyes have been found to be attracted to light dominated by smaller wavelengths (white spectrum) (Van Langevelde et al., 2011) a finding confirmed by this study. According to Prokopy and Owens (2011) and Qiu and Arikawa (2011) possible reason for this is that moths have compound eyes which are sensitive to wavelengths ranging from UV-light to red light. Moths are an important part of many birds' diet (Van Langevelde et al., 2011; Washburn et al., 2011) with adult birds found to feed their young large moth species of the families Cossidae, Sphingidae, Noctuidae and Geometrida (Van Langevelde et al., 2011) as a result of their relative lack of exoskeletal and mandibular protection.

The presence of predatory arthropods, of the orders Hemiptera (family, Reduviidae and Nepidae), Mantodea (family Hymenopdidae, Mantidae and Empusidae), Coleoptera (family Carabidae and Dytiscidae) (*Table 1*), indicates that not all arthropods were attracted to the light source. Predators were likely attracted to the abundance of photophilic insects, as found with other studies (Bruce-White and Shardlow, 2011; Davies et al., 2012.; Meyer, 2012; Wang et al., 2014).

Biomass was found not to be a reliable measure of attractiveness as a result of the substantial variance in the dry mass of different arthropod species. For example, arthropods from the Order Coleoptera, family Scarabaeidae, were caught in some of the light traps, varying in size from 6-50 mm (Scholtz and Holm, 1996) with a mass of up to 88 g while individuals of the order Lepidoptera would weigh very little after drying, even though individuals vary in wingspan size from 3mm-180mm (Scholtz and Holm, 1996).

The study suggests that arthropod abundance at Hosea Kutako airport is an important factor which can be reduced by filtering the airport apron lights (white) with yellow or orange filters, provided the primary security function of the lights are not compromised. This factor is however not significant at the urban Eros airport. An up-scaled study is underway to determine arthropod abundance at the apron lights themselves.

Acknowledgements. The Namibia Airports Company, and the Trumper family are thanked for providing their properties as study areas. The National Museum of Namibia is thanked for providing access to their study collections for identification of arthropod families.

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ECOPHYSIOLOGY OF SELECTED CANDIDA SPECIES ISOLATED FROM DIFFERENT TYPES OF UTILITY WATER UNDER LABORATORY CONDITIONS

BIEDUNKIEWICZ, A.

Department of Mycology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn
1A Oczapowskiego St. 10-917 Olsztyn, Poland
(phone/fax: +48-89-523-42-95)

**Corresponding author*
e-mail: alibi@uwm.edu.pl

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Abstract. The ability of four microfungi species from the potential pathogens group (*Candida albicans, C. guilliermondii, C. krusei* and *C. tropicalis*) to proliferate utility water was examined under controlled laboratory conditions and the enzymatic activity of these microfungi was tested. The experiment found that all microfungi retained their capacity for growth under controlled laboratory conditions. The enzymatic activity of the investigated species was at a high level before multiplication and was reduced by approximately 5% after controlled multiplication under laboratory conditions. All microfungi formed blastospores and pseudomycelium and their arrangement was characteristic for each species. Studies have shown that water can also be a "*habitat*" for microfungi as numerous fungal species may find favorable trophic and growth conditions in water, which directly affect the rate of metabolic processes.

Keywords: *ecophysiology of microfungi, Candida sp., survival in the water utility*

Introduction

The presence of fungi, especially yeast-like, in different types of water is well-documented (Biedunkiewicz et al., 2007; Biedunkiewicz, 2011; Biedunkiewicz and Schulz, 2012; Biedunkiewicz et al., 2013). Many authors have evaluated diverse aspects of purity in freshwater (Wurzbacher et al., 2010), seawater (Vogel et al., 2007), surface waters (Parveen et al., 2011) and across the whole water profile (Bis et al., 2013; Shearer et al., 2007), in outdoor (Biedunkiewicz et al., 2007; Vogel et al., 2007) and indoor baths (Rasti et al., 2012), in drinking water (Biedunkiewicz and Schulz, 2012) and even in municipal fountains (Biedunkiewicz, 2009).

To date, preliminary monitoring studies on the biological and species diversity of yeast-like fungi have evaluated the mycological purity in different types of water resources in a sanitary and epidemiological context (Biedunkiewicz, 2007; Dynowska, 1995; Dynowska et al., 2001). A successive increase in the number of yeast-like fungi in water is most often associated with progressive eutrophication and accumulation of organic and inorganic contamination of a different origin (Dynowska, 1995). Fungi adapt very quickly to new and frequently changing conditions in the aquatic environment thanks to an enzymatic capacity allowing them to produce adaptation enzymes. In waters destined for recreation (for instance, in different types of baths) among allochthonous microorganisms that are important from a medical perspective, there are also fungi that present a risk to humans and animals (Dynowska et al., 2013). In many cases, they may be regarded as general indicators of water quality

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(Biedunkiewicz, 2007; Dynowska, 1995; Dynowska et al., 2001; Dynowska, 1993a; Kiziewicz, 2007).

Admittedly, there is a lack of officially-published epidemiological data on the correlation between microbial density in water and infections in humans. However, the presence of species featured on the lists of potential anthropogenic pathogens suggests such potential and water reservoirs may be a serious source of fungal infections of different etiology (Dynowska et al., 2013).

A question arises: do fungi “treat” water as a specific *habitat* for multiplication, growth and development or only as a site of migration between the individual parts of the biosphere (aerosphere, human onthosphere or lithosphere)?

With this in mind, it was attempted to investigate under controlled laboratory conditions the capacity for the multiplication of selected potentially-pathogenic fungi originating from lake water, an indoor swimming pool, a drinking tap and bottled water. In addition, the enzymatic activity of the tested fungi was evaluated to determine the degree of their pathogenicity. This parameter was studied both before and after culturing.

Materials and methods

Biological material

Four fungal species were analyzed from potential pathogens that are most frequently isolated from different types of water. These were: *Candida albicans* (Robin) Berkhout (1923) – BSL - 2, *Candida tropicalis* (Castellani) Berkhout (1923) – BSL 1, *Candida guilliermondii* (Castellani) Langeron and Guerra (1938) – BSL 1, and *Candida krusei* (Castellani) Berkhout (1923) – BSL 2.

Diagnostic pathway on modified media

Cultures on media with lake water, a swimming pool water and drinking water filtered through sterile membrane filter with a 0.22 µm pore diameter were used to assess the multiplication capacity of the selected fungi under laboratory conditions. Based on such types of filtered water, solid Sabouraud’s medium and Nickerson’s medium were prepared for microcultures. Some of the filtered water was stored at +4°C for 48h and isolates of the tested fungi were added directly into it. All observations of cultures in filtered water were performed supravitally in a suspended drop on a slide placed in a humid incubation chamber. To verify the survivability of the investigated strains, supravital mounts with methylene blue were prepared after 48 h (2 days), 72 h (3 days), 154 h (7 days), 328 h (14 days) and 720 h (30 days).

For each type of water, another diagnostic pathway was developed, including a correct culture temperature and photoperiod appropriate for the analyzed environment.

To preserve the conditions that were the most comparable to the natural conditions in a bath water in summer, cultures were incubated at 20°C. This is a temperature that is typical of water on hot days. The length of a photoperiod typical of summer was retained: 17 day-hours and 7 night-hours. For the samples requiring the conditions of a swimming pool, artificial light was provided for 16 hours a day. The incubation temperature was maintained at 27°C (which is typical of a swimming pool) and at 35°C (which is characteristic for a warm Jacuzzi). In order to create conditions found in hot tap water distribution systems, a culture was run in complete darkness and at 40°C. To meet the criteria typical of storage conditions for medium-mineralized bottled water, cultures were kept at 23°C in daylight.

A technique for fungal counting and evaluation of gross morphological and microscopic morphological features

A 5° suspension (according to McFarland's scale) was prepared from each initial culture of the tested species and a 1:1,000 dilution was then performed. From the last dilution, 10 µl was sampled on another 5 Petrie dishes with solid Sabouraud's medium prepared based on the tested water types. A drop of the suspension was distributed evenly with a sterile spreader and left to be absorbed. The plates were incubated at 20°C, 23°C, 27°C, 35°C and 40°C. The cultures were stored for a maximum of 30 days and the growth rate of the colonies was assessed. Microcultures on Nickerson's agar (which was also prepared with the tested water types) were also set from the initial cultures. Microcultures were incubated at 20°C, 23°C, 27°C, 35°C and 40°C maintaining conditions that were analogous with those for Sabouraud's agar. After 48 h (2 days), 72h (3 days), 154 h (7 days), 328 h (14 days) and 720 h (30 days), the growth rate and the capacity for blastospore, mycelium and chlamydospore formation were evaluated (Biedunkiewicz-Ziomek and Dynowska, 2004). Gross and microscopic features were observed and compared with descriptions of species depicted in the keys for identification of fungi (de Hoog et al., 2000; Lodder and Kreger-van Rij, 1965; Kurtzmann and Fell, 2000; Kurtzmann et al., 2011; Howard, 2003).

Evaluation of enzymatic activity

An API ZYM (bioMérieux) containing the substrates for detecting 19 hydrolases was used to evaluate the enzymatic activity of strains before and after culturing. The level of separated hydrolases was determined based on a five-degree color scale according to the manufacturer's recommendations.

Results

The experiment found that all microfungi retained their capacity for growth under controlled laboratory conditions.

The highest number of colony-forming units was recorded on the cultures set on Sabouraud's medium at 35°C and 40°C. A slightly slower growth rate was detected at 20°C, 23°C and 27°C with the slowest growth being demonstrated at 4°C. All fungi produced blastospores and pseudohyphae. The arrangement of blastospores on pseudohyphae was typical of the individual species. *C. tropicalis* (*Fig. 1*) and *C. krusei* (*Fig. 2*) produced longer pseudohyphae than under standard culture conditions at 37°C. *C. albicans* (*Fig. 3 and 4*) and *C. guilliermondii* (*Fig. 5 and 6*) produced a lower number of blastospores although they retained a full capacity for growing typical pseudohyphae. Within a 4°C to 40°C incubation range, *C. albicans* (*Fig. 7*) did not produce intercalary and lateral chlamydospores, whereas terminal chlamydospores appeared after 7 days of incubation at lower temperatures: between 4°C and 23°C. At 20°C - 23°C, fungi multiplied, producing blastospores on shorter-than-usual pseudohyphae. The temperatures 35°C and 40°C resulted in a typical micromycelium morphology. Microfungi stored in filtered utility water types as a basis for cultures retained at 4°C a full capacity to produce biomass (vegetative cells). After 30 days, the level of cell survival was 30% for *Candida albicans* and *Candida krusei* and 10% for *Candida guilliermondii* and *Candida tropicalis*, regardless of incubation temperature.

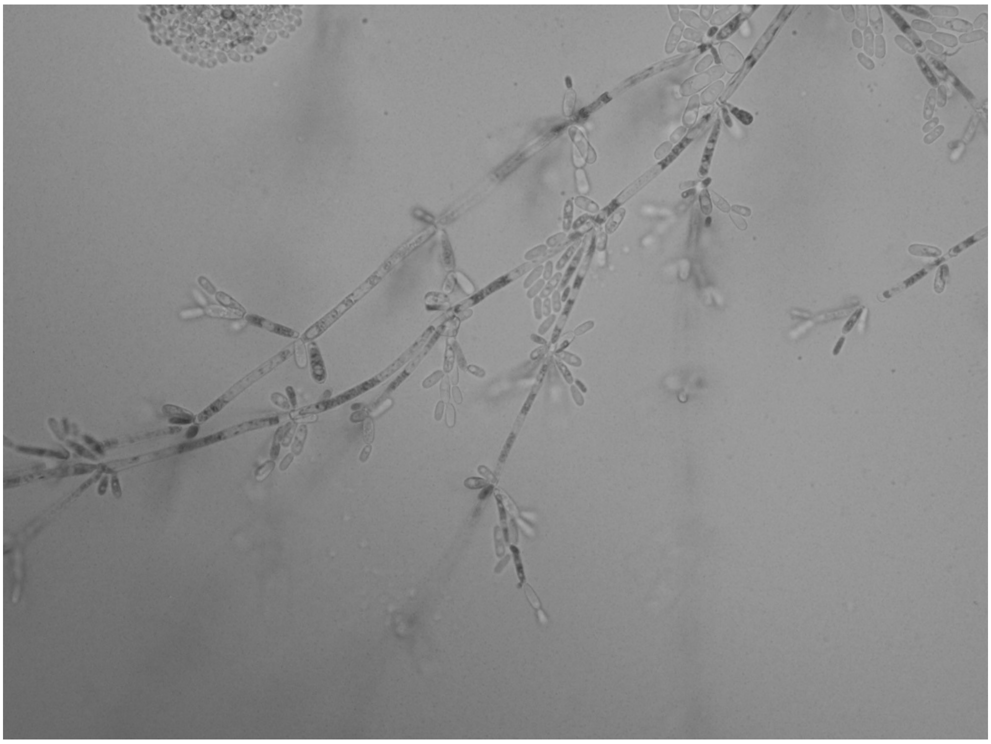

Figure 1. *Candida krusei – microculture on the modified agar Nickerson, after 154 h incubation.*

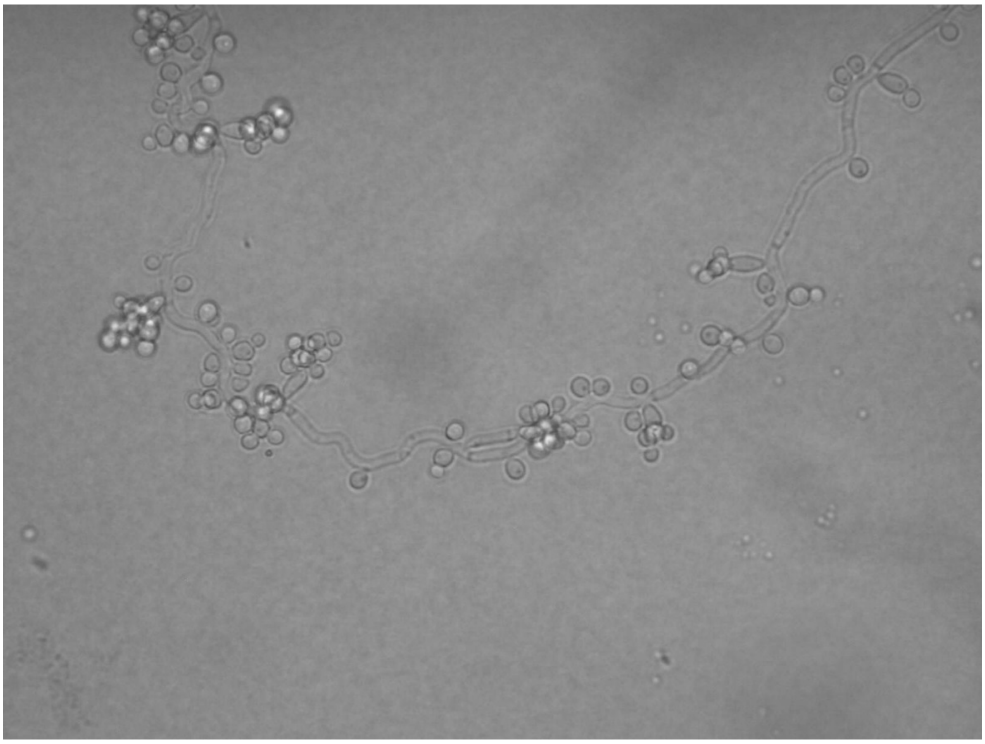

Figure 2. *Candida tropicalis– microculture on the modified agar Nickerson, after 154 h incubation.*

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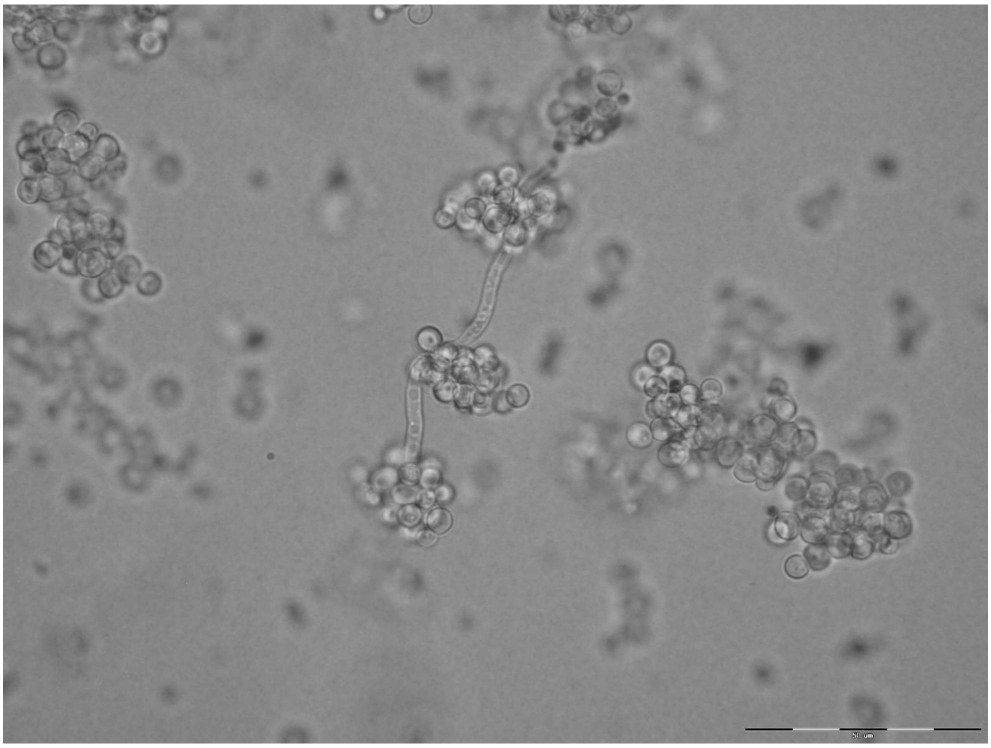

Figure 3. *Candida albicans – microculture on the modified agar Nickerson, after 72 h incubation.*

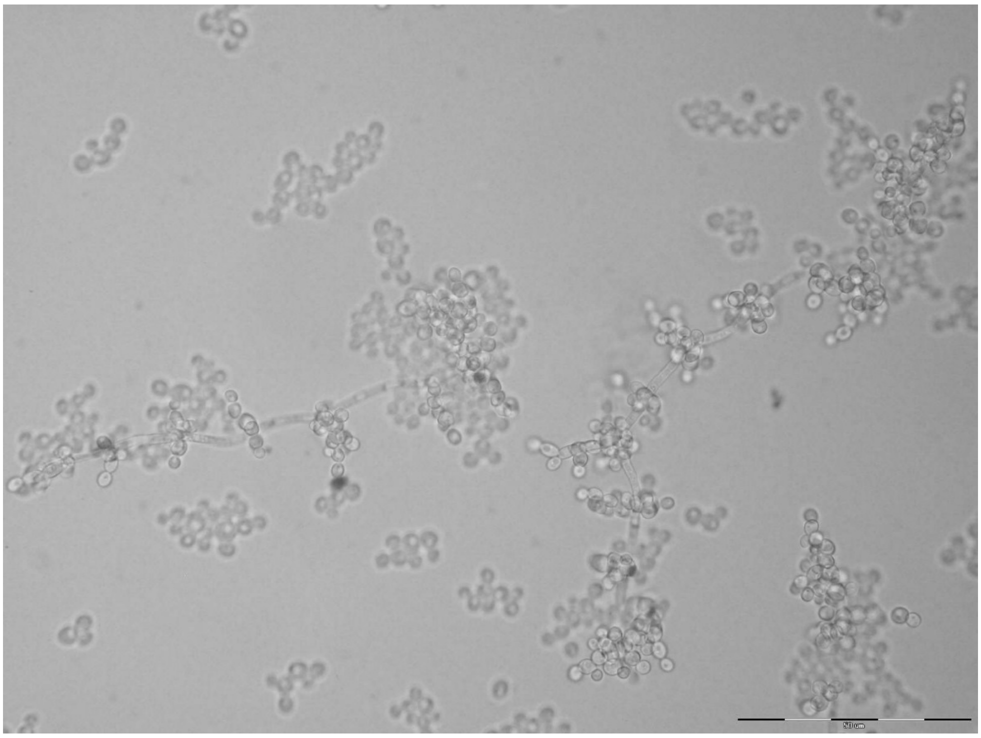

Figure 4. *Candida albicans – microculture on the modified agar Nickerson, after 144 h incubation.*

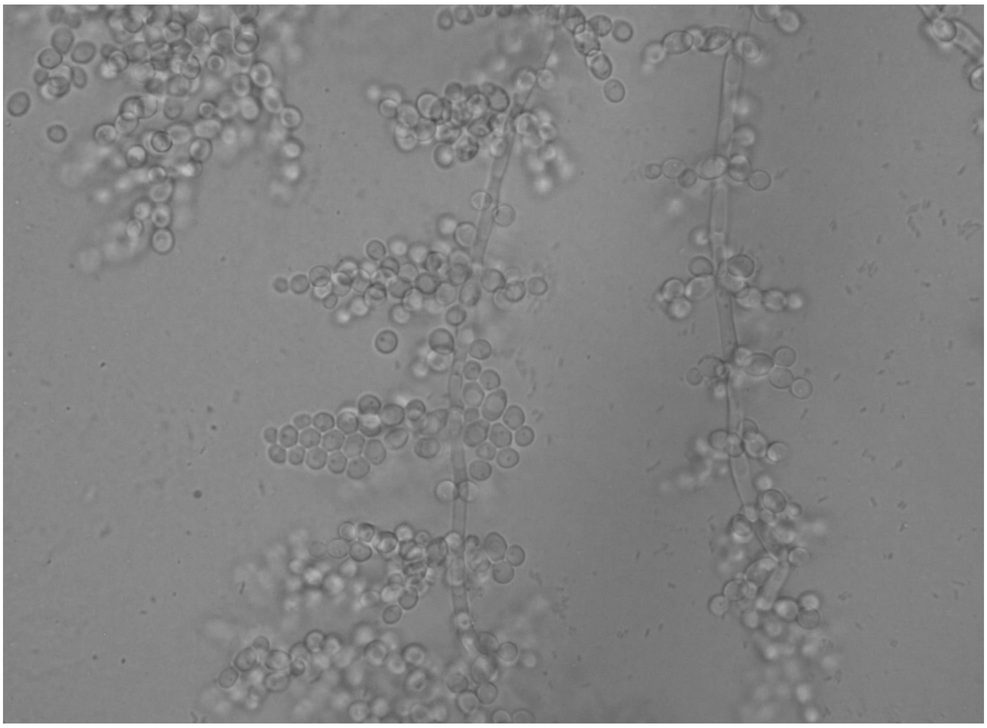

***Figure 5.** Candida guilliermondii – microculture on the modified agar Nickerson, after 72 h incubation.*

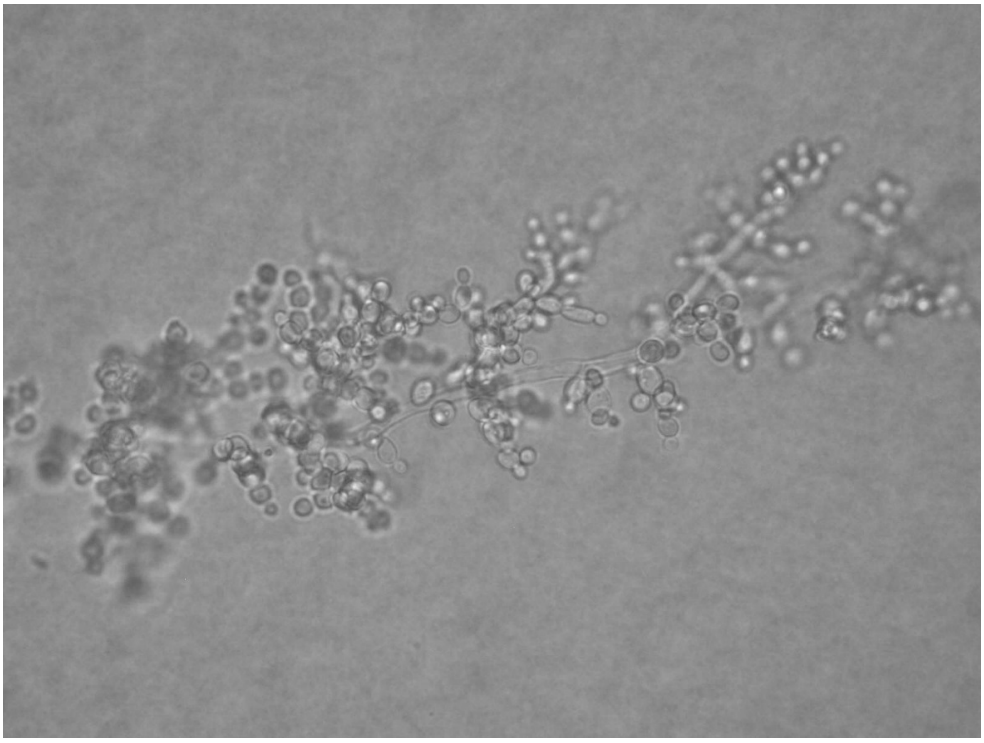

***Figure 6.** Candida guilliermondii – microculture on the modified agar Nickerson, after 154 h incubation.*

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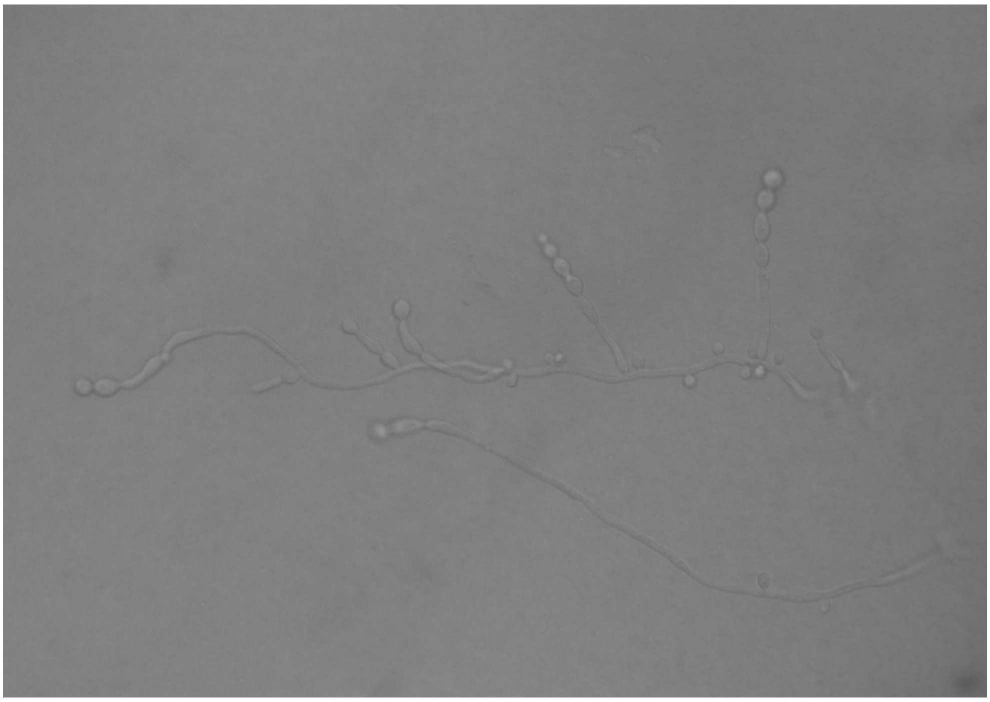

Figure 7. *Candida albicans – microculture on the modified agar Nickerson, after 154 h incubation (pseudomycelium with chlamydospores).*

The enzymatic activity of the investigated species was at a high level before multiplication and was reduced by approximately 5% after controlled multiplication under laboratory conditions. In all tested strains, the highest activity was recorded for acidic and alkaline phosphatase both before (20-35 nmol) and after the experiment (15-25 nmol). A slightly lower activity was detected for phosphohydrolase and esterase (21-30 nmol and 15-20 nmol, respectively, and 15-20 nmol and 10-20 nmol) as well as for leucine, valine and cysteine (from 15-20 nmol and 10-15 nmol). The other enzymes demonstrated activity at a levels lower than 5-10 nmol (*Table 1*).

Discussion

Similar to all living organisms, fungi need water for vital biological activities and metabolic processes (Karch, 2008). Pollution of surface waters may negatively impact the condition and livability of the cells, causing stress and a change in the rate of biological processes; in extreme cases, they may destroy the cells and eliminate the entire population. The conducted studies provide an answer to the question of the degree to which water-inhabiting fungi are adapted to a changing environment and whether the same species that are regarded as potential pathogens in medical mycology are capable of surviving in waters with different physicochemical parameters.

Table 1. *Enzymatic activity of fungi tested before and after the multiplication in controlled laboratory conditions*

	Isolates	***Candida albicans***					***Candida tropicalis***					***Candida guilliermondii***					***Candida krusei***				
	Type of water	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
ESTERASE	**ALP**	(3/3)	(3/3)	(4/4)	(5/4)	(3/3)	(2/1)	(3/3)	(4/4)	(4/3)	(2/1)	(2/1)	(2/1)	(4/3)	(3/3)	(2/1)	(2/2)	(2/2)	(4/4)	(4/4)	(2/2)
	AcP	(0/0)	(0/0)	(4/4)	(4/3)	(3/3)	(0/0)	(0/0)	(3/3)	(0/0)	(0/0)	(0/0)	(0/0)	(3/3)	(0/0)	(0/0)	(0/0)	(0/0)	(4/3)	(3/2)	(0/0)
	Ph	(0/0)	(0/0)	(4/3)	(4/4)	(0/0)	(0/0)	(0/0)	(4/3)	(0/0)	(0/0)	(0/0)	(0/0)	(2/2)	(0/0)	(0/0)	(0/0)	(0/0)	(3/2)	(0/0)	(0/0)
	Est	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	El	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	Lip	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
PROTEASE	**Leu**	(3/2)	(2/2)	(3/2)	(3/3)	(2/2)	(3/2)	(0/0)	(3/2)	(0/0)	(2/2)	(2/2)	(2/1)	(3/3)	(0/0)	(2/1)	(2/2)	(0/0)	(3/3)	(3/3)	(2/2)
	Val	(0/0)	(2/2)	(0/0)	(0/0)	(2/2)	(0/0)	(0/0)	(0/0)	(3/2)	(2/2)	(0/0)	(0/0)	(0/0)	(3/3)	(0/0)	(0/0)	(0/0)	(3/2)	(0/0)	(0/0)
	Cys	(3/2)	(2/1)	(3/2)	(0/0)	(0/0)	(0/0)	(2/2)	(0/0)	(0/0)	(0/0)	(0/0)	(2/1)	(3/3)	(0/0)	(0/0)	(2/2)	(2/2)	(3/2)	(0/0)	(2/2)
	Try	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	αChy	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
GLUCOSIDAS E	**αGa**	(0/0)	(0/0)	(1/0)	(1/1)	(0/0)	(0/0)	(0/0)	(1/0)	(1/1)	(0/0)	(0/0)	(0/0)	(1/0)	(1/1)	(0/0)	(0/0)	(0/0)	(1/0)	(1/1)	(0/0)
	βGa	(0/0)	(0/0)	(1/0)	(1/0)	(0/0)	(0/0)	(0/0)	(1/0)	(1/0)	(0/0)	(0/0)	(0/0)	(1/0)	(1/0)	(0/0)	(0/0)	(0/0)	(1/0)	(1/0)	(0/0)
	βGk	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	αGl	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	βGl	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	Nac	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	αMa	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)
	αFu	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)	(0/0)

Legend: *I – lake, II - swimming pool, III – Jacuzzi, IV - drinking tap, V - bottled water; (on/off) - (before/ after the end experiment); 0 - lack of activity (0 nmol), 1 - very low activity (1-10 nmol), 2 - low activity (11-20 mol), 3 - medium activity (21-30 mol), 4 - high activity (31-40 mol), 5 - very high activity (41 nmol)*

The hydrosphere is the biggest reservoir of fungi. Apart from harmless saprophytes, species thought to be pathogenic are also isolated from reservoirs which they enter together with surface flows or municipal sewage. The numbers of fungi and their enzymatic activity are very important in mycological analyses of aquatic ecosystems. These features allow a determination of the capacity of fungi to become involved in ecophysiological relations with other microorganisms, which is of fundamental importance in a description of aquatic ecosystem dynamics and the functioning of the entire microbiological loop (Dynowska and Biedunkiewicz, 2013).

Potential pathogens may also enter the water from the skin and mucosal membranes of asymptomatic carriers (Biedunkiewicz, 2007). Adherence to the host's cells is essential for the survival of fungi on the mucosal membrane in a symbiotic relation or by causing infections. A strong contact between the surface of epithelial cells and the layers of an adhering fungus then develops. Forms with higher metabolic activity adhere more strongly, which is important in the case of pathogenic fungi. Dimorphism, observed in some *Candida* species, plays an important role in fungi survival in different environments. Two forms may occur: a more enzymatically-active mycelial form (M) which is thought to be more pathogenic, and a yeast-like form (Y) that is primal and has a high capacity for multiplication, adherence and biofilm formation (Ejdys, 2009). Under microaerophilic conditions, blastospores elongate and a blastospore form transforms into a pseudomycelial form. Until recently, it has been thought that one of the above-mentioned forms is isolated from the natural environment (from the hydro-, litho- or aerosphere) or from the mucosal membranes in humans. In recent years, it has been observed that both developmental forms are detected in samples from different ecosystems. Furthermore, their presence in healthy individuals – asymptomatic carriers (Biedunkiewicz, 2007) and in diseased persons (Cierech et al., 2012) cannot be excluded, which may result in their occurrence in waters used for recreational purposes (Biedunkiewicz et al., 2007; Biedunkiewicz et al., 2013; Biedunkiewicz, 2007). When assessing the purity of waters for their bathing suitability, the presence of fungi poses a significant epidemiological risk.

Although to date, no cases of fungal infection in the aquatic environment have been documented, such a situation cannot be excluded because microfungi display high ecophysiological flexibility. Previous personal studies indicated the rate and intensity of fungal growth across a wide range of temperatures (Biedunkiewicz and Ejdys, 2011). Yeasts from cold environments (the psychro- or cryosphere) demonstrated relatively rapid and abundant growth at approximately 1,500 CFU/dm^3 after 24-hour incubation at 37ºC (human body temperature) (Biedunkiewicz and Ejdys, 2011). This demonstrates the rapid acclimatization of these fungi to new thermal conditions, which is more proof of their high adaptive capacity.

Yeasts and yeast-like fungi are regarded as expansive microfungi which are very active in transforming an occupied ecological niche (Dynowska, 1995). High hydrolytic activity is a reliable indicator of conquering new habitats (Dynowska et al., 2002). In conjunction with livability and the growth rate of fungal multiplication, this activity describes the adaptive capacities of individual fungal species in changing environments (Dynowska and Biedunkiewicz, 2013). The ability of fungi to adapt to new conditions by increasing the growth rate of biomass, formation of pseudomycelium and the production of a variety of hydrolytic enzymes is particularly important when fungi penetrate into organ onthocenoses or onto the surface of mucosal membranes through an infection gate. Previous personal studies (Dynowska et al., 2005) demonstrated the high production of the same groups of enzymes isolated from different types of water which had been cultured on standard media. In the present study, despite using modified media with different types of filtered water, a comparably high level of hydrolytic activity was recorded. This indicates the need to investigate microfungi through their capacity to cross protective barriers and to infect humans. A high level of hydrolytic enzyme production is of special relevance for the analysis of pathogenicity of *Candida* fungi, which includes most potential pathogens. The high level of enzymes categorized as acidic and alkaline phosphatases and proteases, as evidenced in the present study, is particularly important since they may initiate damage to the epithelial cells, thus enabling invasion and penetration of fungi deep into the tissues. A relatively high level of esterase (classified in the group of lipases) is also important in the first phase of infection (Borst and Fluit, 2003; Schaller et al., 2005; Deorukhkar et al., 2014).

Studies of different physiological capacities in fungi isolated from the aquatic environment allow determining whether a given species poses a real epidemiological threat to water users. On the surface of mucosal membranes, fungi may coexist with other microorganisms in different biological systems as opportunist, parasitic or pathogenic forms. Conditions in human onthocenoses allow microfungi to survive and multiply and, in extreme situations, to penetrate the tissues and to cause mycosis.

The variability of fungal ecophysiology in their adaptation to the environment is the best survival strategy (Dynowska, 2008). Long-term observations carried out to date in laboratories have been limited to an evaluation of the survival rate of microfungal cultures in sterile distilled water with the method by Castellani (Hartung de Capriles et al., 1989). These observations conducted at 24-28ºC for 20 years revealed a survival rate of microfungi at 62%. The species that were investigated in the present study (*Candida albicans, C. guilliermondii, C. tropicalis* and *C. krusei*) according to Hartunda de Capriles et al. (1989) demonstrated survival lasting from zero (*C. krusei*) up to 19-20 years (*C. guilliermondii* and *C. tropicalis*). *C. albicans*, the most commonly-detected pathogen, survived for only 3 years. However, in cultures capable of further growth, there was 100% livability of the examined strains. When testing *Candida albicans* under starvation in different types of water and at 25ºC (seawater, drinking water and precipitation water) in each water type, a reduction in livability was recorded after approximately one month (Chaieb et al., 2011).

Under conditions simulating the natural environment, the capacity of microfungi for an increased growth rate of biomass and an ability to produce pseudomycelium were observed. This indicates an extended capacity to conquer new substrates, including living organisms. Standard cultures (run in the recommended rage of temperatures: at 25°C and 37ºC and on media as suggested in mycological diagnostics) allowed the gross features of grown colonies to be determined. The observations of inoculates in microcultures on Nickerson's agar in humid chambers reveal morphological features. Shorter distances than those usually seen in cultures between blastospores being formed on pseudohyphae indicate an increased rate of vegetative cell biomass production. The production of intercalary and lateral chlamydospores was inhibited at incubation across the whole range of temperatures while terminal chlamydospores occurred after a week of incubation at 4°C-23°C. This indicates favorable conditions for the growth of *Candida albicans* that was capable of chlamydospore production.

Photoperiod is another factor impacting living organisms. The available literature lacks long-term studies on this phenomenon and fungi that are relevant in medical mycology (Dynowska, 1993b). There are only reports on the impact of day and night duration on phytopathogenic microfungi in correlation with the growth of a host (Jerzy, 2003).

The different culture variants that were adopted in the present study allowed us to select habitats in which attention should be paid to the presence of fungi. These conditions arguably include waters with increased temperatures, such as water in a warm Jacuzzi or tap water. The capacity for growth typical of a given species, multiplication and formation of well-developed pseudomycelium at higher temperatures provides a basis for including selected *Candida* species as proposed bioindicators of warm aquatic ecosystems. Although one should not indiscriminately relate the results recorded under laboratory conditions to the natural aquatic environment, it is possible to determine in which types of water fungi retain all ecophysiological features that are typical of potential pathogens.

The ecophysiological features (multiplication rate, enzymatic activity, capacity for pseudomycelium formation) selected for analyses indicate that microfungi should be considered to be pathogenic factors whose presence in thermal and recreational waters disqualifies them from public use. Based on the conducted experiment, it was found that regular mycological monitoring should be performed in swimming pool water, especially in a warm Jacuzzi and in drinking tap water. Moreover, constant mycological studies of baths during summer due to their massive use by people should be provided. In this context, water is a site of fungal migration. However, one cannot exclude the role of water as a “habitat” for potential pathogens since, depending on the type and volume as well as the availability of organic matter, numerous fungal species may find favorable trophic and growth conditions, which directly affect the rate of metabolic processes.

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SIMULATION OF N_2O EMISSIONS FROM A SUGARCANE FIELD IN OKINAWA, JAPAN

BELLIDO, J.[1] – SAKAI, K.[2*] – NAKAMURA, S.[2] – KAZURO, M.[3] – OKAMOTO, K.[1] – NAKANDAKARI, T.[2]

[1]*The United Graduate School of Agricultural Sciences, Kagoshima University*
Korimoto 1-21-24, Kagoshima 890-8580, Japan.

[2]*Faculty of Agriculture, University of the Ryukyus*
Senbaru 1, Nishihara, Okinawa 903-0213, Japan.

[3]*Faculty of Agriculture, Kagoshima University*
Korimoto 1-21-24, Kagoshima 890-8580, Japan.
(phone: +81-98-895-8783, fax: +81-98-895-8734)

**Corresponding author*
e-mail: ksakai@agr.u-ryukyu.ac.jp

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Abstract. We measured N_2O emissions from a sugarcane field planted in spring 2011 in Okinawa, and examined their variations during the summer growing period (13 July to 4 August). Then we performed simulations with the DeNitrification and DeComposition (DNDC) and Agricultural Production Systems Simulator (APSIM) models to estimate N_2O emissions and compared the results with the observed data from the field experiment. The results showed that (i) nitrification was the dominant process affecting N_2O emissions and nitrifier denitrification occurred under high soil moisture conditions after rainfall; (ii) there was large spatial dispersion of N_2O emissions even in the same field, and it was necessary to consider this dispersion to estimate total N_2O emissions from the field; (iii) the emission factor of the N_2O emissions might be larger than that used by the National Greenhouse Gas Inventory of Japan; (iv) the DNDC model overestimated, and the APSIM model underestimated, the observed N_2O emissions; and (v) simulation of N_2O emissions associated with nitrification by the DNDC model might be improved by modifying coefficients in the equation used to calculate N_2O production during nitrification.
Keywords: *nitrous oxide, nitrification, denitrification, global warming*

Introduction

Global warming is one of the most serious problems facing the world today. The increased heat is trapped in the atmosphere by high concentrations of greenhouse gases (mainly CO_2, N_2O, and NH_4), which reflect the sun's rays back down to the earth (IPCC, 2006; Murray et al., 2005). According to the IPCC (2013), a human influence on the climate system is evident in most regions of the planet. For instance, the combustion of fossil fuels for energy (coal, oil, etc.) produces CO_2 emissions, which have accumulated in the atmosphere. Therefore, substantial reductions of greenhouse gas emissions are essential to mitigate global warming.

The use of biofuels as an alternative to fossil fuels can reduce greenhouse gas emissions. Sources of biofuels include crops such as corn or sugarcane. Although combustion of biofuels releases carbon into the atmosphere as CO_2, these emissions are considered carbon neutral because the carbon is continuously recycled by the emerging plants (Bengston, 2013). Consequently, biofuel use would help to reduce the accumulation of CO_2 emissions from the energy sector in the atmosphere. Crutzen et al.

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(2008) has argued, however, that even if CO_2 emissions are reduced by using biofuels, total greenhouse gas emissions might not be reduced because nitrous oxide (N_2O) is emitted during cultivation of these crops .

N_2O is a powerful greenhouse gas that has been calculated to have 300 times the global warming potential of CO_2 over a 100-year period (Reilly et al., 2003). Agricultural soils are the major source of anthropogenic N_2O emissions (Bouwman, 1996; Akiyama et al., 2000; Smith et al., 2002; Forster et al., 2007). According to the National Greenhouse Gas Inventory Report of Japan (hereafter, NIR; NGGI, 2012), about 25% of the N_2O emissions from agricultural soils is due to the use of fertilizers. Emission factors (EFs) for N_2O emissions due to the application of chemical and organic fertilizers have been calculated. Akiyama et al. (2006) compared EFs for N_2O from agricultural soils in Japan used for various crops and reported that the EF for tea is relatively high (2.9%) and that for rice (0.31%) is relatively low than the EFs for other crops. However, Nonaka (2007) reported that the amounts of nitrogen fertilizers applied to crops have been increasing from year to year. Such increases are expected to directly influence N_2O EFs.

Sugarcane, a typical crop in tropical and subtropical regions, is one of the most demanding crops in terms of fertilizer use (FAO, 2003; Shanthi et al., 2013). Miyakojima in Okinawa Prefecture is one of 13 localities selected by the Regional Revitalization Bureau of Japan as an Eco-Model City (RRB, 2013), and the Eco-Model City Project has promoted the use of sugarcane as the raw material for bioethanol for fuel (Kawai et al., 2011). In the bioethanol production process, however, the project considers only the CO_2 balance; it does not take into account the total greenhouse gas balance or N_2O emissions from sugarcane fields. Direct measurements can be used to characterize N_2O emissions during sugarcane cultivation, but from planting to harvest the sugarcane cultivation period is a year to a year and a half long. Thus, much time is required to obtain enough experimental field results to characterize N_2O emissions during sugarcane production. Therefore, having a properly validated model that can accurately simulate N_2O emissions from the sugarcane fields of Okinawa is important.

The aim of this study was first to measure N_2O emissions from a sugarcane field in Okinawa, and then to conduct simulations of N_2O emissions with two models: the Denitrification and Decomposition (DNDC) and the Agricultural Production Systems Simulator (APSIM) models. We then compared the model estimations with the observed results and examined considerations important for accurate estimation of N_2O emissions from sugarcane fields in Okinawa with these models.

Materials and methods

This study consisted of two parts. The first part was a field experiment for the collection of N_2O emissions data, and in the second part, simulations of N_2O emissions were carried out with the DNDC and APSIM models.

Field experiment and measurement of N_2O emissions

The field experiment was carried out in a sugarcane field at the University of the Ryukyus, Okinawa Island, Japan (26°14′N, 127°45′E). Meteorological data collected from 1981 to 2010 at the Naha weather station of the Japan Meteorological Agency (10 km southwest of the study site) show that the research site receives an average rainfall of 2000 mm. The mean annual humidity is 79%, and the average annual temperature is

23.3°C. January is the coldest month (average temperature, 14.5°C), and July is the warmest month (26.7°C).

The experimental work was conducted in a lysimeter (3.6 m × 2.1 m) filled with Shimajiri-maji (dark red calcareous soil: USDA soil taxonomy), a local soil in Okinawa. The Japanese sugarcane cultivar NORIN-8 (Okinawa Prefectural Agricultural Research Center; Itoman, Okinawa, Japan) was grown. Seeds were sown and germinated in pots, and then the seedlings were transplanted to two ridges in the lysimeter. The distance between plants was 0.25 m, and the distance between the two ridges was 1.25 m. Ammonium sulfide $(NH_4)_2S$ fertilizer (0.083 kg-N, equivalent to 110 kg-N ha^{-1}), was applied in two doses. The sugarcane cultivation schedule is shown in *Table 1*.

Table 1. *Schedule of sugarcane cultivation and N_2O measurement*

Date (dd/mm/yyyy)	Transplanting	Fertilization dose	N_2O measurement	Harvest
13/05/2011	X			
10/07/2011		110 kg-N ha^{-1}		
12/07/2011			X	
04/08/2011			X	
25/08/2011		110 kg-N ha^{-1}		
28/02/2012				X

Two PVC cylinders (15 cm high, 10 cm inner diameter) were installed, one on a ridge and the other between the ridges, on 10 July (*Fig. 1*).

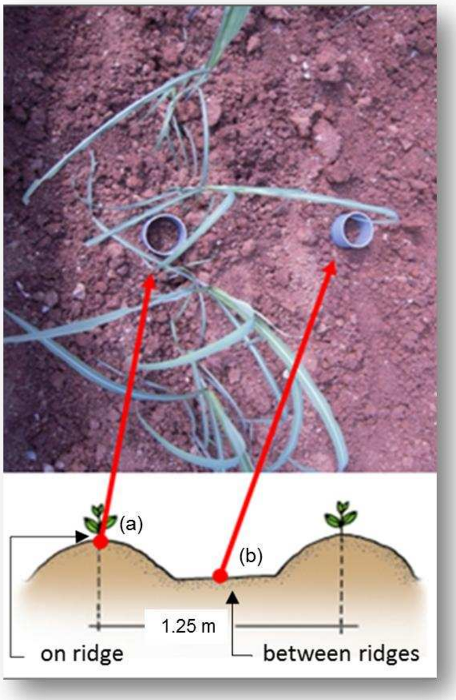

Figure 1. *Installation of chambers in the lysimeter. Chambers were placed in two different locations: (a) on ridge; (b) between ridges.*

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The tops of the cylinders were covered to isolate the air inside the cylinder from the atmosphere, and the airflow was controlled by solenoid valves at defined time intervals. N_2O fluxes were measured by the closed-chamber method, by connecting the closed cylinders to a Thermo Scientific Model 46i Nitrous Oxide Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), which measures ambient N_2O concentrations by non-dispersive infrared spectrometry. N_2O emissions were measured for 30 min every hour, followed by 30 min of ventilation to the atmosphere, alternately in the two cylinders. This procedure was repeated continuously from 13 July to 4 August 2011.

The change in the N_2O concentration, expressed as ppm min^{-1}, was calculated by linear regression using data from the last 7 min of each measurement period. The N_2O flux was then calculated by using the ideal gas law as follows:

$$q = 60 \cdot N \cdot 10^6 \cdot \frac{PV}{RT} \cdot a \cdot \frac{1}{A} \quad \text{(Eq. 1)}$$

where q is the N_2O flux ($\mu\text{g-N} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), N is molecular weight, P is standard atmospheric pressure (101325 Pa), V is the total volume of the closed chamber system (L); R is the gas constant (Pa L mol^{-1} K^{-1}), T is the soil temperature inside the cylinders, a is the change in the gas concentration per minute (ppm min^{-1}), and A is the area of soil surface within the cylinder (m^2).

Simulation of N_2O emissions with the DNDC and APSIM models

DNDC

DNDC (version 9.5) is a process-oriented model consisting of four sub-models: soil, climate, crop, and decomposition and denitrification (Ri et al., 2003; Vogeler et al., 2013). Three sets of data are input: (i) climatic conditions (temperature, precipitation, wind speed, irradiation), (ii) soil parameters (texture, organic matter content), and (iii) farming parameters (crop, fertilization, management). A generic agro-ecosystem modelling framework is used to predict carbon and nitrogen cycling from the input parameters. Model output consists of daily water balance, carbon balance, nitrogen balance, and crop yield. This study focused exclusively on N_2O emissions, and we calculated the total nitrogen flux by summing the calculated daily fluxes of each simulated year.

APSIM

The APSIM is a modelling framework developed to simulate biological and physical processes of cropping systems in response to climate and management (Keating et al., 2003; Delve and Probert, 1998). We used version 7.6, which consists of three components: (i) a set of management modules that allow the user to specify the initial characteristics of the simulation, including data entry options, as well as the format of the output data; (ii) a set of biophysical modules to simulate the biological and physical processes of the selected farming system; and (iii) the simulation engine, which drives the whole simulation process and facilitates communication among the modules. In addition to the modular framework, APSIM provides generic simulations tested for

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several cropping systems in temperate and tropical regions, including a strong framework for simulating sugarcane crops.

Data and parameters used for simulation of N_2O emissions

Both simulations were conducted for the period from 1 January 2011 to 31 December 2012. The cultivation schedule shown in *Table 1* was also used in the model simulations. Climate data used were those observed at Naha weather station of the Japan Meteorological Agency. The soil and crop parameters used in each model are shown in *Table 2*.

Table 2. *Input parameters for the DNDC and APSIM models*

	Soil	
	DNDC	APSIM
Bulk density [g cm^{-3}]	1.325	
Saturated soil moisture content [m^3 m^{-3}]	0.504	
Moisture content at field capacity [m^3 m^{-3}]	0.473	
Moisture content at the first wilting point [m^3 m^{-3}]	0.361	
Moisture content at the permanent wilting point [m^3 m^{-3}]	-	0.282
Cray function [%]	0.40	-
Soil organic carbon [kg-C Kg^{-1}]	0.012	-

	Crop	
	DNDC	APSIM
Maximum biomass production [kg-C ha^{-1} yr^{-1}]		Sugarcane cultivar [a] => nco376
Grain	267.0	
Leaf	2136.0	
Stem	20 025.0	
Root	4272.0	
Annual nitrogen demand [kg-N ha^{-1} yr^{-1}]	400.5	
Thermal degree days for maturity [°C d]	12 000.0	
Water demand [g water g drymatter^{-1}]	350.0	
Nitrogen fixation index [crop-N N-from-soil^{-1}]	1.3	

[a] APSIM does not allow users to set crop parameters, but the cultivar can be chosen from a list.

Results and discussion

Measurement of N_2O emissions in the field

N_2O emissions were clearly detected in the chamber on the ridge during the observation period (*Fig. 2*). Fluxes were relatively larger in the daytime than in the nighttime; and we inferred that they varied synchronously with air temperature changes. N_2O emissions increased from 13 July, when rainfall occurred occasionally, and emissions were highest on 20 July; thereafter, the emissions decreased gradually. We assumed that the decrease in N_2O emissions was due to a decrease in the ammonium content of the soil caused by nitrification. Morimoto et al. (2008) also reported that, in an experiment conducted at a lettuce farm, N_2O emissions were highest soon after the application of fertilizer and then gradually decreased, and they concluded that nitrification was the dominant process affecting N_2O emissions during their experiment. Likewise, Watanabe et al. (2000) reported that the main process affecting N_2O emissions from a maize field in northeastern Thailand was nitrification. Wrage et al. (2001) showed convincingly that nitrifier denitrification, the process by which ammonia (NH_3) is first oxidized to nitrite (NO_2^-) and then the NO_2^- is reduced to nitric oxide (NO), nitrous oxide (N_2O), and molecular nitrogen (N_2), contributes to N_2O emissions. Subsequently, Kool et al. (2011) showed that nitrifier denitrification is a significant cause of N_2O emissions from soil under high soil moisture conditions. On the basis of these previous results, we concluded that nitrification was the dominant process responsible for N_2O emissions in our sugarcane field, and that nitrifer denitrification occurred under high soil moisture conditions after rainfall in our experiment.

In contrast, N_2O emissions measured between ridges were mostly zero during the observation period; N_2O emissions were detected between ridges only after a rainfall (*Fig. 2*). Because the fertilizer was applied only on the ridges, there was little nitrogen from the fertilizer between the ridges. Therefore, we inferred that N_2O emissions occurred between the ridges only after rainfall had washed nitrogen (as ammonium) from the ridges.

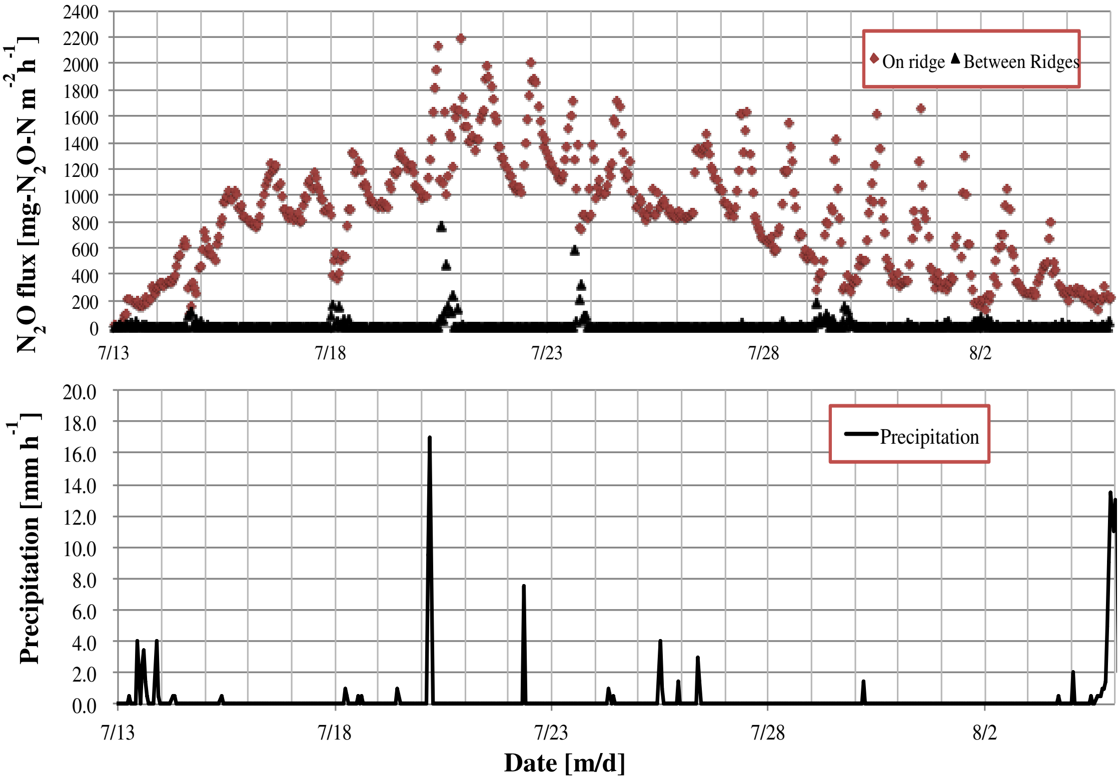

Figure 2. *Field measurement results: (a) On-ridge and between-ridge N2O emissions; (b) precipitation.*

These results show that there was large spatial difference in emissions even in the same field. Therefore, to estimate total N_2O emissions from the field, it was necessary to take dispersion into account. Therefore, we calculated EFs (i.e., the percentage of nitrogen in the N_2O emissions attributable to the added nitrogen in fertilizer) for three cases. Because N_2O emissions from between the ridges were mostly zero, we considered the N_2O emissions from the field without fertilizer to be zero. We then calculated N_2O emissions for three different cases: Case 1, ridge width = 10 cm; Case 2, ridge width = 20 cm; and Case 3, the average of on-ridge and between-ridge emissions. The EFs calculated for the chamber placed on-ridge, for the chamber placed between-ridge and for the Cases 1–3 are shown in *Table 3*. The NIR EF for upland fields is 0.62% (NGGI, 2012). The calculated between-ridge and Case 1 EFs were lower than the NGGI value of 0.62%. Because the diameter of the chamber in our experiment was 10 cm, we assumed that Case 1 represented the minimum width of the fertilized area. Therefore, we inferred that the actual width of the fertilized area might be close to or wider than the Case 2 width and that the actual EF for this sugarcane field was larger than the NIR value.

Table 3. *Value of the EF of each case*

Emission factor	On-ridge	Between-ridge	Case 1	Case 2	Case 3
EF (%)	4.43	0.065	0.481	0.897	2.25

Comparison of the DNDC and APSIM model estimations with the experimental results

We show the observed N_2O emissions data and the values calculated by the DNDC and APSIM models in *Figure 3*.

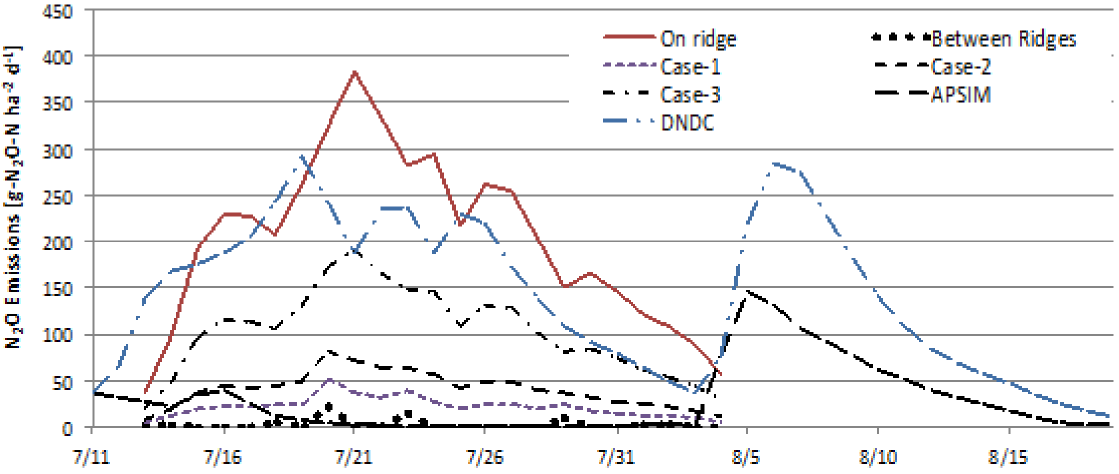

Figure 3*. Comparison of observed N2O emissions with values calculated by the DNDC and APSIM models.*

Because the output of both models is given on a daily basis, we calculated the total daily N_2O emissions from the measured data and then compared the simulated emissions with the observed values. Considering the spatial dispersion mentioned above, we compared the simulation results with the N_2O emissions of Cases 1–3 as well as with the observed on-ridge and between-ridge emissions.The observed data collected from 13 July to 4

August were compared against the simulation for the period from 10 July to 19 August. The pattern of N_2O emissions simulated by the DNDC model was similar to that of the field data, although a time lag was slightly detected in the simulation. In contrast, the N_2O emissions simulated by APSIM were smaller than the observed emissions, and the emissions peak was simulated soon after the 10 July fertilizer application.

Total N_2O emissions for on-ridge chamber, between-ridge chamber, and for each of the three cases (Case 1, ridge width = 10 cm; Case 2, ridge width = 20 cm; and Case 3, the average of on-ridge and between-ridge emissions) were then calculated from the sum of each N_2O emission, separatelly, during 23 days (from 13 July to 4 August). Similarly, total N_2O emissions simulated by two models over the same time period, are shown in *Table 4*.

Table 4. *Total measured and simulated N_2O emissions*

N_2O emission	**On-ridge**	**Between-ridge**	**Case 1**	**Case 2**	**Case 3**	**DNDC**	**APSIM**
Total (kg)	4655.3	68.7	550.5	897.5	2249.5	3778.6	246.0

The emissions simulated by DNDC were between the Case 3 and on-ridge values. We considered that the most realistic N_2O emissions value was probably close to the Case 2 value or between the Case 2 and Case 3 values. Therefore, we thought that the DNDC model overestimated actual N_2O emissions. In contrast, the emissions simulated by APSIM were smaller than the Case 1 emissions. Thus, we considered that APSIM underestimated N_2O emissions. Vogeler et al. (2011) showed that N_2O emissions simulated by the DNDC model from a urine patch in a pasture were larger than the emissions simulated by the APSIM model. Considering that N_2O emissions from agricultural soils are influenced by many factors, including soil moisture, soil temperature, and inorganic nitrogen and organic carbon contents (Akiyama et. al., 2010), it is clear that the DNDC and APSIM models must be validated to determine the most appropriate model for simulating N_2O emissions from sugarcane fields in Okinawa Prefecture.

Characteristics of the DNDC and APSIM models in the simulation of N_2O emissions

We showed above that the DNDC model overestimated and the APSIM model underestimated N_2O emissions. Next, we examined the characteristics of the two models in the simulation of N_2O emissions, focusing on nitrification and denitrification processes. Daily changes in the nitrification and denitrification rates calculated by the DNDC and APSIM models are shown in *Figure 4*.

Comparison of the ranges of the nitrification and denitrification rates confirmed that in the simulation results of both models the nitrification rate was larger than the denitrification rate. Moreover, in both models, the denitrification rate was influenced by precipitation events. Some differences in the nitrification rate were detected in the two models, however. In the APSIM model, the nitrification rate was highest soon after the fertilization, whereas in the DNDC model nitrification rate peaks occurred after rainfall events. This difference reflects that fact that the nitrification equation in the DNDC model takes into account the effect of soil moisture, whereas that in the APSIM model

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does not. N_2O production during nitrification is simulated in the two models by using the following equations:

$$\text{DNDC}: N_2O_{ni} = 0.006 \cdot R_{ni} \cdot W_{fps} 2.72^{34.6-9615/(T_s+273.15)} \quad \text{(Eq. 2)}$$

$$\text{APSIM}: N_2O = k_{ni} \cdot R_{ni} \quad \text{(Eq. 3)}$$

where N_2O_{ni} is the N_2O production during nitrification; R_{ni} is the nitrification rate (kg ha^{-1} d^{-1}); W_{fps} is the water-filled pore space (%), T_s is the soil temperature (°C), and k_{ni} is a coefficient for estimation of N_2O production.

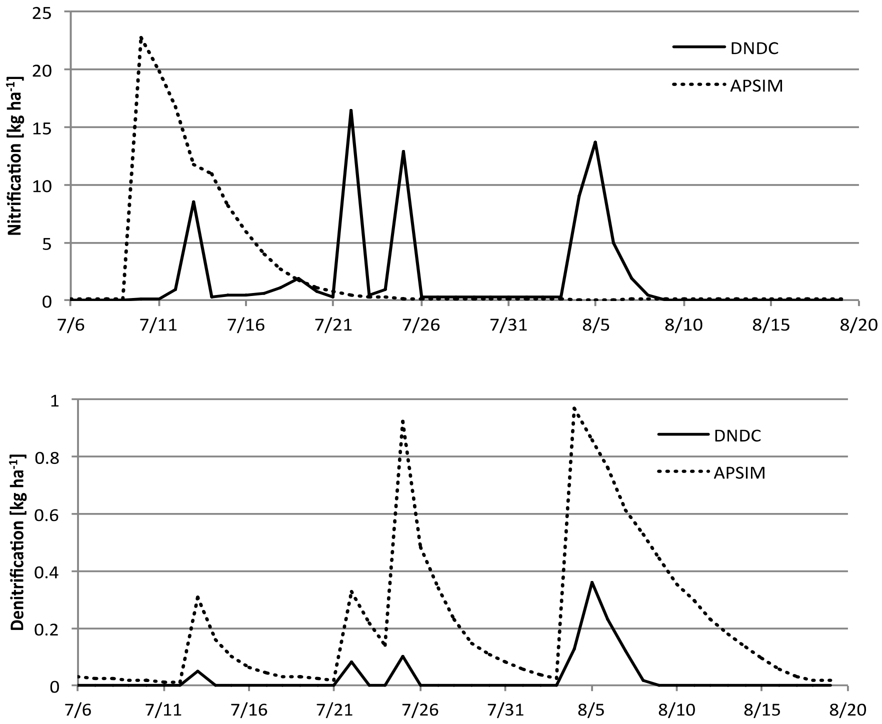

Figure 4. *Daily changes of the (a) nitrification rate and (b) denitrification rate calculated by the DNDC and APSIM models.*

The DNDC model also takes into account the fraction of N_2O emitted to the atmosphere. As a result, in the simulation, nitrification rate peaks and rainfall events are synchronous with each other and with N_2O emissions.

In the APSIM model, we used 0.002 as the default value of k_{ni}, following Li et al. (2007). However, this value may be soil-specific, so a larger value might have been a more appropriate value in our study. In future studies, different k_{ni} values should be evaluated.

Our results suggest that the simulation of N_2O emissions associated with nitrification by the DNDC model might be improved by modifying the coefficients in Eq. (2) such that total simulated N_2O emissions agreed with the total observed N_2O emissions.

Conclusions

We measured N_2O emissions from a sugarcane field in Okinawa Prefecture to examine the characteristics of N_2O emissions in the field. Then, we simulated N_2O emissions using the DNDC and APSIM models and compared the results with the observed data in order to examine the applicability of the two models.

From the results of this study, we concluded as follows:

1) Nitrification was the dominant process affecting N_2O emissions, and nitrifer denitrification occurred under high soil moisture conditions after rainfall.
2) There were large spatial variations of N_2O emissions even in the same field, so it was necessary to consider dispersion to estimate N_2O emissions from the field.
3) The emission factor for N_2O emissions from this field might be larger than the emission factor for upland fields proposed by the NIR.
4) The DNDC model overestimated the observed N_2O emissions and the APSIM model underestimated them.
5) It might be possible to improve simulations of N_2O emissions associated with nitrification by the DNDC model by modifying the coefficients in the equation used to calculate N_2O production during nitrification.

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INFLUENCE OF ULTRAVIOLET IRRADIATION AND ACID PRECIPITATIONS ON THE CONTENT OF ANTIOXIDANTS IN WHEAT LEAVES

BADRIDZE, G.* – KACHARAVA, N. – CHKHUBIANISHVILI, E. – RAPAVA, L. – KIKVIDZE, M. – CHANISHVILI, SH. – CHIGLADZE, L.

Ilia State University Institute of Botany Botanikuri 1, Tbilisi 0105, Georgia
(phone: +99-532-214-7168; mob.: 555-270-698)

**corresponding author*
e-mail:gbadridze@yahoo.com

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Abstract. Combined effect of pre-sowing treatment with UV and post-emergence spraying with acid solution on antioxidant system of wheat species (*Triticum aestivum* L.- soft wheat, *T. durum* Dest. – durum wheat, *T. macha* Dek. Et Men. – macha wheat (endemic of Georgia, partly wild, relict species), and three varieties of *T. persicum* Zhuk. - dika: *T. persicum var. stramineum, T. persicum var. fuliginosum* and *T. persicum var. rubiginosum* (white, black and red dika respectively) has been studied. The combination of two stresses induced full stimulation of the enzymatic system (catalase, peroxidase), caused increase of phenolic substances content (16-58%) and anthocyanins synthesis in leaves of tested species. Impact of both stresses increased content of proteins in leaves of Macha wheat, soft wheat and white and red varieties of dika, while in rest species content of proteins diminished. Influence of two stresses diminished the activity of the protective ascorbate system in leaves of all tested species, except the durum wheat. Normal termination of vegetation cycle and grain harvest of all tested species points to their resistance to the applied stresses.

Keywords: *UV irradiation of seeds, artificial acid precipitations, antioxidants, wheat.*

Introduction

Under natural conditions plants are usually attacked by several stress factors simultaneously. According to investigations the plant's response to the combined effect of several stressors differs from its reaction on the same factors separately (Craufurd and Peacock, 1993; Jiang and Huang, 2001; Pnueli et al., 2002). Plants resistance to simultaneous effect of different stresses is of particular interest in selection. This problem is very popular today, though little is known about the molecular mechanisms responsible for plant adaptation to combined effect of different stressors (Rizhsky et al., 2004).

Mostly data on simultaneous influence of drought and high temperature on agricultural plants are given in literature (Craufurd and Peacock, 1993; Jiang and Huang, 2001). It is established that the combination of these two stressors comparatively negatively influences plant growth and development, than each of them separately (Jagtap et al., 1998; Wang and Huang, 2004). The ultraviolet irradiation (UV) and acid precipitations (AR) are among those abiotic factors, which significantly influence plant. Effect of each of these stressors separately on different plant species was investigated by a number of scientists. Though, there is no data on the combined influence of UV and AR on plants.

The combined effect of pre-sowing treatment with UV and acid spraying on antioxidant system of some wheat species has been studied in the given work. The

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purpose of the investigation was: 1. To establish the reaction of the antioxidant system of plants on the successive impact of two stressors. In particular, how the effect of one stressor (UV irradiation) stimulates or diminishes the tolerance of plant towards another stress (acid precipitation). 2. To reveal comparatively stress-resistant species of wheat. We supposed that combination of UV irradiation and acid precipitations may have positive influence on physiological and biochemical indices of plant connected with stress resistance. In case of positive results they could be taken into account in the optimization of agriculture.

Review of literature

Generally it is known that influence of any stress induces formation of reactive oxygen species (ROS) in plant. Therefore the mechanism of stress-resistance to different stressors seems to be similar in plant (Kreps et al., 2002; Cheong et al., 2002). Although, investigations have revealed activation of various genes in response to ROS formed as a result of influence of different stressors in Arabidobsis (Mittler et al., 2004). According to these data we must suppose that adaptation to separate stress-factor is unique in plant and is realized following the plant's demand at a particular moment. Thus, combined effect of two or more stressors seems to induce diverse response in plant (Mittler, 2006). Moreover, these responses may be antagonistic (Mittler, 2002; Barcelo and Poschenrieder, 1990). In literature we meet examples on synergetic and antagonistic relations between the responses to different stresses (Walter, 1989; Sandermann, 2004).

Thus, the appropriate respond is needed for plant adaptation to combinated affect of different stress-factors, which would fit each of stressors and at the same time must compensate the antagonistic aspects of stress-factors combination.

UV irradiation is one of the stresses attacking plants during the whole life. It is known that high doses of UV-B and UV-C irradiation negatively influence plant growth and development, photosynthesis and other vital processes. UV irradiation stimulates formation of ROS and oxidative stress, which have undesirable effect on macromolecules and may lead to the cell death (Toncheva-Panova et al., 2010; Zu et al., 2010; Pradedova et al., 2011; Schreiner et al., 2012). Though, low doses of UV-B and UV-C may increase the stress-adaptive reactions in plant, which considers activation of the enzymatic and non-enzymatic defense systems (Lavola et al., 2003; Katerova and Todorova, 2011; Rai et al., 2011).

A number of investigations deal with the positive role of pre-sowing treatment of seeds with different type of electromagnetic irradiation (among them is UV irradiation) on yield amount and quality, also in protection against different diseases; because treatment of seeds with chemicals against diseases is inconvenient both ecologically and economically (Dubrov, 1977; Ghallab and Omar, 1998; Delibaltova and Ivanova, 2006; Aladjadjiyan, 2007).

In our early experiments irradiation of some vegetable seeds with full spectrum of UV affected the metabolism of plants developed from these seeds, and caused activation of stress-adaptive mechanisms, by stimulating the synthesis of antioxidants in leaves. The results depended on irradiation intensity, plant species and the type of antioxidant system (Kacharava et al., 2013).

Recently acidic precipitation ("acid rain") has been considered to be one of the natural stressors endangering the ecological balance in the biosphere. In spite of the

efforts of the international community, acidification of the environment still remains a significant ecological problem (Rogizhin et al., 2000; Dukhovskiy et al., 2003; Chupakhina and Maslennikov, 2004). Acidic precipitations induce the formation of free radicals and active oxygen species—deleterious to plants and limiting their tolerance against different environmental factors (Merzliak, 1999; Munzuroglu et al, 2004).
According to our previous observations spraying leaves of different species of wheat with simulated acid precipitations (pH2.5 and pH1.5) caused changes in the qualitative characteristics of the elements of antioxidative system and in many cases had stimulative effect. Though, revealed changes were not equal and depended on plant species (Chkhubianishvili et al., 2008; Rapava et al., 2010).

Materials and methods

Plant material

Following species of wheat were selected for testing: winter wheats - *Triticum aestivum* L.- soft wheat (as widely cultivated and popular species of wheat), *T. durum* Dest. – durum wheat (valuable species of high nutritional quality), *T. macha* Dek. Et Men. – macha wheat (endemic of Georgia, partly wild, relict species), and spring wheats – three varieties of *T. carthlicum* Nevski: *T. carthlicum var. stramineum, T. carthlicum var. fuliginosum*, and *T. carthlicum var. rubiginosum* (white, black and red dika respectively, endemics of Georgia). Seed material was received from the laboratory of genetic resources of the Institute of Botany of Ilia State Uniuversity, Georgia.

UV irradiation and acid spraying of the experimental material

Dry seeds of experimental plants were irradiated with full spectrum of UV rays during 3h from an artificial source (lamp ДРТ-400, Russia) which was situated at 30cm from seeds. The intensity of irradiation was measured with a UVP radiometer (UVP Inc. USA) and exposure was 1.19mW/cm^2, 1.3mW/cm^2 and 1.84mW/cm^2 for A, B and C sections of UV spectrum respectively. Irradiated seeds were sowed on the experimental plot of the Institute of Botany of Ilia State University, Georgia, under field conditions. Plants were sowed in rows. Three row for each variant. Control, UV-irradiated and UV irradiation+acid sprayed variants were situated in separate blocks. The tested plants were sprayed with a water solution of a mixture of sulfuric and nitric acids 2:1, pH1.5, since the stem rising phase (Z30 by Zadok scale), at intervals of 5 days, till the end of vegetation (Z77 by Zadok scale) (totally 9 sprayings). Plants sprayed with distilled water served as controls for each studied species or cultivar. Analyses were made in the flowering phase (Z60-65 by Zadok scale), one week after the last spraying. Material for analysis was picked randomly for all three variants. Analyses were made in five biological replicates.

Antioxidant enzyme assay

Activity of peroxidase was determined spectrophotometricaly, using guaiacole. Optical density of guaiacole oxidized products was measured at wavelength of 470 nm over a period of 2 min (Ermakov et al., 1987). Results are given in conditional units per one gram of fresh weight.

Catalase activity was measured gasometrically: the volume of the oxygen released after adding H_2O_2 (30%) to water extract of experimental leaves was determined (Pleshkov, 1985).

Anthocyanins

One g of fresh leaves was placed in a mix of 20 ml of 96% ethanol and 2 ml of 1% HCL for 24 h. The extinction of the obtained extract was measured at 529 nm (Ermakov et al., 1987).

Ascorbic acid

A titration method was used to measure the content of ascorbic acid. 2 g of fresh leaf material was mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov et al., 1987).

Proline

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer at 520 nm (Bates et al., 1973).

Total phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris et al., 1987).

Total protein assay

Content of proteins was determined after Lowry (1951).

Soluble carbohydrates

Anthrone reagent was used for determining the content of soluble carbohydrates (Turkina and Sokolova, 1971). To 100 mg of air-dry leaf material was added 96° alcohol for extraction (3-fold extraction). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5 ml of distilled water. To 0.5 ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10 min. After this procedure the test-tubes were placed in a cold water bath and 15 min later the optical density of the solution was measured at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

Statistical analysis

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 12.5.

Results

Catalase and peroxidase

High activity of peroxidase was characteristic for soft wheat among the tested species (*Figure 1*). Seed irradiation caused diminishing of the enzyme activity in leaves of macha wheat and durum wheat, while in soft wheat enzyme's activity significantly increased ($p < 0.05$). UV irradiation appeared to be stimulative for peroxidase activity in leaves of spring wheats as well. Here white dika distinguished with high peroxidase activity in control variants. Irradiation of seeds with UV for 3 h increased the enzyme activity in all varieties of dika ($p < 0.05$).

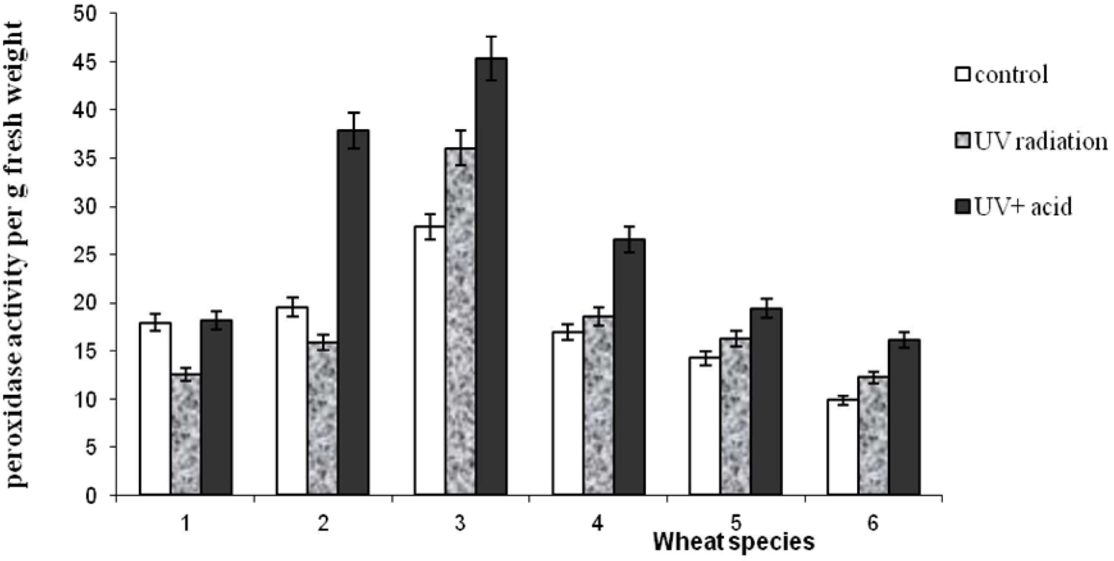

***Figure 1.** Combined effect of seed UV treatment and acid spraying of leaves on the activity of peroxidase in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p < 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)*

Effect of both stressors has stimulated activity of peroxidase in leaves of all experimental species. This time stimulation was higher, than in case of seed irradiation.

Observations have revealed that catalase activity in leaves of all tested plants was similar (*Figure 2*). Both treatments – seed pre-sowing irradiation with UV and acid spraying of plants, developed from the irradiated seeds, have stimulated catalase activity in leaves of experimental plants. Though, combination of UV and acid spraying appeared to have more stimulative effect on enzyme's activity, than only seed UV treatment.

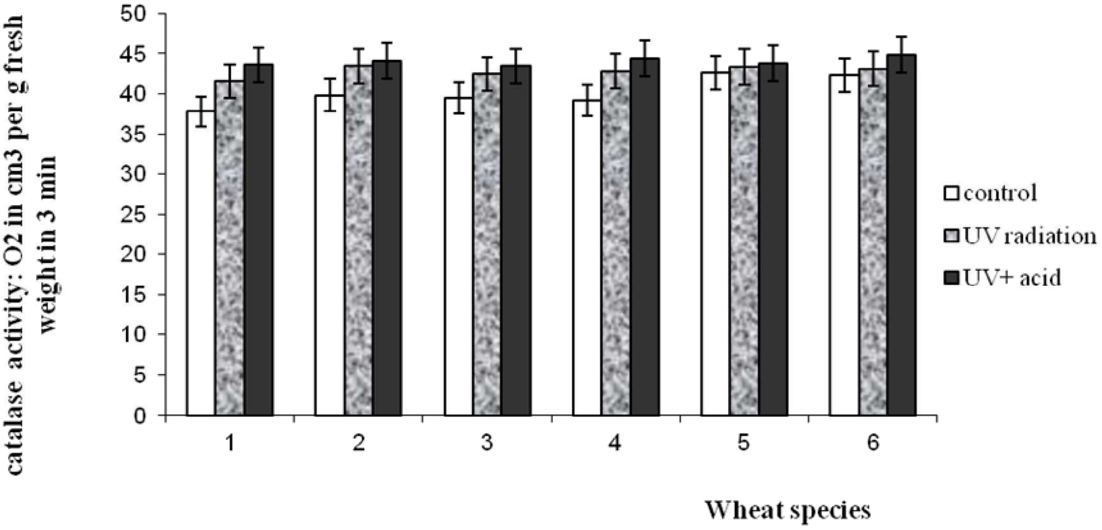

Figure 2. *Combined effect of seed UV treatment and acid spraying of leaves on the activity of catalase in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)*

Ascorbic acid

Experimental results demonstrate between-specious and intra-specious differences in reactions to one and the same stressors among the experimental plants.

With high content of leaf ascorbate were distinguished black and red varieties of dika (*Figure 3*). Seed irradiation has changed amount of the vitamin in all tested plants but at different levels. Content of ascorbic acid increased in leaves of macha wheat, durum wheat and red dika, but decreased in leaves of soft wheat and black dika. Combinations of two stressors (seed irradiation and spraying with acid of plants developed from the irradiated seeds) inhibited ascorbic acid synthesis in all species, except the durum wheat. In the last in contrary, increase of the index was mentioned (*Figure 3*). Moreover, in irradiated and later sprayed with acid variants the content of the ascorbate diminished with higher extent than in irradiated variants.

Proline

High content of proline was established in species of winter wheat (*T. macha, T. durum, T. aestivum*). Pre-sowing irradiation of seeds decreased content of the substance in leaves of all experimental plants (macha wheat was exception) (*Figure 4*). Spraying leaves with acid appeared to be more depressing for proline content in all spring species, compared with control and irradiated variants; while in winter species increase of proline took place, compared with irradiated variants. Macha wheat and durum wheat made exception: here the index was lower than in control, while in soft wheat content of proline exceeded the control.

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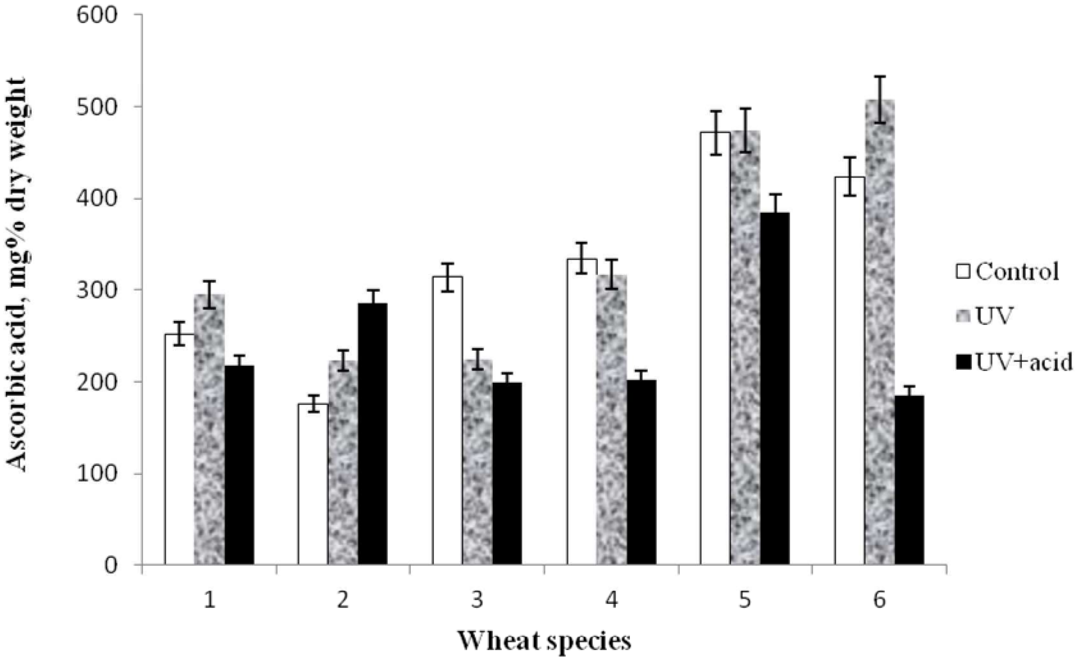

Figure 3. Combined effect of seed UV treatment and acid spraying of leaves on the content of ascorbic acid in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)

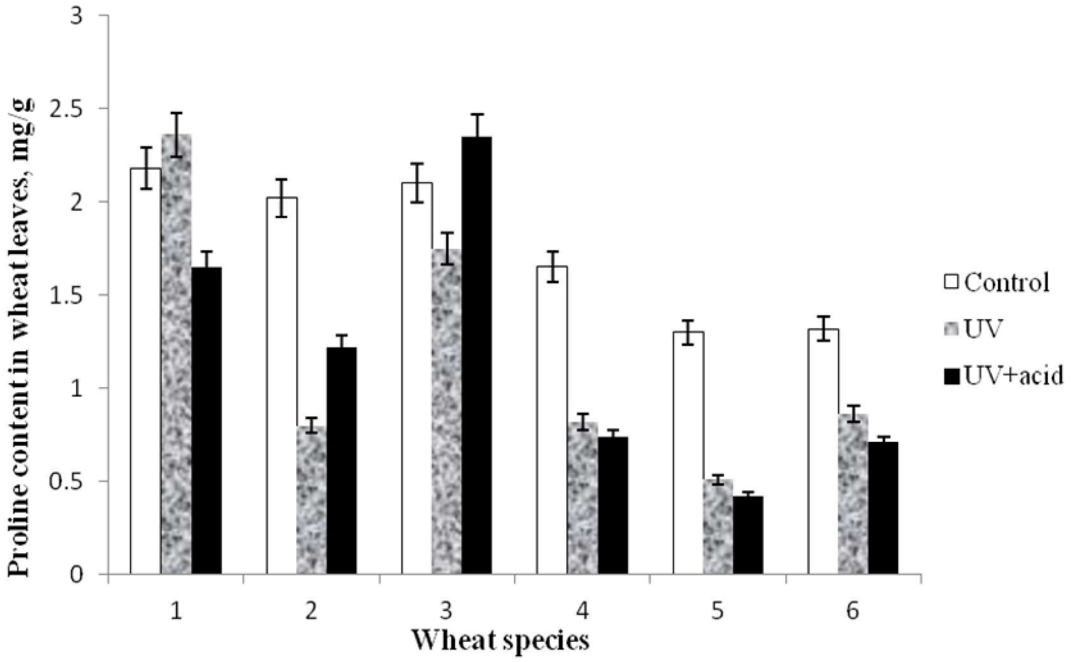

Figure 4. Combined effect of seed UV treatment and acid spraying of leaves on the content of proline in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p = 0.003$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p = 0.022$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p < 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)

Soluble phenols

Investigation of the content of soluble phenols has revealed the high extent of accumulation of these substances in macha wheat, and white and black dika. Comparatively low was this index in soft wheat and red dika (*Figure 5*). Both, seed irradiation and spraying with acid precipitations appeared to be stimulative for synthesis of phenols in leaves of all studied species. Moreover, the percentage of stimulation was higher in winter wheats (29-33%) compared with varieties of dika (11-16%). Combination of two stresses was more stimulative for phenol synthesis, than only UV irradiation (*Figure 3*). The percentage of the increment of phenolic substances in case of double stress varied between 16-58%.

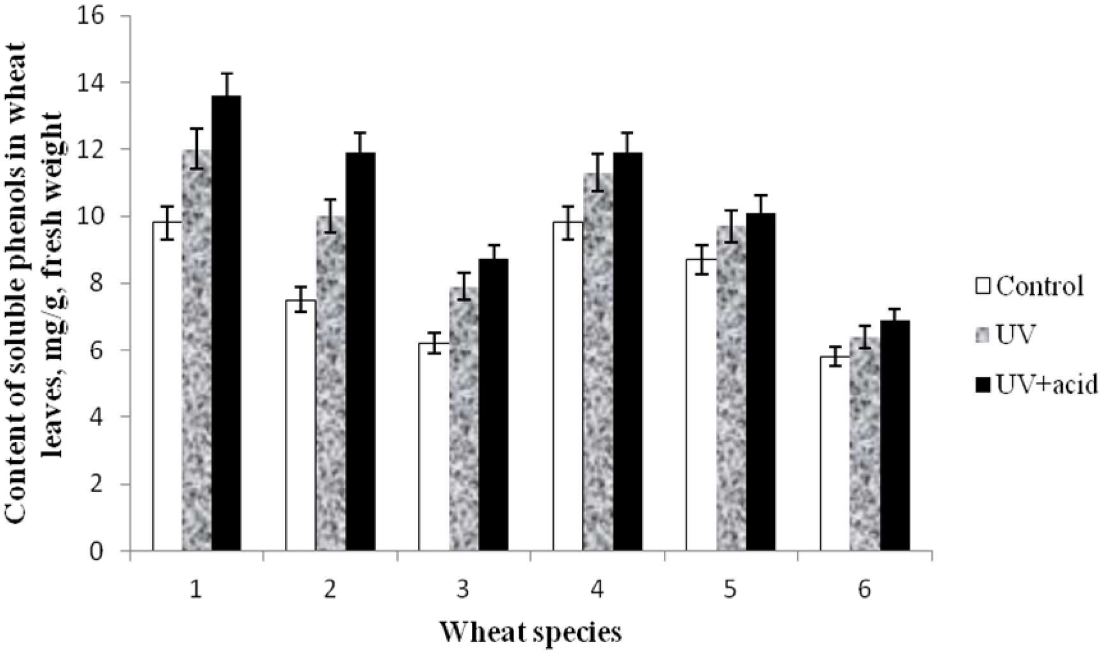

Figure 5. *Combined effect of seed UV treatment and acid spraying of leaves on the content of soluble phenols in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p < 0.001) 3. T. aestivum L. (p = 0.002). 4. T. persicum var. stramineum Zhuk. (p = 0.009) 5. T. persicum var. fuliginosum Zhuk. (p = 0.01) 6. T. persicum var. rubiginosum Zhuk. (p = 0.01)*

Anthocyanins

According to obtained results high content of antocyanins among control variants was mentioned in leaves of durum wheat and black and red varieties of dika. In other species data were alike. Pre-treatment of seeds with UV inhibited anthocyanins synthesis in leaves of macha wheat, soft wheat and white dika. Stronger was the effect in case of spraying these species with acid solution (*Figure 6*). In durum wheat and black and red varieties of dika incontrary, UV treatment and spraying with acid as well, stimulated the process of accumulation of anthocyanins in leaves. Though, the index of double stressed (UV treated+acid sprayed) variants was lower, compared with UV treated ones, but prevailed the control variants.

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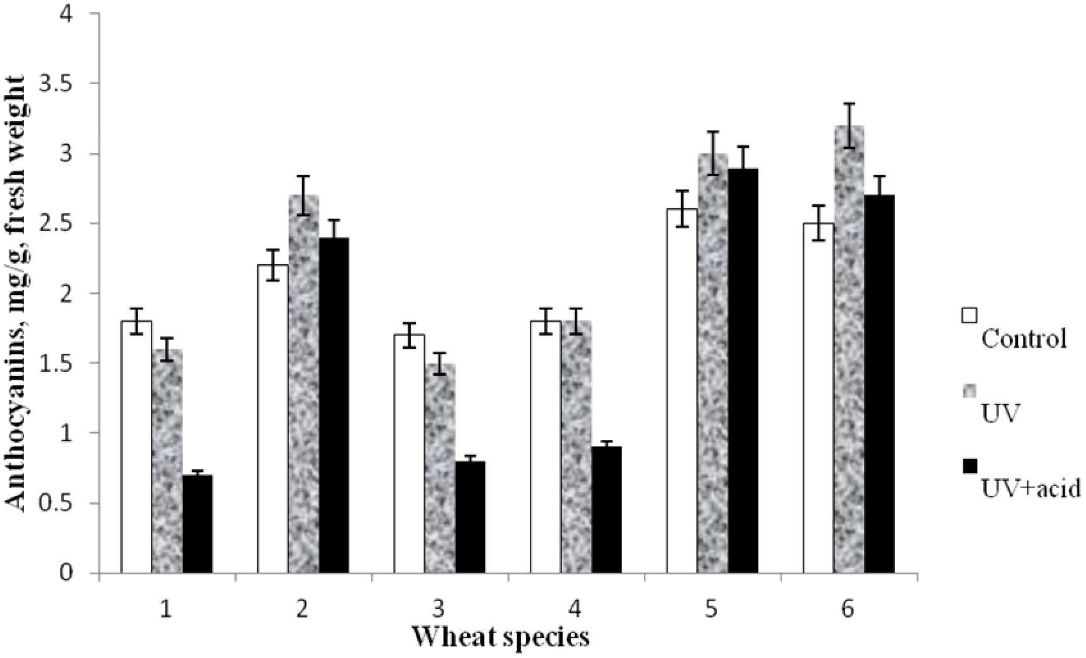

Figure 6. *Combined effect of seed UV treatment and acid spraying of leaves on the content of anthocyanins in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p = 0.004$) 2. T. durum Desf. ($p = 0.01$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.01$) 6. T. persicum var. rubiginosum Zhuk. ($p = 0.006$)*

Total proteins

Macha wheat and durum wheat appeared to have comparatively high level of proteins in leaves (*Figure 7*). Pre-treatment with UV diminished the content of total proteins in the named species. Irradiation stimulated synthesis of proteins in soft wheat and all varieties of spring wheat. Impact of both stressors increased content of proteins in leaves of macha wheat, soft wheat and white and red varieties of dika, while in rest species content of proteins diminished.

Soluble carbohydrates

Among the tested wheats macha wheat and white and red varieties of dika were distinguished with high content of soluble carbohydrates in leaves (*Figure 8*). UV treatment of seeds and impact of two stress combination as well, appeared to be suppressing for soluble carbohydrates synthesis in leaves; especially the double stress did. The sharp decrease of the soluble carbohydrates under stress conditions was mentioned in those species, which were distinguished with high content of these substances in leaves (macha wheat, white dika. 63% and 50% respectively).

Discussion

Pre-sowing treatment of seeds with UV irradiation supplies the dormant seeds with extra energy, which is stress for them. Free radicals produced during this process, changes in cell membrane permeability and electric potential, presumably initiate

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diverse metabolic responses including biosynthesis of antioxidants. This, from its side, must be reflected on further development of plant and formation of adaptive mechanisms to unfavorable environmental conditions (Dubrov, 1977).

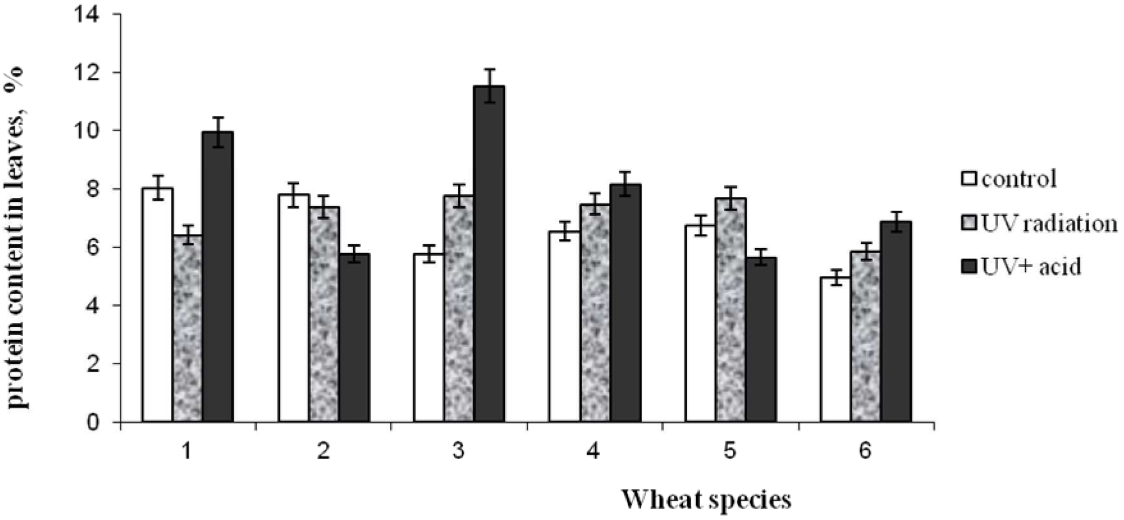

Figure 7. *Combined effect of seed UV treatment and acid spraying of leaves on the content of total proteins in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p < 0.001) 3. T. aestivum L. (p < 0.001). 4. T. persicum var. stramineum Zhuk. (p < 0.001) 5. T. persicum var. fuliginosum Zhuk. (p < 0.001) 6. T. persicum var. rubiginosum Zhuk. (p = 0.01)*

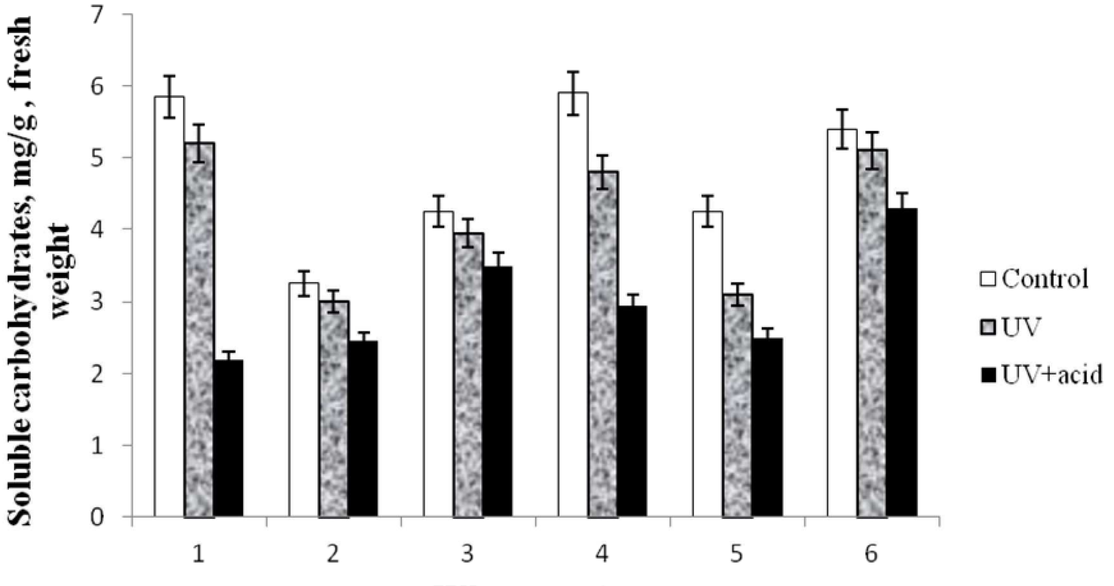

Figure 8. *Combined effect of seed UV treatment and acid spraying of leaves on the content of soluble carbohydrates in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p = 0.04) 3. T. aestivum L. (p = 0.047). 4. T. persicum var. stramineum Zhuk. (p = 0.001) 5. T. persicum var. fuliginosum Zhuk. (p < 0.001) 6. T. persicum var. rubiginosum Zhuk. (p = 0.05)*

It is established that the concentration of ROS serves as a signal for switching on of different genes and signal systems, controlling the responses to stress (Dalton et al., 1999; Foyer and Noctor, 2003). Evidently UV-treatment of seeds must have stimulated such type of signaling systems in seeds; besides, this stimulation was so strong, that clearly influenced plant's vital processes during a long period after emergence. In particular, leaves of plants, emerged from the irradiated seeds were tested in flowering phase, while the effect of irradiation on the antioxidant system still was evident.

Catalase and peroxidase

Enzyme catalase (CAT, EC 1.11.1.6) accumulates in peroxisomes and is responsible for destruction of hydrogen peroxide. During stress conditions the number of these organelles increases in the cell. This plays an important role in detoxication of hydrogen peroxide, diffused in peroxisome from other parts of the cell (Mittler, 2002).

According to the classical view on peroxidase, its main function is to protect the organism from damaging effect of peroxide. Peroxidases (EC 1.11.1) are a big group of enzymes, met everywhere in the cell and fulfilling multilateral function in plant metabolism (Passardi et al., 2005). In particular, they neutralize the hydrogen peroxide, formed during stress and take an active part in adaptation of plant to unfavorable environmental conditions; peroxidases effect processes of plants growth and development, regulate content of auxins and phenolic substances, etc (Andreeva, 1988; Cevahir et al., 2004; Graskova et al., 2010). Moreover, cell wall peroxidases take part in formation of ROS, which play a protective role against abiotic stresses and have signaling function in case of some stresses, manifested in activation of stress-defensive mechanisms of plants (Mika et al., 2004).

Increased activity of catalase in all irradiated variants of our tested plants once again proves the fact that the activation of catalase-synthesizing genes takes place in case of different, among them radiation stresses (Scandalios et al.,1997). If we suppose that UV treatment of seeds caused intensification of respiration in mitochondria and generally increased the content of ROS in plant, activation of catalase for neutralizing of stressors would be a logical result (*Figure 2*).

In case of peroxidase experimental results were rather different (*Figure 1*). By the opinion of scientists decrease of peroxidase activity in plant leaves may be caused by the low energetic potential of metabolic reactions, while activation of the enzyme is the result of essential shifts in metabolic, in particular the respiration system, aiming adaptation of the plant and retention of cell homeostasis (Sergeichik and Sergeichik, 1988; Tucic et al., 2007). Accordingly, we may suppose that seed irradiation changed the metabolic processes of macha wheat and durum wheat to rather different direction, by diminishing the activity of the enzyme, than in the rest species, where increase of peroxidase activity took place; i.e. from the point of view of peroxidase activity macha wheat and durum wheat may be considered as comparatively sensitive to irradiation, than other species.

Spaying of irradiated plants with acid precipitations revealed a synergistic effect with UV-irradiation and caused further activation of catalase in leaves of experimental plants (*Figure 2*).

Spraying with acid appeared to be stimulative for peroxidase as well. Activity of this enzyme increased in leaves of all tested species (*Figure 1*). Presumably, combination of two stresses fully activates the enzymatic system of the given species, demonstrating the adaptivity of their enzymatic systems.

It must be mentioned that results of combined effect of irradiation and acid spraying in some cases differ from our early experimental data, obtained with artificial acid rains (Kacharava, 2013). In particular, spraying of leaves with pH1.5 acid solution inhibited activity of catalase in red dika, and of peroxidase – both, in red and black dika (Kacharava, 2013). We may suppose that pre-treatment of seeds with UV has stimulative effect on these antioxidant enzymes and increases adaptivity of plants to acid stress.

It must be mentioned that in spite of significant role in ROS detoxication the enzymatic antioxidative system is not able to provide full protection of the cell, conditioned by a number of factors. That is why the opinion on the effective role of low molecular antioxidant substances (ascorbic acid, proline, anthocyanins, etc.) in protection of cell metabolism from ROS has appeared (Blokhina et al., 2003).

Ascorbic acid

L-ascorbic acid or vitamin C is an important metabolite both in plants and animals. Its antioxidative activity stipulates stress resistance and life span in plants. According to great number of recent investigations besides signaling function, vitamin C takes an active part in plant protection against environmental stresses (heavy metals, salinity, temperature, UV-B radiation, etc.) by activation of corresponding genes (Shalata and Neumann, 2001; Vwioko et al., 2008). Moreover, ascorbic acid directly neutralizes ROS, and plays a role of secondary antioxidant in the reduction cycle of oxidized form of α-tocopherol and some carotenoids (Noctor and Foyer, 1998; Potters et al., 2002). At the same time, ascorbic acid is a co-factor of many enzymes (Arrigioni and De Tullio, 2000). The response of ascorbic acid to stresses is regulated by a complex chain of successive biochemical reactions, comprising activation or inhibition of key enzymatic reactions, synthesis of stress-responsible proteins and other protective substances (Khan et al., 2011). As the concentration of ascorbic acid prevails content of other antioxidants in plant, it is regarded as the main antioxidant in plant (Gallie, 2013). Thus, the content of vitamin C in leaves may be regarded as the main indicator for stress resistance and adaptation.

Literary data exist on ascorbate concentration increase in plants under stress conditions (Zengin and Munzuroglu, 2005; Mogren et al., 2012; Priyanka et al., 2014). According to our experimental data it may be concluded that the increase or maintenance of ascorbate at a control level in response to applied UV radiation in tested species (macha wheat, durum wheat, black and red varieties of dika) is indication to their resistance to the used doses of radiation (*Figure 3*).

Our early experiments on the influence of simulated acid rains on different plant species demonstrate that in most experimental plants spraying with acid solution stimulated synthesis of ascorbic acid in leaves, though in some cases opposite effect was observed (Chkhubianishvili et al., 2008).

Spraying leaves of experimental plants with acid solution has revealed the synergistic effect with UV-radiation stress in tested species. Exclusions were macha wheat, black and red varieties of dika. In these cases, in contrary, antagonistic effect was observed. Thus, acid stress diminished content of ascorbic acid in leaves of tested plants (*Figure 3*). Only in durum wheat combination of two stresses appeared to be stimulative for ascorbate synthesis; but discussion about stress-tolerance of experimental species only by ascorbate content would not be reasonable.

Proline

From literary data it is known that proline protects plants aginst stress as osmolite, by regulation of water retention in the cell (Saradhi et al., 1995). Besides, it plays a role of proteins stabilizer (Anjum et al., 2002) and regulates NAD/NADH ratio of the cell (Alia and Saradhi, 1993). Recently a multilateral function of proline in stress adaptation of plants and signal transduction has been established. Stimulation of proline synthesis in chloroplasts presumably supports stabilization of the redox balance by dissipation of extra energy and retains cell homeostasis. Moreover, by means of modulation of responses to biotic or abiotic stresses proline plays a role of metabolic signal, which regulates the metabolic pull and expression of great number of genes, effecting this way plant's growth and development (Szabados and Savoure, 2009).

According to literary data, amount of endogenous proline increased under stress conditions in different plants. This is the indication to stress resistance (Szabados and Savoure, 2010; Kaur et al., 2011). Diminishing of proline content in irradiated variants of the tested plants in our experiments may be expression of the proline pull sensitivity to the applied doses of UV irradiation (*Figure 4*).

It is known that the reductive equivalent must be formed as a result of fast catabolism of proline, which promotes oxidative phosphorilation in mitochondria and synthesis of ATP to recover stress-induced lesion (Hare et al., 1998). Presumably, mitochondria, as the main functioning organelles of dormant seeds, are most of all affected by UV stress, in pre-sowing treatment experiments. Pre-sowing UV-irradiation of experimental seeds must have switched on the stress-protective mechanisms, presumably with the assistance of proline, which would defend the stressed mitochondria. Apparently it was the reason of proline decline in UV-treated variants.

Combination of two stresses diversely changed proline content in leaves of experimental plants (*Figure 4*). Spraying with acid solution stimulated proline synthesis in durum and soft wheats. Apparently, under the influence of acid stress additionally were activated those genes, which are associated with proline synthesis.

Results of our early experiments on acid spraying of wheat plants, once more prove the great influence of UV pre-treatment of seeds on vital processes of the developed plants. In particular, spraying of leaves with pH1.5 acid solution activated proline synthesis in all three varieties of dika (Kacharava et al., 2013). Apparently, the effect of UV-treatment on proline synthesis in tested plants was so intensive, that spraying leaves with the same acidity (pH1.5) solution could not change the effect of irradiation.

Soluble phenols

Phenols are the most active secondary metabolites in plant. The antioxidant properties most of them prevails that of such antioxidants, as ascorbic acid and tocipherol (Hernandez et al., 2009). Phenols neutralize the ROS till they manage to damage the cell (Lovdal et al., 2010). Activation of many phenol-synthesizing genes takes place under stress conditions. Significant increase of the content of phenolic substances under different biotic or abiotic stresses has been established (Winkel-Shirley, 2002).

Activation of the synthesis of phenolic substances under UV-stress in all tested species of wheat may be regarded as a confirmation of resistance of the experimental plants to the applied doses of radiation (*Figure 5*). They are surely protected by the phenilpropanoid pathway of synthesis of phenolic substances (Lapshin and Zagoskina, 2004). Spraying leaves with acid solution has increased the effect of irradiation and intensified the synthesis of phenolic substances in all tested plants.

Obtained results support the idea, which explains the promotion of phenolic metabolisms in wheats under stress conditions by the activation of synthesis of those enzymes, which support convertion of the primary products of photosynthesis into necessary for phenolics synthesis ones (Chumachenko, 1965; Dubrov, 1968).

In early, acid spraying experiments the stimulative effect of acid on the synthesis of phenolic substances in varieties of dika has been established (Kacharava et al., 2013). Thus, in case of phenolic substances the combination of two stressors revealed synergism and increase stress-resistance of studied species (*Figure 5*).

Anthocyanins

They belong to the group of phlavonoids, which are concentrated mainly in vacuole and possess strong antioxidant properties (Kahkonen and Heinonen, 2003). The antioxidant properties of anthocyanins are stipulated by transport of electrons, belonging to hydroxyl groups, or hydrogen atoms to ROS (Blouin and Peynaud, 2005). Anthocyanins are able to join metal ions as well, which take an active part in oxidation process (Tita, 2004). Accumulation of anthocyanins in vacuole prevents to their direct contact with the sites of ROS formation. In spite of it, increase of anthocyanins content under metal or other type of stresses has been established (Mobin and Khan, 2007).

As it is known, ascorbic acid is the co-factor of those enzymes, which support anthocyanins synthesis (Gallie, 2013). Though, according to our experimental results, the evident relation between the content of ascorbic acid and synthesis of anthocianins was not revealed. Increase in ascorbate content did not always coincide with stimulation of anthocyanins synthesis. Evidently, the specific peculiarities of stress-protective strategy are being revealed here too.

Pre-sowing UV-treatment of seeds has activated anthocyanins synthesis in most tested species (exceptions were macha wheat and soft wheat) (*Figure 6*). The same effect was observed in case of double stress, compared with the control variants. Thus, the phlavonoid pathway of stress-protection was activated in these species, together with the phenilpropanoid pathway, indicating to more adaptability to unfavorable conditions.

Total proteins

Recently, bioactive peptides from enzymatic hydrolysis of various food proteins such as soy protein, casein, whey protein, gelatin and wheat gluten have been shown to possess antioxidative activity (Elias et al., 2008). So called heat shock proteins are also synthesized in plant. This is a general name of protein substances, formed in plant during different stresses (cold, heat, drought, light, heavy metals, salinity, etc.) (Timperio et al., 2008; Kochhar and Kochhar, 2005). Shock proteins protect other proteins from stress damage.

According to literary data the content of total proteins in case of different stresses increases in stress tolerant and adapted plants (Ali and Basha, 1998; De Britto et al., 2011; Kosakivska et al., 2008; Kamal et al., 2010). Thus, increase of total protein amount in leaves of dika varieties and soft wheat in our experiments may indicate to more adaptivity of these plants compared with macha wheat and durum wheat (*Figure 7*). In the last ones the index decreased.

Stimulation of proteins synthesis in macha wheat by the acid spraying may be the result of activation of any shock protein synthesis, increasing plant's resistance to stress.

Soluble carbohydrates

Though the soluble carbohydrates are related to the metabolic ways of ROS formation, they play important role in their neutralization as well. Increase of glucose may cause intensification of NADPH synthesis (by the pentoso-phosphate cycle), which is a significant intermediate product of ascorbate-glutathione cycle (Couee et al., 2006). It was demonstrated that in some species of plants abiotic stress increased the level of glucose and sucrose as well. Though their role in protective mechanisms is not confirmed (Couee et al., 2006). Van den Ende and Valluru (2009) suppose that sucrose may protect membrane against drought and cold stress. Accumulation of soluble carbohydrates in response to different stresses in various parts of a plant has been demonstrated in a number of works (Prado et al., 2000; Finkelstein and Gibson, 2001; Nayer and Reza, 2008). Generally it is established that the metabolism of soluble carbohydrates under stress conditions is a dynamic process and comprises reactions of catabolism and synthesis simultaneously (Hilal et al., 2004).

In all experimental variants of wheat, both irradiated and irradiated and then sprayed with acid, amount of soluble carbohydrates in leaves decreased (*Figure 8*), which may be explained by the intensification of their respiration (activation of catalase may also serve as confirmation to it). Besides, soluble carbohydrates may take part in intensive synthesis of phenolic compounds, since the link of fructose with lignin and phenolics synthesis has been demonstrated (Hilal et al., 2004).

In our early experiments artificial acid precipitations caused activation of the synthesis of soluble carbohydrates in wheat leaves (Rapava et al., 2010). Presumably, inhibitory effect of the UV-irradiation on seeds is so significant, that spraying with acid solution could not decline it, or, obtained results demonstrate the effect of the double stress.

Summarizing it may be concluded:

1. The protective system of catalase is resistant to UV stress in all studied species. According to peroxidase activity macha wheat and durum wheat may be regarded as more sensitive to UV stress, compared with other species.

   The combination of two stresses induced full stimulation of the enzymatic system in tested species and adaptivity of the latter to the applied stresses may be concluded.

2. Increase or the same level of the ascorbic acid content in response to the applied dose of UV in some studied species (macha wheat, durum wheat, black and red varieties of dika) may indicate to their resistance to radiation stress. Combination of two stresses diminished the activity of the protective ascorbic acid system in leaves of the tested plants, except the durum wheat. In the latter double stress appeared to be stimulative for ascorbate content and presumably enhanced its stress tolerance.

3. The protective proline system of the tested species seems to be sensitive towards UV irradiation and UV-acid spraying stress. Acid stress presumably caused activation of genes associated with proline synthesis in durum wheat and soft wheat, stipulating increase of their resistance to double stress.

4. Activation of the synthesis of phenolic substances in response to applied stresses in all tested plants may be regarded as a confirmation of their resistance and well protection against stress with the phenilpropanpid way of phenolics synthesis.
5. Increase of anthocyanins synthesis in response to seed irradiation and irradiation-acid spraying treatments in most tested species is demonstration of stress-protective phlavonoid pathway activation as well.
6. Gain of total protein content in response to UV stress in dika varieties and mild wheat may serve as indication to more adaptability of these species to UV radiation. Acid stress caused stimulation of protein synthesis in macha wheat, which may be linked with the activation of stress-protective shock-proteins.
7. Decrease of soluble carbohydrates content in UV-irradiated variants of the tested species may caused by the intensification of leaves respiration (catalase activation also indicates to it). Moreover, soluble carbohydrates may take part in intensified synthesis of phenolic substances, as the relation between fructose and synthesis of lignin and phenolic substances has been demonstrated.

Thus, if we discuss about stress-tolerance of plants by the amount of activated parameters of the antioxidant system, relative resistance of the studied wheat species to UV and acid stresses may be supposed. According to our scheme, high sensitivity to pre-sowing treatment with UV demonstrates macha wheat, and mild wheat (four parameters were activated or retained at control level under the stress). Dika varieties seem to be less sensitive to the same stress (especially red and black varieties. Six indices were activated).

Sensitivity of macha wheat to combined influence of UV-acid stresses remained similar to radiation stress; while in other species it was not so. In particular, white and black varieties of dika were sensitive to double stress (four parameters were activated), while in durum wheat in contrary – stress-tolerance increased (six indices were activated).

Finally we can say that normal termination of vegetation cycle and grain harvest of all tested wheat species point to their resistance to the applied types of stresses.

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BIOMASS PRODUCTION OF SELECTED GRASSLAND, WETLAND AND CROPLAND VEGETATION COMMUNITIES

HAKROVÁ, P.[1] – KŘOVÁKOVÁ, K.[1,2]*– NOVOTNÁ, K.[1]– VINCIKOVÁ, H.[1]– PROCHÁZKA, J.[1]

[1]*Laboratory of Applied Ecology, Faculty of Agriculture, University of South Bohemia in České Budějovice*
Studentská 13, 370 05 České Budějovice, Czech Republic
(phone: +420-387772738; fax: +420-389032520)

[2]*Department of Applied Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague*
Kamýcká 129, 165 21 Praha 6 – Suchdol, Czech Republic

**Corresponding author*
e-mail: cariadwr@gmail.com

PHOTOGRAPHIC DOCUMENTATION OF THE STUDIED SITES

Figure 1. *Alluvial Alopecurus meadow (Alop-3), 28.5.2013*

Figure 2. *Alluvial Alopecurus meadow (Alop-3), 16.7.2013*

Figure 3. *Intensively managed meadows (IntensM-8), 1.4.2013*

Figure 4. *Intensively managed meadows (IntensM-8), 14.5.2013*

Figure 5. *Maize (Maize-21), 3.9.2013*

Figure 6. *Mesic Arrhenatherum meadows (Arhen-20), 16.7.2013*

Figure 7. *Oilseed-5, 30.4.2013*

Figure 8. *Oilseed-5, 6.8.2013*

Figure 9. *Oilseed-5, 1.10.2013*

Figure 10. *Reed beds of eutrophic still waters (Glyc-10), 1.4.2013*

Figure 11. *Reed beds of eutrophic still waters (Glyc-10), 3.9.2013*

Figure 12. *Reed beds of eutrophic still waters (ReedL-9), 1.4.2013*

Figure 13. *Reed beds of eutrophic still waters (ReedL-9), 11.6.2013*

Figure 14. *Reed beds of eutrophic still waters (ReedT-9), 14.5.2013*

Figure 15. *Reed beds of eutrophic still waters (ReedT-19), 12.11.2013*

Figure 16. *Reed beds of eutrophic still waters (Typha-11), 11.6.2013*

Figure 17. *Reed beds of eutrophic still waters (Typha-14), 16.7.2013*

Figure 18. *Tall-sedge beds (Carex-12), 15.5.2013*

Figure 19. *Tall-sedge beds (Carex-12), 11.6.2013*

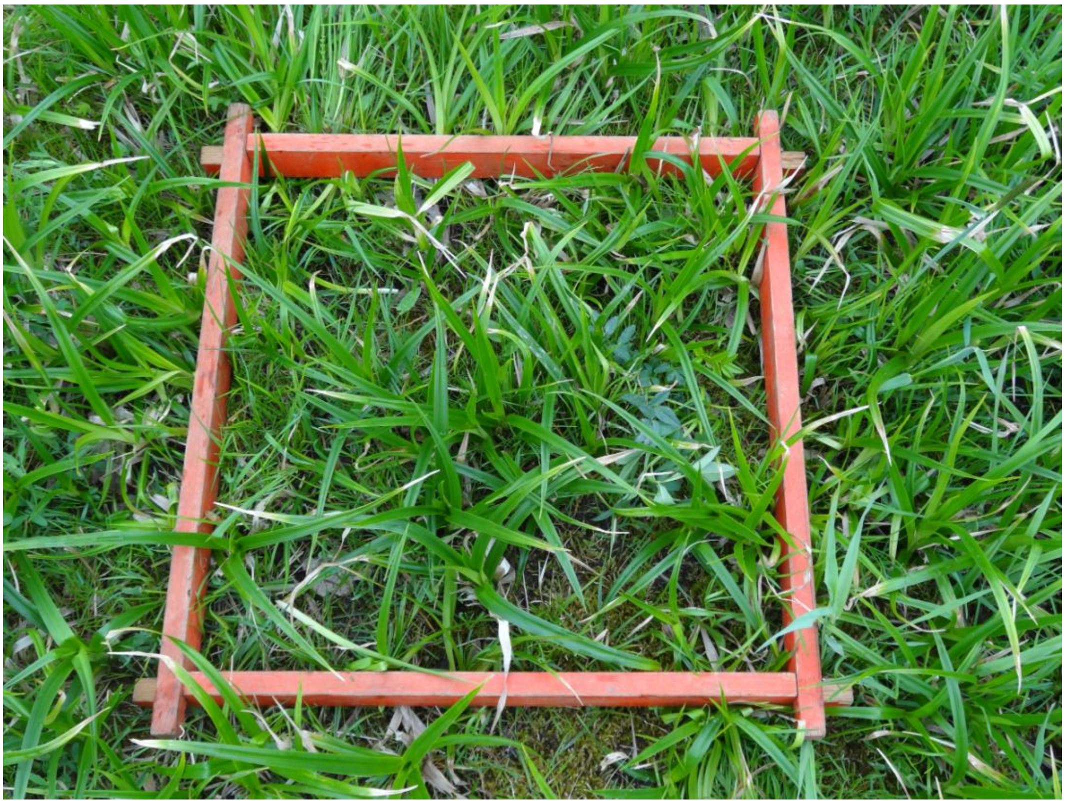

Figure 20. *Wet Cirsium meadows (Cirs-2), 30.4.2013*

Figure 21. *Wet Cirsium meadows (Cirs-2), 11.6.2013*

Figure 22. *Wet Cirsium meadows (Cirs-2), 1.10.2013*

Figure 23. *Wet Filipendula grasslands (Filip-1), 30.4.2013*

Figure 24. *Wet Filipendula grasslands (Filip-1), 16.7.2013*

Figure 25. *Wet Filipendula grasslands (Filip-1), 12.11.2013*

Figure 26. *Winter wheat (WheatW-6), 30.4.2013*

Figure 27. *Winter wheat (WheatW-6), 16.7.2013*

BIOMASS PRODUCTION OF SELECTED GRASSLAND, WETLAND AND CROPLAND VEGETATION COMMUNITIES

HAKROVÁ, P.[1] – KŘOVÁKOVÁ, K.[1,2]*– NOVOTNÁ, K.[1]– VINCIKOVÁ, H.[1]– PROCHÁZKA, J.[1]

[1]*Laboratory of Applied Ecology, Faculty of Agriculture, University of South Bohemia in České Budějovice*
Studentská 13, 370 05 České Budějovice, Czech Republic
(phone: +420-387772738; fax: +420-389032520)

[2]*Department of Applied Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague*
Kamýcká 129, 165 21 Praha 6 – Suchdol, Czech Republic

**Corresponding author*
e-mail: cariadwr@gmail.com

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Abstract. Biomass production is a generally well-studied phenomenon but it often only focuses on simple habitats such as monodominant wetland communities. Information on aboveground biomass production of various managed and unmanaged communities was necessary for a research project called the "Minimization of Radioactive Contamination Impacts on the Landscape in the Emergency Planning Zone of the Temelín Nuclear Power Plant" to create growth models of selected plant communities to estimate the amount of biomass potentially contaminated by radiation in case of a nuclear power plant accident. In the present paper we introduce the results of biomass sampling carried out in the vegetation season of 2013 and compare it with the previously published data. Especially the curve shapes could be well compared where the relevant data was found in literature, namely in the case of monodominant wetland communities. In cases where the data on seasonal production was not available (e.g. Filipendula stands) the peak values were compared and found to correspond well, too. There was no relevant published data to be found for 9 stand types (some rich-in-species grasslands and crops); together with detailed description of the sampled stands the original data on the aboveground biomass production is published for the first time.

Keywords: *production curves; grassland stands; wetland stands; arable croplands*

Introduction

Primary production of plant biomass is a crucial basis for other ecosystem processes. It is strongly related to the flow of matter through an ecosystem (Mooney, 1991) and thus plays a key role in landscape functions (Wiegand et al., 2004). Besides the physiology of plants forming a plant community and their ecologic strategies biomass production is significantly influenced by environmental factors such as climate and microclimate and the resulting water supply (Palmer and Yunusa, 2011), soil properties, especially the nutrient regime (Čížková et al., 2001) and pressure from the herbivores (Moise and Henry, 2012) or – in case of agricultural ecosystems – an extra supply of nutrients, harvest and other farming practices (Heggenstaller et al., 2009).

The methods to determine biomass production can be generally divided into destructive and non-destructive types. Belowground (root) biomass is usually determined by destructive methods such as monoliths (dug samples) or sampling tubes where the soil is subsequently washed out (Dykyjová, 1989; Rychnovská, 1987). Destructive sampling of aboveground biomass is based on the removal of plants from a square plot of a precisely defined surface (for grass communities it is generally between

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0,25 x 0,25 and 1 x 1 m, cf. Dykyjová, 1989; Rychnovská, 1987). With helophytes, which often tend to form clusters, the sampling plots have to be larger than the average clusters (Ondok and Dykyjová, 1973; Ondok and Květ, 1978). Sampling plots can be distributed either randomly on the site, which requires more samples to capture the differences in plant size or density in various conditions (especially when it comes to nutrients and water availability). The second approach uses sample plots located at transects along the ecological gradients; this method is more advantageous as it requires less sampling plots.

Non-destructive methods – or rather a combination of both approaches – are based on the estimation of the aboveground biomass counting the number of stalks and/or plant leaves in a sampling plot, and multiplying it by the average weight of the relevant plant parts harvested from the neighbouring plants outside the plot (Ondok and Květ, 1978; Květ and Westlake, 1998). If the plots are held as permanent and the outside harvest is carried out in regular (usually monthly) intervals, the seasonal dynamics of biomass production can be observed (Ondok and Dykyjová, 1973).

There are also indirect methods to estimate the biomass production. One of the indices describing both the plant productivity and biogeochemical fluxes between vegetation and the atmosphere is the leaf area index (LAI) (Bréda, 2003). Together with the above described in-situ biomass sampling it is also often a subject of remote-sensing based estimations of biomass production in the landscape (Na et al., 2003; Cook et al., 2009) or the global scale.

The biomass production values can be obtained in various forms depending on the aim of the study, selected method and other circumstances influencing the experimental design. The biomass amount can be expressed as peak (i.e. the maximum) value or total (sum) value of the biomass produced by a plant community throughout the growing season (Scurlock et al., 2002). The seasonal variability of biomass production and its dynamics can be observed using growth models (e.g. Rosef and Bonesmo, 2005), which requires regular harvest of accumulated biomass during the growing season.

Biomass production and plant productivity were initially studied as a part of an overall ecological research of selected important biotopes. A broad research on productivity of terrestrial, freshwater and marine biotopes was launched as one of the chapters of the International biological program (IBP). A great amount of measurements of biomass production and productivity, vegetation density, LAI and other indicators (such as shoot length, inflorescence number, etc.) of both individual species and communities was gathered in the Czech Republic under this research project (cf. e.g. Hejný et al., 1970; Rychnovská, 1972; or Dykyjová and Květ, 1978). The campaign of such an extent and aim is rather unimaginable nowadays: biomass production is studied as a component of crop yield (Heggenstaller, 2009), an indicator of the plant potential as an energetic source (Bentsen et al., 2014) or a carbon sink (Aosaar et al., 2013).

The data on biomass production presented in this paper were collected in order to create the growth models of selected plant communities to estimate the amount of biomass potentially contaminated by radiation in case of a nuclear power plant accident (Brom et al., 2013); the actual amount of contaminated biomass which would be necessary to harvest, transport and decontaminate or safely store and its spatial distribution are some of the key parameters to design later phases of emergency planning aimed at landscape decontamination. As one of the objectives of the research project “Minimization of Radioactive Contamination Impacts on the Landscape in the Emergency Planning Zone of the Temelín Nuclear Power Plant” the grassland, wetland

and crop vegetation communities at selected sites located in the Emergency Planning Zone of the Temelín Power Plant (south of the Czech Republic) were measured during the growing season 2013 in suitable intervals. There are a few studies covering this diversity of biotopes and presenting the primary values. The preliminary results of the aboveground biomass productivity of the studied biotopes are also compared with the relevant values obtained through a literature review.

Methods

Selection of permanent sample plots for aboveground biomass sampling

Preliminary plot selection was restricted to three model catchments of Žimutice, Knín and Krč (*Fig. 1*) located in the Temelín Nuclear Power Plant Emergency Planning Zone; it was based on the screening of the Natura 2000 maps and Land Parcel Identification System (LPIS) database. The final selection of the plots was done during the field survey in March 2013.

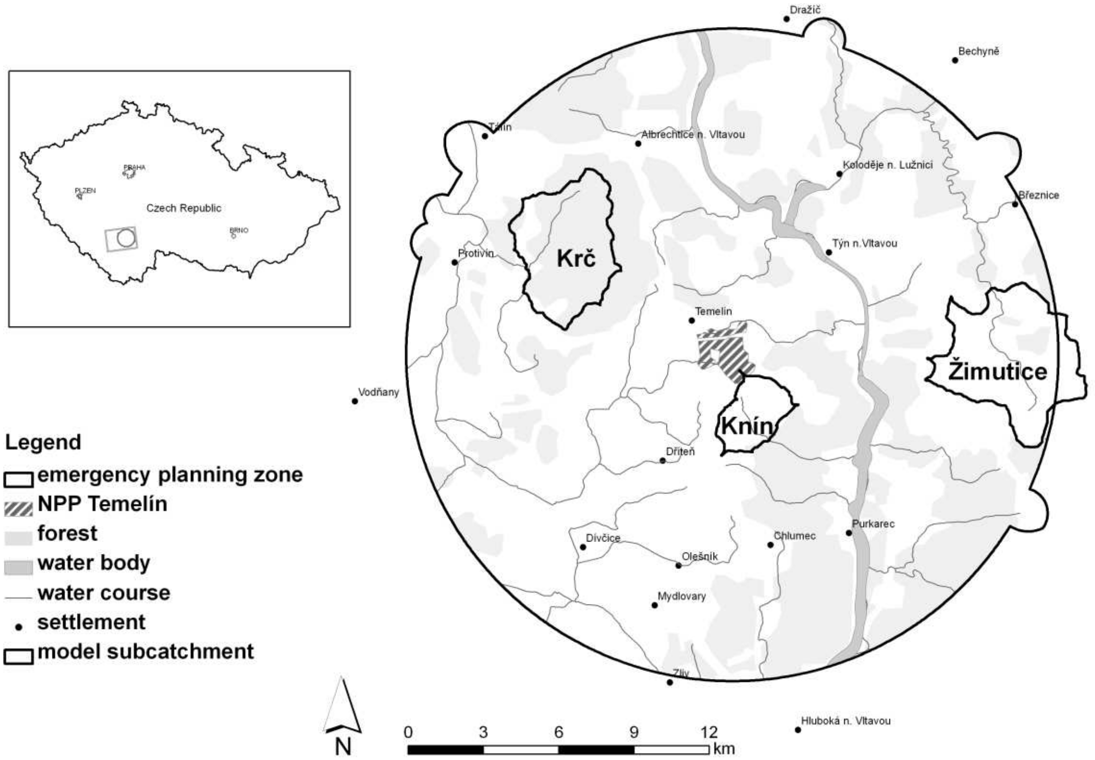

Figure 1. Localization of the model catchments in Emergency planning zone of NPP Temelín and position of the zone in Czech Republic. The sampling sites of Filip-1, Cirs-2, Allop-3, ReedT-4, Oilseed-5, WheatW-6, WheatS-7, IntensM-8 and Maize-21 are situated in the Žimutice catchment, the ReedL-9, Glyc-10, Typha-11 and Carex-12 belong to the Knín catchment, and Filip-13, Typha-14, Carex-15, Cirs-16, Glyc-17, Allop-18, ReedT-19 and Arrhen-20 to the Krč catchment.

The overall climatic parameters of the studied area during the 2013 vegetation season are given on *Fig. 2*.

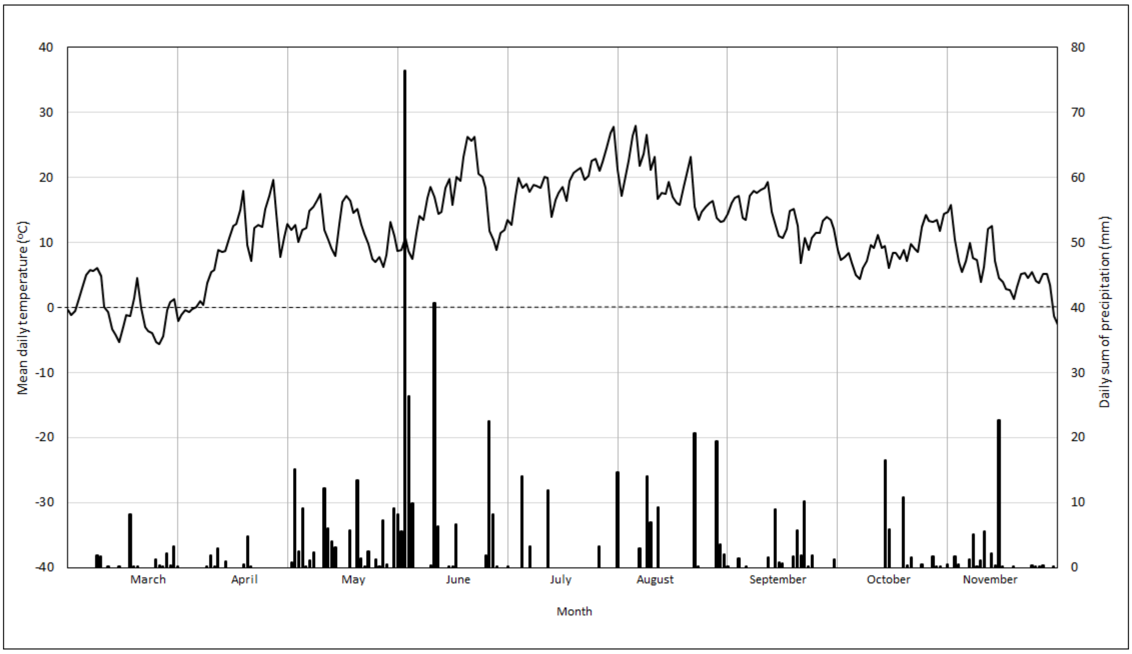

***Figure 2**. Daily sum of precipitation (columns) and mean daily temperatures (curve) in the Emergency planning zone area in the vegetation season of 2013 (source: Czech Hydrometeorological Institute)*

21 vegetation stands in total were selected covering a wide variety of natural conditions (from freshwater to terrestrial biotopes) and types of land use (agricultural arable and grassland stands and non-agricultural semi-natural and natural non-forest vegetation). Agricultural crops were sampled on a one-plot-per–crop basis given their low production variability. Four crop species were selected covering the largest area of the arable land in the Emergency Planning Zone, i.e. winter crop (the "WheatW-6" sampling site), spring crop ("WheatS-7"), oilseed rape ("Oilseed-5") and maize ("Maize-21").

The grassland stands were sampled along the gradient from a dry to wet grassland. The sample sites were selected to cover each wetness level by two stands (e.g. dry grasslands are represented by the "Intensively managed meadows"[1] ["IntensM-8"] and the "Mesic *Arhenatherum* meadows" ["Arhen-20"]). Pairs of the sites are also situated to cover the "agricultural" (Žimutice and Knín) and "natural" (Krč) model catchments.

In the present work we studied unmanaged stands such as the "Wet *Filipendula* grassland" ("Filip-1", "Filip-13") and "Wet *Cirsium* meadow" ("Cirs-16") which was not mowed. The managed stands were represented by a mowed "Wet *Cirsium* meadow" ("Cirs-2"), "Alluvial *Alopecurus* meadow" ("Alop-3", "Alop-18"), "Mesic *Arrhenatherum* meadow" ("Arrhen-20") and "Intensively managed meadow" ("IntensM-8"). All these meadows were mowed twice in the observed period, and the "IntensM-8" was even cropped in the autumn.

Natural and semi-natural grassland communities were represented by the "Wet *Cirsium* meadows", T1.5 ("Cirs-16"), "Wet *Filipendula* grasslands", T1.6 ("Filip-1", "Filip-13"), "Tall-sedge beds", M1.7 ("Carex-12", "Carex-15") and "Reed beds of

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[1] The nomenclature and codes of the vegetation stands follow the Habitat catalogue of the Czech Republic (Chytrý et al., 2010), designed for the NATURA 2000 habitat mapping.

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eutrophic still waters", M1.1 ("ReedT-4", "ReedL-9", "Glyc-10", "Typha-11", "Typha-14", "Glyc-17", "ReedT-19"). A short description of the communities both according to Chytrý et al. (2010) and our relevés performed on the studied habitats together with type of management of the sites is given in the following text and in *Tab. 1*.

Table 1*.Management type and phytocenologic characteristic of the sampled stands.M, J, S – cover (in percent) and number of species in May, July and September.Ratio (G:L:F) – proportion of grasses, legumes and herbaceous plants; the grasses group besides the Poaceae grasses involves also Cyperaceae, Juncaceae and Typhaceae. The percentage at the individual species represents the average cover calculated from all the three relevés. Diagnostic species are listed after Chytrý et al.(2010).*

	Management	**Cover (M, J, S, %)**	**Number species (M, J, S)**	**Ratio G:L:F**	**Dominant species (%)**	**Species with cover higher than 5%**	**Diagnostic species (%)**
ReedL-9	unmanaged	30, 97.5, 90	1, 2, 1	1:0:1	*Phragmites australis* (72.3)		*Phragmites australis* (72.3)
ReedT-4	unmanaged	10, 51, 95	1, 3, 1	1:0:2	*Phragmites australis* (51.7)		*Phragmites australis* (51.7), *Solanum dulcamara* (0.2)
ReedT-19	unmanaged	17.8, 97.2, 96.6	9, 7, 9	3:0:9	*Phragmites australis* (68.3)		*Phragmites australis* (68.3), *Equisetum fluviatile* (0.2)
Typha-11	unmanaged	12, 46.5, 41.5	3, 5, 4	3:0:2	*Typha angustifolia* (29.3)		*Typha angustifolia* (29.3), *Glyceria maxima* (2.5), *Typha latifolia* (1)
Typha-14	unmanaged	71.3, 65.9, 70.8	7, 6, 5	3:0:5	*Typha angustifolia* (48.3)	*Lemna minor* (20)	*Typha angustifolia* (48.3), *Typha latifolia* (0.5), *Equisetum fluviatile* (0.2)
Glyc-10	unmanaged	70.5, 80.5, 70.5	2, 3, 2	2:0:1	*Glyceria maxima* (72.7)		*Glyceria maxima* (72.7), *Phragmites australis* (1)
Glyc-17	unmanaged	65.9, 78.5, 90	9, 8, 7	3:0:9	*Glyceria maxima* (74)		*Glyceria maxima* (74)
Carex-12	unmanaged	31, 90.5, 76	2, 3, 4	3:0:2	*Carex acuta* (62.7)		*Carex acuta* (62.7), *Phalaris arundinacea* (0.2), *Lysimachia vulgaris* (0.2)
Carex-15	unmanaged	92, 83, 86.1	10, 12, 11	5:1:10	*Carex acuta* (68.3)	*Phalaris arundinacea* (15)	*Carex acuta* (68.3), *Phalaris arundinacea* (15), *Lysimachia vulgaris* (0.5), *Lythrum salicaria* (0.3), *Galium palustra* (0.3), *Peucedanum palustre* (0.1)
Filip-1	unmanaged	92, 95.5, 89	11, 9, 7	4:0:8	*Filipendula ulmaria* (83.3)		*Filipendula ulmaria* (83.3), *Caltha palustris* (0.8), *Scirpus sylvaticus* (0.8), *Lysimachia vulgaris* (0.5), *Alopecurus pratensis* (0.3)
Filip-13	unmanaged	76.2, 89, 95.5	7, 5, 4	1:0:7	*Filipendula ulmaria* (56.7)	*Phalaris arundinacea* (18.3), *Urtica dioica* (9.3)	*Filipendula ulmaria* (56.7), *Caltha palustris* (1.8)
Cirs-16	unmanaged	71, 92.2, 91.2	18, 18, 19	7:1:15	*Scirpus sylvaticus* (46.7)	*Phalaris arundinacea* (10), *Glyceria maxima* (8.3)	*Scirpus sylvaticus* (46.7), *Carex nigra* (2.3), *Deschampsia cespitosa* (2.2), *Cirsium palustre* (1), *Caltha palustris* (0.5), *Sanquisorba officinalis* (0.4), *Galium palustre* (0.3), *Lathyrus pratensis* (0.3), *Poa trivialis* (0.2), *Juncus conglomeratus* (0.2)
Cirs-2	mowing	92.5, 87, 52.5	17, 15, 8	7:3:14	*Scirpus sylvaticus* (36.7)	*Poa trivialis* (28.3)	*Scirpus sylvaticus* (36.7), *Poa trivialis* (28.3), *Carex nigra* (3), *Holcus lanatus* (2), *Alopecurus pratensis* (1.7), *Juncus effusus* (0.7), *Filipendula ulmaria* (0.5), *Ranunculus acris* (0.3), *Rumex acetosa* (0.3), Lychnis *flos-cuculi* (0.3), *Angelica sylvestris* (0.2), Lathyrus *pratensis* (0.2), *Sanquisorba officinalis* (0.2), *Cardamine pratensis* (0.2)
Allop-3	mowing	94, 65.5, 69.5	18, 17, 16	10:2:12	*Poa trivialis* (35)	*Ranunculus repens* (10), *Alopecurus pratensis* (6.7), *Festuca pratensis* (6)	*Poa trivialis* (35), *Ranunculus repens* (10), *Alopecurus pratensis* (6.7), *Festuca pratensis* (6), *Holcus lanatus* (4), *Ranunculus acris* (2.8), *Trifolium hybridum* (2), *Carex hirta* (1.2), *Sanquisorba officinalis* (0.5), *Lychnis flos-cuculi* (0.3), *Cardamine pratensis* (0.2), *Geranium pratense* (0.2)
Allop-18	mowing	74.1, 78.1, 88.3	25, 28, 31	15:3:17	*Festuca rubra* (13.3)	*Poa trivialis* (10), *Ranunculus repens* (8.3), *Lathyrus pratensis* (6.7), *Alopecurs pratensis* (5.8)	*Poa trivialis* (10), *Ranunculus repens* (8.3), *Lathyrus pratensis* (6.7), *Alopecurs pratensis* (5.8), *Holcus lanatus* (4.3), Poa pratensis (4), *Deschampsia cespitosa* (3.3), *Carex hirta* (3), *Ranunculus acris* (1.2), *Lysimachia nummularia* (0.5), *Sanquisorba officinalis* (0.5), *Lychnis flos-cuculi* (0.2), *Scirpus sylvaticus* (0.2), *Festuca pratensis* (0.2), *Glechoma hederacea* (0.1)
Arrhen-20	mowing	81.1, 94, 79.5	23, 21, 18	10:4:12	*Dactylis glomerata* (15)	*Holcus lanatus* (10), *Poa pratensis* (6.7), *Arrhenatherum elatior* (6), *Alopecurus pratensis* (6), *Trifolium pratense* (6), *Festuca rubra* (5),	*Dactylis glomerata* (15), *Holcus lanatus* (10), *Poa pratensis* (6.7), *Arrhenatherum elatior* (6), *Trifolium pratense* (6), *Festuca rubra* (5), *Trisetum flavescens* (5), *Festuca pratensis* (4.3), *Achillea millefolium* (4), *Plantago lanceolata* (3.3), *Rumex acetosa* (1.2), *Anthoxanthum odoratum* (1.2), *Ranunculus acris* (1), *Lotus corniculatus* (0.3), *Saxifraga granulata* (0.2), *Veronica chamaedrys* (0.2), *Lathyrus pratensis* (0.2)
IntensM-8	mowing, grazing	100, 96.5, 36	16, 14, 8	7:2:10	*Taraxacum* sect. *Ruderalia* (40)	*Trifolium repens* (12), *Trifolium pratense* (8.5), *Poa pratensis* (7.3)	
WheatW-6	arable land	80.6, 86, 0	3, 3, 0	2:0:2	*Triticum aestivum* (82.5)		
WheatS-7	arable land	50.3, 82.1, 0	4, 6, 0	2:0:6	*Triticum aestivum* (65)		
Oilseed-5	arable land	98.5, 99.1, 0	3, 4, 0	4:0:0	*Brassica napus* (97.5)		
Maize-21	arable land	5, 16.6, 61.5	1, 9, 3	4:0:6	*Zea mays* (26.7)		

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Reed beds of eutrophic still waters (ReedT-4, ReedL-9, Glyc-10, Typha-11, Typha-14, Glyc-17, ReedT-19)

The littoral vegetation of fishponds, dead river arms and banks of slow-moving river parts, poor in species. They are characteristically dominated by one species determining the physiognomy of the stand; the stands can reach 0,5–4 m in height and the adequate diverse amount of biomass (Chytrý et al., 2010). That is why the community was further classified according to the dominant species into three classes: Reed beds of eutrophic still waters with dominant Phragmites australis (ReedT-4, ReedL-9, ReedT-19), Reed beds of eutrophic still waters with dominnt *Typha angustifolia* and *T. latifolia* (Typha-11, Typha-14) and Reed beds of eutrophic still waters with dominant *Glyceria maxima* (Glyc-10, Glyc-17).

Both the littoral and terrestrial *Phragmites* stands of the ReedL-9, ReedT-4 and ReedT-19 classes belong to this group. The first two stands were relatively typical in terms of their species composition (see *Tab. 1*), with some untypical accompanying species only present in the ReedT-19 class, although their proportion was not high. These were species belonging to the Wet *Cirsium* meadow, e.g. *Scirpus sylvaticus*, *Cirsium palustre*, *Galium palustre*, and also ruderal species (*Urtica dioica*).

The Glyc-10 was a typical stand of its biotope type according to Chytrý et al. (2010) (see *Tab. 1*). The Glyc-17 species composition was more variable with *Glyceria maxima* as a dominating species but there was also a small proportion of the Tall sedge bed species such as *Carex rostrata*, *Peucedanum palustre* or ruderal species (*Urtica dioica, Cirsium arvense*).

Tall-sedge beds (Carex-12, Carex-15)

The stands dominated by tall sedges are typically situated on littoral shallows and banks of fishponds, river arms in late stages of succession, inundated river and stream alluvia or waterlogged depressions on meadows. Frequently they adjoin the eutrophic *Phragmites* stands (Chytrý et al., 2010).

Carex-12 and Carex-15 were both adjacent to the littoral reed stands. The water level had been under the soil surface for most of the year, which probably led to the absence of wetland species and the dominance of some ruderal species such as the *Urtica dioica* and *Galeopsis bifida*. In other aspects both of the stands with the dominant *Carex acuta* and other accompanying species corresponded with the biotope classification according to Chytrý et al. (2010).

Wet Filipendula grasslands (Filip-1, Filip-13)

The community usually arises from fallow Wet *Cirsium* meadows; it is often a monodominant stand of *Filipendula ulmaria* with an admixture of other tall plants.

Whereas the Filip-1 class corresponded well with this biotope type description according to Chytrý et al. (2010), the Filip-13 stand was partially ruderalised (with the presence of the *Urtica dioica*, *Galium aparine*).

Wet Cirsium meadows (Cirs-2, Cirs-16)

Wet meadows rich in species on waterlogged gleysols in stream and smaller river alluvia or springs ranging from lowlands to mountainous areas. The groundwater level is permanently high. The meadows are mown once or twice a year; they rapidly change in line with the change in management.

The Cirs-16 is situated in the estuarine part of a fishpond. It is a rich-in-species stand dominated by the *Scirpus sylvaticus* and including 22 more species representing up to 45% (cf. Tab. XY). The stand generally corresponded with the description according to Chytrý et al. but the proportion of the species from neighbouring biotopes (e.g. the *Glyceria maxima*, *Phalaris arundinacea* and also ruderal species such as the *Urtica dioica*, *Cirsium arvense*, *Galium aparine*, etc.) was higher.

Alluvial Alopecurus meadows (Alop-3, Alop-18)

Fresh wet meadows in inundated areas of stream alluvia, on deep soils rich in nutrients situated from lowlands to submontane areas. These meadows were mown at least once a year.

Mesic Arrhenatherum meadows (Arhen-20)

Meadows of lowlands and hilly areas rich in species on nutrient-rich soils dominated by the *Arrhenatherum elatius* or submontane meadows on oligotrophic Cambisols poorer in nutrients dominated by the *Agrostis capillaris* and *Festuca rubra*. The stands were usually mown twice a year and occasionally grazed.

Intensively managed meadows (IntensM-8)

The meadows or clover/grass mixtures poor in species, well manured and occasionally ploughed. The prevailing species are grasses (*Alopecurus pratensis, Dactylis glomerata*) and nitrophilous broadleaf herbaceous plants (*Taraxacum* sect. *Ruderalia*, *Rumex obtusifolius*, *Antriscus sylvestris*).

The mowed meadows with their structure and proportion of diagnostic species corresponded well with the biotope description according to Chytrý et al. (2010); the IntensM-8 was a standard trefoil-grass mixture with the dominant *Taraxacum* Sect. *Ruderalia* and presence of a small proportion of many field weed species. The stand was mown twice in the 2013 season and after the second mowing it was grazed until November.

Sampling of aboveground biomass

The amount of aboveground biomass was estimated by destructive sampling from sample plots situated along the moisture-based transect (to cover the variability of the stands as much as possible) at regular 5–50 m distances (according to the area of the stand). Five squares comprising the surface of 0,5 x 0,5 m were cut from each stand. The total of 105 plots (5 plots from each of 21 vegetation stands) was sampled during each sampling term. The biomass was cut just above the ground by garden scissors. In case of non-managed stands dead last year's shoots (i.e. at reed stands) were removed. The biomass was weighted, dried at 85 °C and weighted again (Dykyjová, 1989; Ondok and Květ, 1978; Rychnovská, 1987). The sampling was carried out from the beginning of March to mid-November 2013 (the exact values are stated in *Figs. 3 – 9* and *Tab. 2*). The intervals between sampling dates were chosen according to the growth intensity of the stands (the highest intensity in spring; less frequent sampling in the autumn). 11 samplings were done altogether. Relevés of all stands (Moravec et al., 1994) were done during the sampling carried out on the 14th May, 16th July and 3rd September. One control sampling was done on the 15th July 2014 on the WheatW-6 and Oilseed-5 sampling sites.

Results

The production values are presented both in the form of production curves (*Fig. 3 – 9*) and the average values of the individual sampling dates of all the studied stands (*Tab. 2*).

The *Phragmites* production of biomass (*Fig. 3*) have similar curve shapes in all the locations; the minimum values were observed on the first sampling date in April and the maximum was reached in October, whereas the maximum value of the ReedL-9 littoral stand in Knín was higher (2,309 g DW*m^{-2}) than that of the ReedT-19 terrestrial stands in Krč (1,544 DW*m^{-2}) and the ReedT-4 stand in Žimutice (1,819 g DW*m^{-2}). The development of the ReedT-4 and especially the ReedT-19 terrestrial stand was notably slower in springtime than that of the ReedL-9 littoral stand; the differences between the values reached up to 1,300 g DW*m^{-2} in May and June. The decline of the curves is of a different intensity - the November values drop below the spring (ReedL-9), summer (ReedT-4) or only autumn (ReedT-19) values.

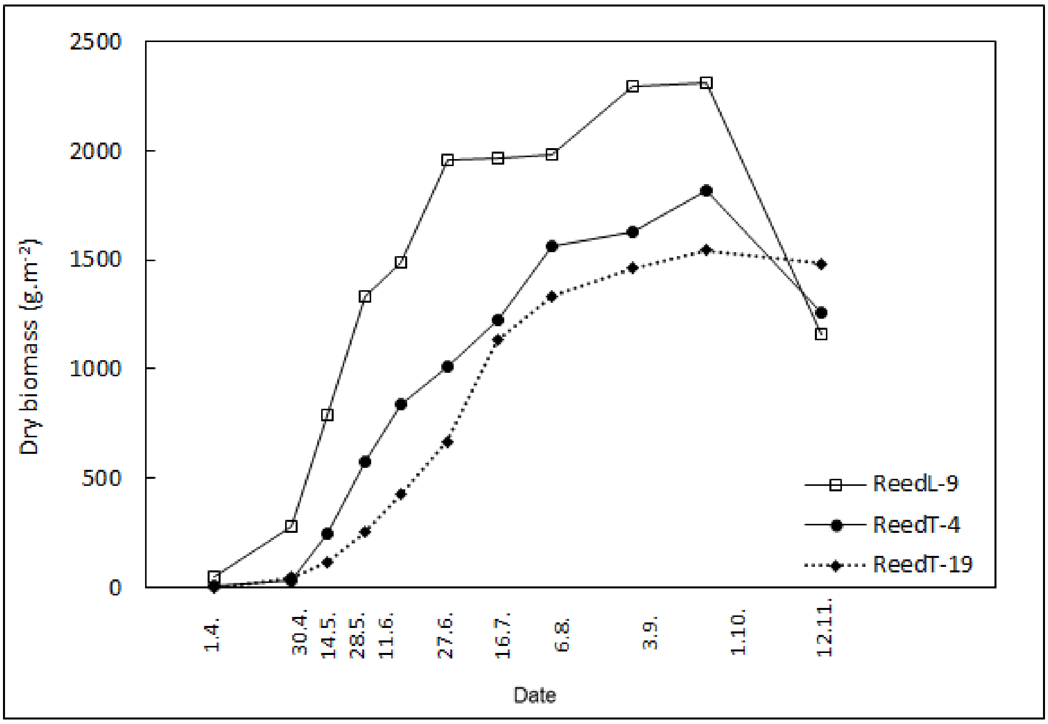

Figure 3. *Dry biomass production of the Phragmites reed bed community*

The *Glyceria* stands (*Fig. 4*) display a similar curve development in both locations. The Glyc-10 performed in general lower values than the Glyc-17. There is a greater difference between the maximum values at the Glyc-17 (1,291 g DW*m^{-2}) and Glyc-10 (940 g DW*m^{-2}), both reached in July. The Glyc-10 curve also declines faster and lower than the Glyc-17 one.

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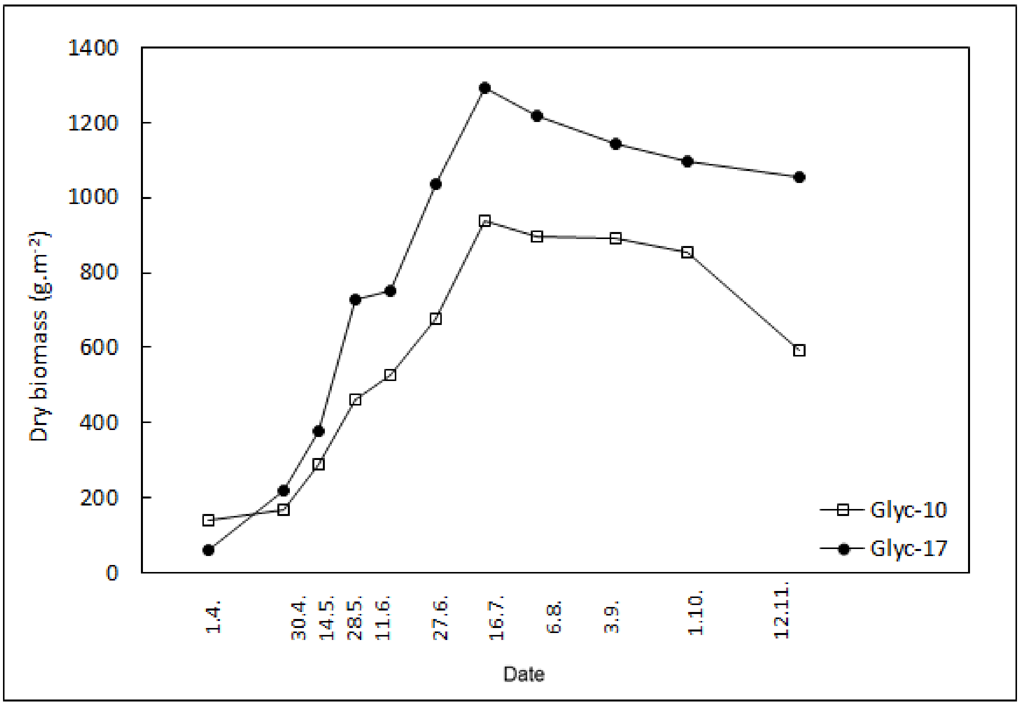

Figure 4. *Dry biomass production of the Glyceria reed bed community*

The curves of tall sedges stands (*Fig. 5*) follow a similar trend until the June sampling, when the Carex-12 stand reached its maximum (970 g DW*m^{-2}) and then slowly declines; the Carex-15 stand, on the other hand, increases until the beginning of August and then declines fast and low. The maximum value reached was almost identical (963 g DW*m^{-2}).

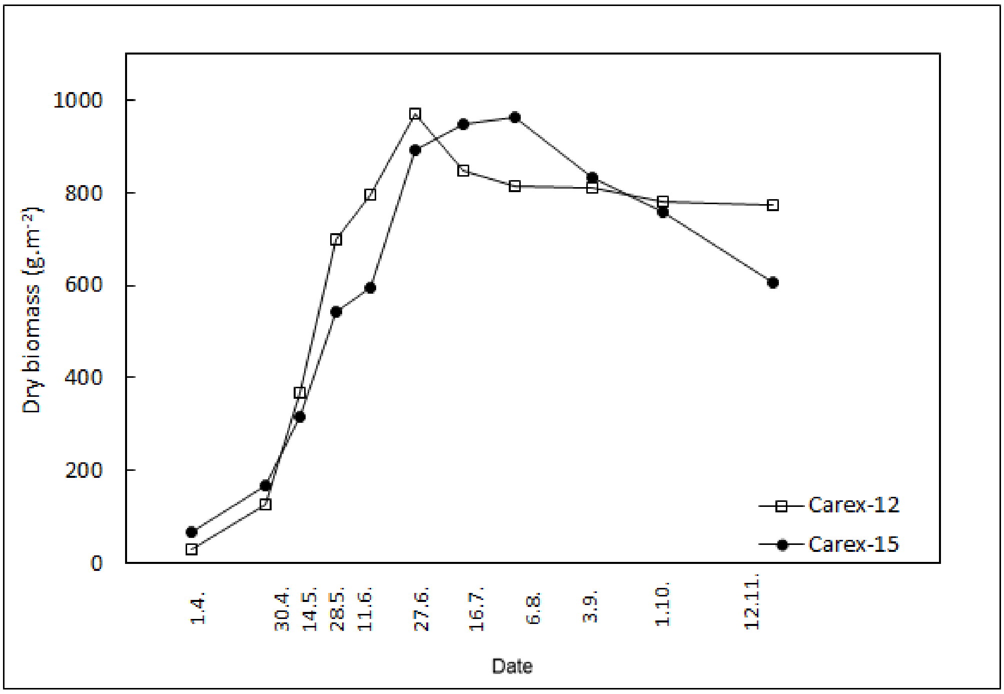

Figure 5. *Dry biomass production of the Carex reed bed community*

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The *Typha* curves (*Fig. 6*) have again a very similar development in both locations. Typical is a long, relatively slow increase and maximum values occurring in late September (1,461 g DW*m^{-2} at the Typha-11 stand, 1,129 g DW*m^{-2} at the Typha-14 stand). There is an insignificant peak at the Typha-14 stand, and the decline towards October sampling date is very slow.

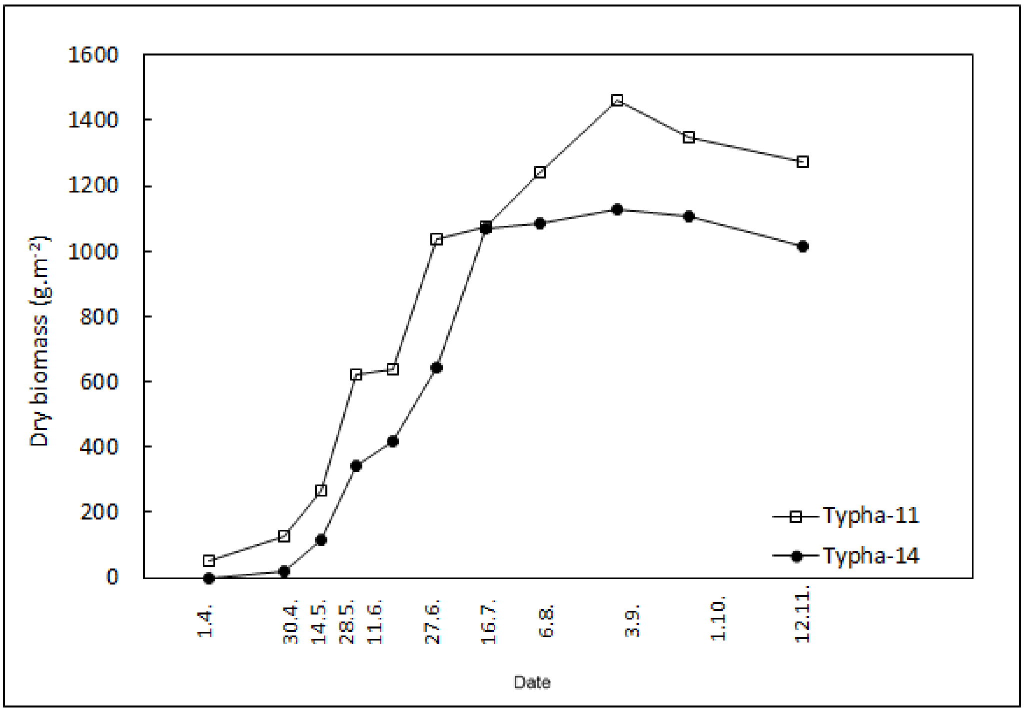

Figure 6. *Dry biomass production of the Typha reed bed community*

The biomass curves of unmanaged wet *Cirsium* and *Filipendula* meadows (*Fig. 7*) show an interesting similar trend between the Filip-13 and Cirs-16 stands which also reached the maximum values on the same date in August (1,248 g DW*m^{-2} at the Filip-13 stand, 922 g DW*m^{-2} at the Cirs-16 stand), then significantly declined. The Filip-1 stand had a different development – after a rapid increase between May and mid-June there were no dramatic changes in values. The maximum (1,172 g DW*m^{-2}) was reached in July.

The curves for managed (mowed) meadows of various types (*Fig. 8*) show many similarities in their development. In general, the first mowing (between 11 and 27 June) was obviously done after the first period of the post-peak decline; all the maximum values were reached at the end of May except for the Cirs-2 stand (11 June, 380 g DW*m^{-2}). Surprisingly, the highest biomass value was not observed in the intensively managed and nutrient-rich stand of the IntensM-8 class (540 g DW*m^{-2}) but in the Alop-3 stand (577 g DW*m^{-2}). The Alop-18 and Arrhen-20 stands had lower peak values (471 and 389 g DW*m^{-2} respectively).

The second peak was reached in August in all cases. All the locations except the Alop-18 were mown just before the sampling date of the 3rd September; the Alop-18 stand had not been mown until the 5th September. The highest values of the second peak occurred at the Cirs-2 stand (361 g DW*m^{-2}), which is comparable to the first peak value. The biggest difference was observed in the Alop-18 stand (by approx. 350 g DW*m^{-2}). Other locations show an even decline between the first and the second mowing by approx. 150–200 g DW*m^{-2}.

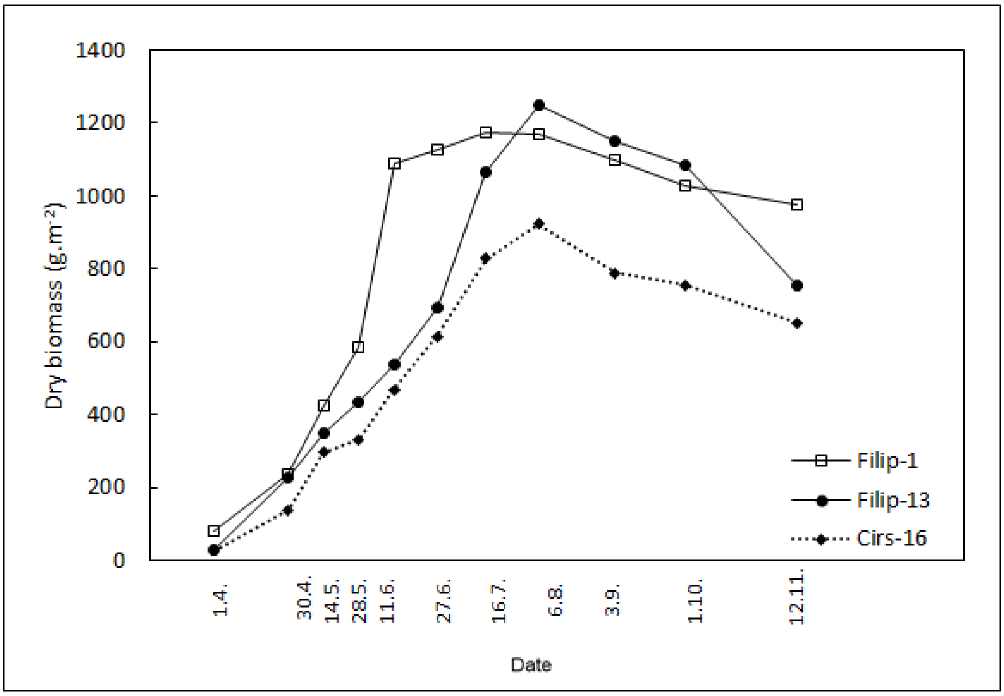

Figure 7. Dry biomass production of unmanaged wet meadows

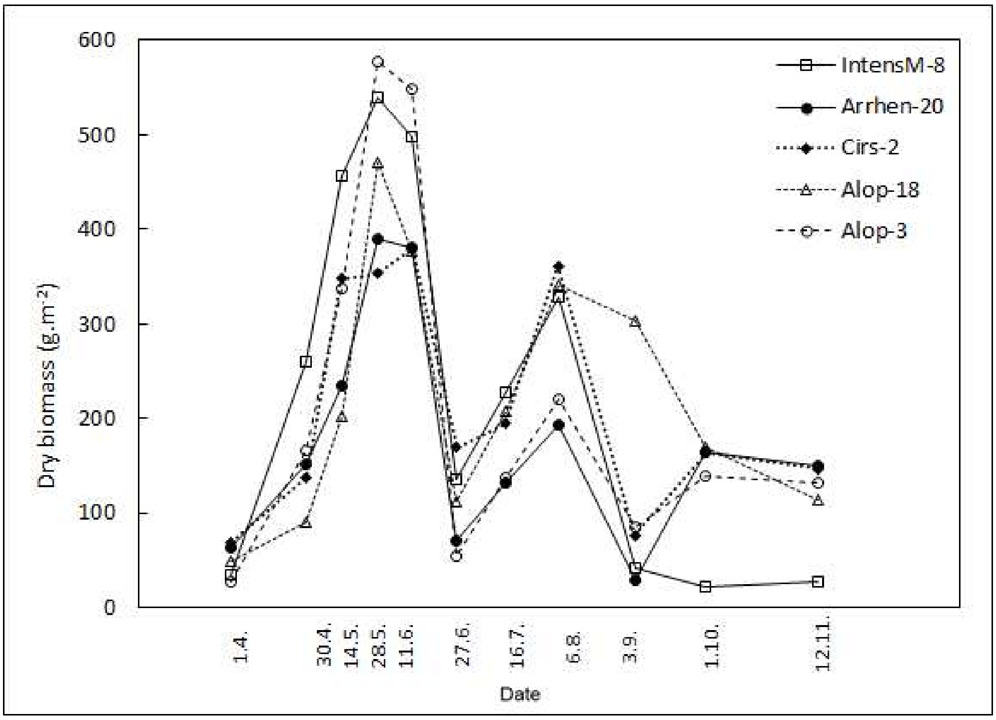

Figure 8. Dry biomass production of mowed meadows

The Arrhen-20, Cirs-2 and Alop-3 stands also formed the third peak in October; the biomass amount was relatively even on these localities (165, 164, and 140 g DW*m^{-2} respectively). The continuing decline of the IntensM-8 curve is caused by autumn grazing.

Biomass curves of crops (*Fig. 9*) differ significantly both as to their trends and values.

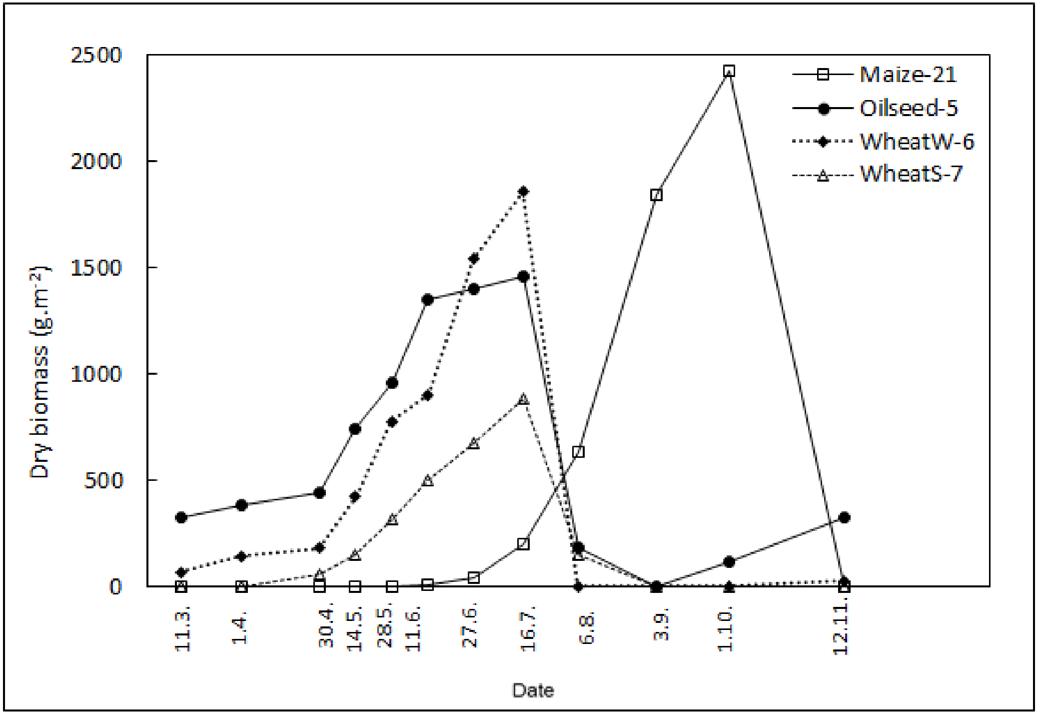

Figure 9. *Dry biomass production of agricultural crops*

Table 2*. Average values of average biomass values (g DW*m-2) of all stands in all sampling terms.*

	11.3.2013	1.4.2013	30.4.2013	14.5.2013	28.5.2013	11.6.2013	27.6.2013	16.7.2013	6.8.2013	3.9.2013	1.10.2013	12.11.2013
ReedL-9	0,0	47.7	282.8	793.6	1,334.4	1,487.0	1,953.8	1,964.9	1,980.0	2,291.8	2,308.7	1,162.5
ReedT-4	0.0	5.8	36.1	248.0	579.0	836.9	1,009.3	1,222.5	1,560.0	1,628.7	1,819.2	1,256.0
ReedT-19	0.0	0.0	48.4	116.4	252.3	427.8	669.5	1,133.8	1,334.5	1,460.8	1,543.6	1,480.4
Typha-11	0.0	52.7	108.0	269.4	621.4	636.6	1,038.2	1,076.7	1,242.6	1,460.8	1,346.2	1,275.5
Typha-14	0.0	0.0	21.4	116.1	341.9	420.1	641.1	1,068.6	1,085.8	1,128.6	1,107.7	1,016.0
Glyc-10	0.0	139.4	168.0	287.6	464.2	527.0	674.8	940.3	859.2	890.4	853.0	591.7
Glyc-17	0.0	58.6	218.5	376.3	726.2	753.3	1,036.7	1,291.1	1,216.2	1,141.2	1,094.8	1,053.1
Carex-12	0.0	30.7	126.8	367.2	699.3	795.5	970.2	847.3	815.2	811.3	781.2	772.7
Carex-15	0.0	66.3	166.3	314.5	540.8	596.3	893.3	947.5	963.3	831.4	759.7	606.2
Filip-1	0.0	78.6	237.6	423.3	584.0	1,087.2	1,125.8	1,172.4	1,167.8	1,096.6	1,025.3	972.9
Filip-13	0.0	29.1	226.4	349.3	431.3	538.1	691.5	1,066.8	1,247.6	1,151.5	1,081.7	754.9
Cirs-16	0.0	26.8	138.3	295.3	330.2	466.5	614.1	827.4	922.2	786.8	755.5	650.9
Cirs-2	0.0	69.1	138.0	347.4	353.7	379.8	169.0	194.3	361.1	76.2	163.9	147.0
Alop-3	0.0	27.2	166.3	338.1	576.9	548.2	53.9	137.1	219.8	85.4	139.7	131.1
Alop-18	0.0	49.7	91.2	202.1	470.6	376.7	112.8	207.6	340.6	303.8	169.7	114.0
Arrhen-20	0.0	63.6	152.3	234.4	389.4	380.7	70.5	131.7	192.5	29.7	164.9	150.4
IntensM-8	0.0	33.7	260.0	457.2	540.0	497.8	136.3	228.0	327.6	40.9	21.8	27.2
Maize-21	0.0	0.0	0.0	0.0	1.9	2.7	38.8	201.2	636.5	1,840.2	2,429.7	0.0
Oilseed-5	324.6	380.1	439.4	739.1	956.7	1,349.4	1,401.3	1,457.8	180.9	0.0	115.3	324.6
WheatW-6	65.3	140.5	181.0	423.5	778.4	902.4	1,538.8	1,856.3	0.0	0.0	0.0	25.2
WheatS-7	0.0	0.0	58.2	151.3	312.0	500.6	678.1	878.9	144.7	0.0	0.0	0.0

The Maize-21 curve shows a rapid increase between July and October and the maximum value was reached in this late period just before the harvest (2,430 g DW*m^{-}

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^{2}). The Oilseed-5 curve evenly increases until the harvest time in July (the maximum value is 1,458 g DW*m^{-2}); since it was grown as a winter crop, the curve declines after harvest (6th August), then falls to zero (3rd September – ploughing) and slowly increases again in the autumn. The wheat curves, interestingly, have a different development – the WheatW-6 curve shows more similarities with the oilseed biomass growth and reaches even a higher maximum value (1,505 g DW*m^{-2}) before the harvest, then it declines and only in November a slight increase could be observed. The WheatS-7 spring wheat curve increases slowly and evenly until the pre-harvest maximum also in July, which is only almost a half of the winter crop peak value (817 g DW*m^{-2}). It was possible to capture the biomass value of stubble in August (145 g DW*m^{-2}), then the field was ploughed.

Discussion

Discussion on methodology

The methodical approach of this study was defined to balance three factors – the needs of the above mentioned research project, representativeness of the samples and obtained data, and the possibility and requirements of the biomass sampling procedure and processing.

The studied biotope types present representative samples of non-forest landscape elements in the Emergency Planning Zone; they cover 51% of the non-forest area of the zone (34.1% of the total area of the zone) (Vinciková et al., 2010). The sampling locations were selected as typical stands of their type (Chytrý et al., 2010) in the studied catchments which were chosen as the sample areas of the project; nevertheless, it would be more accurate to sample at least five stands of each biotope to cover the nutrient, moisture and other variability of the environment and its impact on the biotope. This method would be of course much more demanding and time-consuming (sampling must be carried out on the same date) and it is generally applied for detailed studies of one or a few biotope types (e.g. Kaplová et al., 2011).

Similar limits defined the sampling method itself. Three sampling points is the minimum number to acquire representative data (Dykyjová, 1989), five samples present a good enhancement of the representativeness; situating the sampling points along (in most cases) the moisture transect helped cover the diversity of the stands even better. Also the sampling dates were chosen in sufficient intervals, covering more precisely the estimated peaks of the biomass amount than the end of the season. This approach is very important especially in case of wetland communities, with a rapid growth in the first half of the vegetation season such as the *Phragmites* stands (e.g. Čížková et al., 2001; Dykyjová and Květ, 1970).

The procedure of sampling the plots and further biomass processing was carried out according to the standard methodology (Dykyjová, 1989; Ondok and Květ, 1978; Květ and Westlake, 1998).

Discussion on results

The results of the biomass production sampling are generally difficult to compare with other authors regardless the fact the methods used are identical, because the conditions of the stands vary both in space and time. They are influenced especially by nutrient and water supply depending on the micro-, meso- and also macroclimate of the

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area. Comparing the biomass values of the same plot sampled during two different growing seasons can be a bit tricky as well because of the differences in the yearly distribution of precipitation, temperatures and sunny days; spring temperatures and water availability are especially important (Květ and Westlake, 1998). The season of sampling was significant for its very wet springtime and relative lack of precipitation in the second half of the year (cf. *Fig. 2*). In the following text the comparison of measured and reviewed biomass values was done mostly by comparing the trends of the production curves, where available, rather than focusing on the individual values. Especially in case of the rich-in-species stands the variability of values can be higher than in monocultures; that is why we provide a detailed description of the species composition of the stands both in *Tab. 1* and in the text to explain the occurring differences.

Reed beds of eutrophic still waters dominated by the Phragmites australis

The first shoots of the ReedL-9 littoral reed community appeared in March and an intensive growth was observed until the end of June; the maximum (2,309 g DW*m^{-2}) was reached at the beginning of October (*Fig. 3*). The shape of the growth curve is comparable to the results of Dykyjová and Hradecká (1976) from the Opatovice pond situated in the Třeboň region, South Bohemia, although the maximum was reached there in September and the value was lower (1,824 g DW*m^{-2}). Květ and Westlake (1998) published the *Phragmites* growth curves measured in South Moravia (Czech Republic), England and Denmark. These curves followed a similar trend, only the curve from South Moravia increased faster than the other curves, situated in colder areas. Our ReedL-9 littoral stand best corresponds with the curve from Moravia whereas the terrestrial reeds of the ReedT-4 and namely ReedT-19 stands, with their slower increase in spring, rather resemble the English production curve.

Significantly more information was found on the maximum reached values of the biomass of the *Phragmites* stands. Dykyjová and Květ (1970) stated maximum biomass values of eleven South-Bohemian and six South-Moravian fishponds (both locations to be found in the Czech Republic) sampled between 1965 and 1969. They ranged from 890 to 2,730 g DW*m^{-2} (littoral stands of South Bohemia) and from 865 to 1,930 g DW*m^{-2} at terrestrial stands in Moravia. This reflects the variability of biomass production; warmer Moravian locations also reached their maximum up to 1.5 month earlier than the South Bohemian fishponds. Květ and Husák (1978) mention the range of the aboveground biomass between 600 and 3,500 g DW*m^{-2}. In her study on the *Phragmites* biomass production in Estonia Ksenofontová (1988) measured the peak biomass values between 668 and 1,311 g DW*m^{-2}. Our measured maximum values correspond well with these data. The later maximum values (1st October 2013) could have been caused either by the site conditions or the sampling year climate course but are by no means unique – Dykyjová and Květ (1970) measured the peak value as late as 9th October 1966.

Reed beds of eutrophic still waters dominated by the Typha angustifolia

Květ and Westlake (1970) published the growth curves of the *Typha angustifolia* observed in various regions of the Czech Republic. Initially its increase was less steep than that of the *Phragmites* curves, which was confirmed in our locations as well. The Typha-11 curve trend, maximum value and date are in line with these data (Květ and

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Westlake, 1998). Dykyjová and Květ (1970) presented the maximum values measured in littoral stands of the Opatovice fishpond in South Bohemia in August and September, ranging from 1,570 to 3,880 g DW*m^{-2}. Květ and Husák (1978) provided similar results (1,000–3,000 g DW*m^{-2}). Our maximum values (1,461 and 1,129 g DW*m^{-2} respectively) correspond with the published results; nevertheless, the range of maximum values is again relatively wide.

Reed beds of eutrophic still waters dominated by the Glyceria maxima

The Glyc-10 and Glyc-17 growth curves, especially the increasing part until the June maximum, entirely correspond with the results published by Petřík (1972). The maximum values were identically measured in June and are fairly similar (1,090 measured by Petřík, our results are 940 and 1,291 g DW*m^{-2} respectively). The decrease of our curves is rather slower. The curves published by Květ and Westlake (1998) from the Czech Republic and England are similar except that the spring increase occured later and the maximum values were reached in September and October. Again, this can be caused by yearly weather development, general climatic conditions of the area and stand conditions. Květ and Westlake (1998) provided a low maximum stand crop (600–1,100 g DW*m^{-2}) in the mild climate and relatively poor soil conditions of the English location; also the peak is relatively broad, because while old shoots are dying new shoots are emerging from July to September. In the more extreme climate and fertile soils of the Czech Republic the maximum standing crop is higher (1,200–3,200 g DW*m^{-2}) and the peak is much more distinct. The growth strategy of the *Glyceria maxima* is different from the *Phragmites* and *Typha,* some of the green shoots overwinter and increases rapidly in the spring and new shoots grow later. This is also influenced by the maturity of the stand.

As to the maximum values, Květ and Husák (1978) state a range between 600 and 2,600 g DW*m^{-2}, Dykyjová and Květ (1970) measured 652 g DW*m^{-2} at the alluvial pond of the Dyje river (South Moravia) and 1,387 at a South Bohemian fishpond.

Tall-sedge beds

The growth curves of these stands (*Fig. 5*) are very similar until June. The Carex-15 curve continues increasing until August and falls down relatively quickly afterwards. A similar trend was published by Květ and Westlake (1978) for a *Carex rostrata* stand in Minnesota. The Carex-12 curve is more similar to the growth curve described by Novák (1977) who also noted an even growth until the June peak followed by a slow decline. Květ and Westlake state the maximum value for the *Carex rostrata* biomass reaching 700 g DW*m-2; Prach et al. (1996) measured 940 g DW*m-2 in the *C. acuta* stand in the river Lužnice alluvium, which is very similar to our results (*Tab. 2*). Lukavská (1988) stated the maximum biomass between 627 and 1,059 g DW*m-2 for a vegetation community dominated by the *C. acuta* and accompanied by the *Calamagrostis canescens* and other *Carex* species which was reached in August. Also Kuncová (2009) measured the total maximum biomass of this community of Wet Meadows in Třeboň (South Bohemia, Czech Republic) corresponding to 546 or 670 g DW*m-2 respectively where the proportion of the *C. acuta* was 423 and 618 g. Novák (1970) stated the maximum total biomass of a similar community of 644 g where the *C. acuta* biomass only reached 248 g.

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Meadow communities rich in species

A few studies were found focusing on the aboveground biomass production of the rich-in-species meadow vegetation communities. Their high variability poses difficulties when comparing the different stands: there were not many analogies to be found in literature especially for the unmanaged meadows of the Filip-1, Filip-13 and Cirs-16 classes. Prach et al. (1996) stated a maximum standing crop value for the stand dominated by the *Filipendula ulmaria* of 1,009 g in July, which is only slightly lower than our values.

The growth curves of the studied mown meadow stands are quite similar, characteristic by a steep increase until the first mowing (*Fig. 8*). The growth of grassland until the second mowing was less intensive and the maximum values reached approximately from one to two thirds of the first peaks. The maximum values especially before the first mowing were influenced by the stand type, especially its nutrient richness and water availability.

Petřík (1970) observed a biomass growth in the Continental inundated meadows and Intermittently wet *Molinia* meadows in South Moravia during three years. The seasons differed more in terms of the maximum values than the curve trends which were quite similar and are well comparable with our curves.

Crops on arable land

We sampled the crop species that are most common in the studied area nowadays. Only the production of the crop parts which are then used (i.e. grain, straw, tuber or seed) is important enough to measure so it is practically impossible to find relevant data on the total aboveground production of any crop stands. Bureš (1970) observed the growth of oats and the maximum biomass he provides is 890 g DW*m^{-2}; this value and the curve trend is analogous to our values for spring wheat. In case of silage corn, which is harvested as a whole, the yield values of various hybrids ranged from 1,400 to 2,050 g DW*m^{-2} (cf. http://www.zea.cz/). Our measured values are higher due to different cropping practices – whereas we harvested the biomass just above the ground, the harvest machines leave stubble of approx. 30 cm in height.

Surprising was the high production of biomass of winter wheat which far exceeded the seemingly richer production of oilseed. Facing the scarcity of relevant and comparable data in published papers we performed another sampling of both spring and winter wheat and oilseed crops in the estimated peak period of the 2014 vegetation season. Both the values were higher than the peak values of 2013 (2,612 g DW*m^{-2} for wheat and 1,921 g DW*m^{-2} for oilseed) which was probably caused by better growth conditions in 2014 but their ratio was almost identical (56 to 44% in 2013 and 58 to 42% in 2014). This can be understood as an example of a high year-to-year variability of the aboveground biomass production. On the other hand, it means the ratios could serve for comparing the production curves for different seasons and it is a certain proof that the methodological approach we used was correct and suitable.

Conclusion

The paper summarizes the results of the annual biomass production measurement for many different biotopes and vegetation stands. It is generally not common to study biomass production of such wide variety of biotopes, especially when it comes to the stands with high species diversity such as various meadow types; this paper can therefore be used as a reference material for similar vegetation stands. This was also the reason why we provided a detailed description of species composition and abundance of the stands.

The results are compared with literature in cases where the values of adequate stands were available and the measured and reviewed values and/or curve trends were in almost all cases similar. This also supports the credibility of our results for the stands where no comparable data was found during our review.

The outputs of this study will be used in the above mentioned project to model the total amount of biomass which could be potentially contaminated by a radiation leak. Such an event can occur anytime in the year so the estimation of a momentary biomass production of the vegetation stands prevailing in the Temelín NPP Emergency Planning Zone is necessary. Nevertheless, the data can be used in an even broader sense – for the purposes of an estimate of biomass production as a source of renewable energy, observation of carbon balance and nutrient supplement, etc. Another important aspect can be the contribution of these data to broad-scale biomass production models, which are usually based on satellite imaging and often lack the direct in-situ calibration of the computed values.

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APPENDIX

Electronic Appendix: Photographic documentation of the studied sites

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SPATIAL PATTERNS OF TREE SPECIES NUMBER IN RELATIONSHIP WITH THE LOCAL ENVIRONMENTAL VARIATIONS IN KARST ECOSYSTEM

TOURE, D.[1*] – GE, J.[1] – ZHOU, J.[2]

[1]*Institute of Ecology and Environmental Sciences, School of Environmental Studies, China University of Geosciences (Wuhan)*
Hubei, 388 Lumo Lu, 430074, P.R China
(phone: +86-1597-299-3853)

[2]*Faculty of Environmental Geology, School of Environmental Studies, China University of Geosciences, Wuhan, Hubei, P.R. China*

**Corresponding author*
e-mail: touredado@yahoo.fr

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Abstract. Karsts are recognized as fragile environments where, forest degradation is faster and the composition and growth rate of vegetation is considered as a product of the interactions between karst ecological factors. A representative sample of vegetation plots from a systematic sampling was analyzed in Longhushan, southwest China. The sampling combined measurements of species number with extensive information on geological, environmental, and soil factors to establish species response to the single or combined influence of these factors. Both General and Generalized Linear Models predicted richness as a function of the combination of variables from the three groups. Richness increased with elevation, slope, moisture, and in dolomite dominated areas, but decreased with organic matter. Patterns of richness were dependent on complex relationships between soil, geological, and environmental factors, within this complexity it appeared that rock type played an important role. Evidence of significant variations was provided after combining variables from the three groups, suggesting their interactions influence on plants. Our findings have implications for the understanding of these interactions and suggest that plant species can be affected by it, and also carbonate rock type may be an important parameter to consider while examining relationships between plant and environmental factors in karst areas.

Keywords: *trends in plant species richness, environmental habitat factors, interactions, karst forest*

Introduction

Plant communities and biodiversity are believed to have a high degree of spatial variability that is controlled by both abiotic and biotic factors. Many types of environmental changes may influence the processes that can both augment or erode diversity (Sagar et al., 2003). The understanding of patterns and processes of biological diversity in space is a fundamental problem in ecology and conservation (Rosenzweig, 1995; Moritz, 2002), given the increasing rates of species extinction and human alteration of natural habitats.

Richness, the number of species per unit area, is a simple and easily interpretable indicator of biological diversity (Peet, 1974). Plant richness is said to be likely governed by two or more environmental factors (Margules et al., 1987; Pausas, 1994; Austin et al., 1996) and variations in richness are often linked to various environmental gradients (Wang et al., 2002; Sharma et al., 2009). For instance, elevation gradients are one of the most commonly discussed determinative factors in shaping the spatial patterns of

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species richness (Lomolino, 2001; Chawla et al., 2008; Acharya et al., 2011). Studies on the variations in species richness along elevation gradients have resulted in at least five patterns and all trends have been discussed in relation to different environmental variables (Körner, 2002; Grytnes, 2003b; Rahbek, 2005). Topography is correlated with other important environmental variables including the physical and chemical properties of the soils. Topography has also an important role in controlling the distribution of light, heat, moisture, and the strength and frequency of disturbance (McDonald et al., 1996; Shen et al., 2000). However, most researches have mainly focused on the relationships between soil and plants or plants and topography (Wu et al., 2001; Gong et al., 2007; Yue et al., 2008), while further studies of their interrelationships is required (Liu et al., 2003; Ye et al., 2004).

The geology is also an important factor to consider when analyzing the spatial patterns of plant species. The influence of geology can be split into the direct influence of rock type itself (chemistry and physical structure), and the indirect role that it plays in soil formation as well as the development of structures that influence the distribution of plants at a range of scales (Cottle, 2004). Studies have shown associations between geological substrates and tree species distribution and community composition (Reiners, 2002; Tuomisto et al., 2003; Phillips et al., 2003; terSteege et al., 2006; Fayolle et al., 2012). Yet, there are limited examples showing the significance of the direct influence of rock type (its chemistry) on plant distribution (Cottle, 2004).

Properties of bedrock, soil, and topography are thus interrelated and associated with plant species, but the problem is to define what this association is and at what level. Examining simultaneously these factors could improve our understanding of the spatial patterns of plant species in a particular geological environment like karst mountains. Karst ecosystem, described as the ecosystem that is restrained by karst environment (Yuan, 2001), especially by karst geological setting (Cao et al., 2003), is recognized as a highly complex interactive system which incorporates component landforms, life, energy flows, water, gases, soils and bedrock. Perturbation of any one of these elements is likely to impact upon the others (Yuan, 1988; Eberhard, 1994). Recognition and understanding of the importance and vulnerability of this dynamic interaction must underpin the effective management and conservation of karst forest resources.

This research examines patterns in tree species richness and their ecological correlates in the karst forest of Longhushan Nature Reserve (LNR), southwest China (SW China). Located at the northern edge of tropical Asia, the karst landscape of SW China is one of the most typical landscapes developed on limestone in the world (Yuan, 1993; Liu, 2009). These mountains have unique types of vegetation (Zhang et. al., 2010) and evolved into a cluster of distinctive mini-hotspots. However, plant diversity is threatened by rapidly changing land use patterns in tropical Asia (Sodhi et al., 2010), where forests are becoming increasingly disturbed and fragmented (Sodhi et al., 2004; Laurance, 2007), so it is said that more effort should be made to document biodiversity in the region (Webb et al., 2010).

Due to the rapid increase of population and related excessive exploitation of the region’s natural resources (habitat destruction, farming, logging, infrastructures, fuel wood, tourism, deforestation), the karst lands of SW China are faced with serious degradation sequences. The anthropogenic influence combined with the ecological fragility of the karst system often result not only in the loss of biological diversity, but also in forest degradation to shrubs, grasses, or even to rock desertification in some areas, making ecological restoration and reconstruction imperative (Wu et al., 2008;

Song et al., 2008). LNR has the advantage of relatively well established vegetation even retaining the arbor layer, but also under significant anthropogenic influences which may exceed its environmental carrying capacity. The area is hence an ideal research habitat to study plant species, the range of environmental conditions in which they develop, and species patterns in such ecosystem.

The main purpose is to explore patterns in tree species richness and their relationships with the interactions among environmental habitat factors. The study intends to examine: 1) dominant species in plant communities; 2) rock type, soil characteristics, environmental factors, and their single effects on richness; 3) species richness response to the joint effects of rock type, soil and environmental factors; and 4) the dominant species distribution along significant environmental variables. These informations could be useful in the development of management, conservation, and restoration strategies of forest ecology and biodiversity in karst regions.

Materials and methods

Site location and description

The study was carried out in LNR located in the subtropical area of southern China at the northern edge of tropical Asia, Nanning city, Guangxi Zhuang Autonomous Region (*Figure 1*). LNR covers an area of 2255.7 hectares and is bounded between latitudes 22°56′ to 23°00′N and longitudes 107°27′ to 107°41′E. The area has a monsoonal climate characteristic of the subtropical zone and is influenced by the regulation of a maritime climate. The annual average temperature is 21.8C°, with the annual average precipitation of 1500 mm which is mostly centralized in summer. The reserve belongs to Guangxi which is recognized to have one of the key forest areas in southern China with high plant biodiversity. However, aside from being divided by a highway, the status of the area is that of a nature reserve and tourist attraction. Longhushan is also a primate reserve with an increasing impact from both primate population density and anthropogenic impacts through agriculture, facilities and infrastructure developed for tourism.

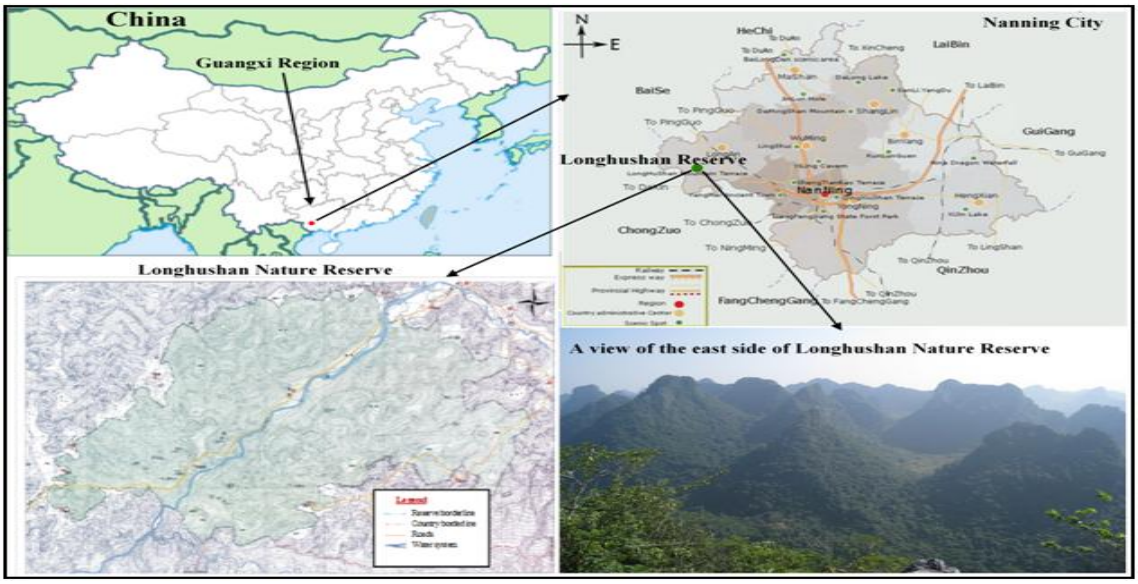

Figure 1. *The location and presentation of LNR*

Sampling design and analysis

A representative sample of vegetation plots was analyzed from a systematic sampling implemented in the reserve, using 30 meters square quadrats randomly located along four south-north transects lines. The sampling combined measurements of plant species number per quadrat, with extensive information on rock type (RT), soil and other environmental variables, allowing for the direct consideration of the interactions between different environmental habitat factors. Rock and soil samples were collected from 17 quadrats, in addition to several environmental variables including elevation (E), slope degree (Sd), canopy cover (CC), soil depth (SDp), ground temperature (GT), and ground cover (GC). Rock samples were collected from the outcrops on the surface of each quadrat, while soil samples were collected from the topsoil layers after the removal of leaf litter.

The basement material of karst is carbonate rock compose of limestone (mainly composed of calcite – $CaCO3$), and dolostone (composed of dolomite – $CaMg (CO3)2$). Rock samples were analyzed by a staining method for the percentage content of calcite and dolomite, using alizarin-red test (Friedman, 1959; Warne, 1962). Soil samples were examined for some major characteristics that have influence on other properties and nutrients availability, including texture (ST), moisture (M), pH, and organic matter (OM) content. Soil M was analyzed using the standard gravimetric method (oven-dry method), while pH was obtained using the soil-water suspension method. The classic rapid dichromate oxidation technique was used to determine soil organic carbon (Nielson and Sommers, 1982). Soil type was examined using the USDA method to determine soil textural classes based on percentage content of sand, silt, and clay.

Statistical analysis

Statistical analysis was conducted at 95% confidence interval ($p<0.05$ CI) using SPSS 19 software. Data were divided into four groups including one group of response variable (species richness or plant index), and three groups of predictors including soil characteristics (SDp, ST, M, pH, OM), geological factor (RT), and environmental factors (E, Sd, CC, GC, GT). Pearson’s simple correlation tested the bivariate correlations between each predictor and richness as well as the correlations among the predictors. Analysis of variance (ANOVA) was applied to examine the effects of RT or ST (categorical variables) on plants by comparing the average species richness between different RT and ST levels. ANCOVA examined the influence of ST while controlling the effects of covariates from soil or environmental factors. Furthermore, predictors from all three groups were tested separately and then in combinations in four multiple linear regressions (MLR) models to find the best fit for richness. Models 1, 2, and 3 were designed to test the single effect of each group, while model 4 was fitted with combinations of variables selected from all three groups to predict richness. The equation to calculate the predicted value of richness for the significant model was established following the general form of the MLR function below.

$$Y = A + B1X1 + B2X2 + \cdots BkXk$$

Where Y is the response value of richness, A is the y-intercept, B_1 to B_k represent the regression coefficients, X_1 to X_k are the different predictor variables.

Moreover, as the effects of environmental variables on plant species may not be linear in nature, the generalized linear model (GzLM) expands the General Linear Model (GLM) so that richness is linearly related to the predictors via a specified link function, and also allows richness to have a non-normal distribution. Species richness being a count variable, Poisson model was selected as a reasonable candidate response distribution with the natural log as the link function. Predictors from all three groups of factors were tested separately and then collectively in several combinations (within and between groups) including and excluding different variables. Based on their goodness of fit (GOF) test statistics, four models were designed to show the effects of the interactions between variables from the three groups. As for the MLR analysis, models 1, 2, and 3 were designed to test richness as a function of the single groups of soil, geological, or environmental factors, while model 4 was fitted with combinations of variables from the three groups to predict richness. The predicted value of richness for the significant model was calculated following the general form of the Poisson regression (PR) function, where the natural log of richness is predicted with a linear combination of the predictor variables.

$$Log\ (richness) = Intercept + B1X1 + B2X2 + \cdots BnXn$$

$$Richness = (e)^{Intercept} \times (e)^{B1X1} \times (e)^{B2X2} \times \ldots\ (e)^{BnXn}$$

Where B_1, B_2... B_n represent the regression coefficients and X_1, X_2 ... X_n the different environmental variables used as predictors in the models.

Results

Characteristics of plant communities, soil and geological factors of the study area

Table 1 presents the basic descriptive statistics of all variables examined. Dolomite dominated in 11 samples varying from 70 to 98%, representing about 65% of the studied plots, from which more than 90% has dolomite content ≥90%. Seventy five percent (75%) of the studied area was dominated by fine and medium textured soil, while 25% was found with coarse and moderately coarse soils. Soil pH was moderately acidic in two plots (5.25 and 5.71) representing 11.76%, while over 88% of the surveyed area was found with pH ranging between near neutral to moderately alkaline (6.66-7.91). This agrees with the results of previous studies (Liu, *et. al.,* 2006; Hu, *et. al.,* 2009) in another southwest China karst area (Guizhou province). OM content interpreted according to Hartz, (2007) showed that nearly 53% of the study site was found with high OM content (>5%) and 47% with low OM content (<5%). Considering the moisture interpretation chart of Harris and Coppock, (1991), about 88.23% of the sampled plots has insufficient available moisture (50% or less), whereas only 11.76% has sufficient available moisture (50 to 75%). Plant communities were generally evergreen with delimitation between arbor layers, shrubs and grasses. A total of 59 species were recorded across the 17 quadrats from which *Sterculia nobilis*, *Ficus sp.*, *Albizia chinensis*, *Liquidambar formosana*, *Teonongia tonkinensis*, and *Bischofia javanica* were the most dominant

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based on their importance value indices (*Figure 2*). Among them *Sterculia nobilis* was by far the most abundant representing about 14% of the total importance value.

Table 1. *Descriptive statistics of all variables of interest from the four groups of factors (N=17)*

Groups of Factors	Factors	Min	Max	Mean	Std. Deviation	Type of Variables
Plant index	Species richness	3	12	7.18	3.11	Response
Soil characteristics	Organic matter content (%)	2.35	12.51	7.11	3.32	Predictors
	Soil depth (cm)	3	100	34.29	36.71	
	pH	5.25	7.91	7.30	0.79	
	Moisture (%)	14.14	57.49	38.13	11.69	
	Soil texture	-	-	-	-	
Geological factor	Rock type	-	-	-	-	
Environmental factors	Ground temperature (°C)	24.00	29.00	26.99	1.42	
	Ground cover (%)	20	95	63.53	20.82	
	Canopy cover (%)	40	90	65.00	16.45	
	Slope degree (°)	5	60	23.71	16.72	
	Elevation (m)	109	243	150.82	35.16	

Rock type was coded as 1 = dolomite, 2 = calcite; Soil texture was coded as 1 = coarse textured soil, 2 = moderately coarse, 3 = medium, 4 = fine

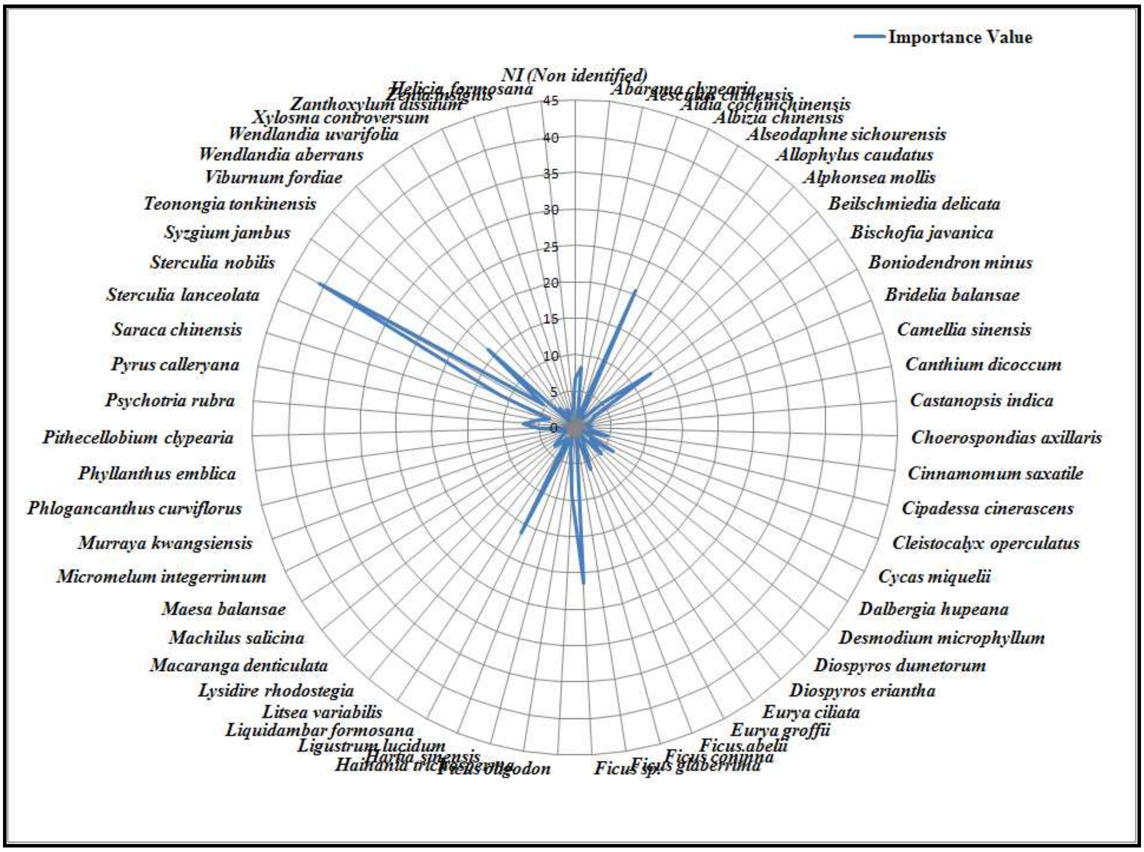

Figure 2. *Importance values of 59 plant species recorded in LNR*

Correlation, ANOVA and ANCOVA results

Correlation analysis indicated no significant association between richness and any predictor except Sd ($r=0.542$, $p<0.05$), while few inter-correlations were found among some of the predictors with the highest between M and OM ($r=0.760$, $p<0.01$), SDp and pH ($r=-0.661$, $p<0.01$). However, no correlation coefficient was equal or greater than 0.8 to fear for serious multicollinearity problem. ANOVA also found no significant changes in richness related to ST or RT, as the null-hypotheses were not rejected. With ANCOVA a fairly significant association was found when the joint effects of ST and RT were examined after controlling the effects of M and GC. ST was found significantly related to richness ($F=4.228$, $p=0.040$) accounting for 58% of its variance (Partial $Eta^2=0.585$), with near 67% chance of finding a significant difference in our case (observed power=0.667). The highest impact was from medium textured soils which also registered the highest mean species richness, followed by moderately coarse, coarse, and fine soils.

MLR models for richness response to the single or combined effects of geological, soil, and environmental factors

Although model 2 was found marginally significant ($F=2.860$, $p=0.068$), MLR analyses (*Table 2*) showed that the models (1, 2, 3) designed with single groups of factors still failed to explain significant variations in richness, since in all three cases none of the null-hypotheses (F-ratio) was rejected ($p>0.05$). In contrast, model 4 fitted with combinations of variables from the three groups produced a correlation coefficient ($R=0.956$) which suggests at first, the existence of strong association between the five predictors and richness. Furthermore, unlike the previous models, model 4 explained significant variation in richness ($F=23.318$, $p<0.001$), accounting for 87.5% of its variance (adj. $R^2 = 0.875$). Compared to models 1, 2, and 3, the amount of variation accounted for in the response greatly increased by over 76%, 100% and 50%, respectively. Moreover, except OM found with a marginal effect ($t=-2.100$, $p=0.060$), each predictor significantly explained richness ($p<0.05$) which increased with E, Sd, M, and in dolomite dominated areas. The t-tests show that RT ($t = -7.083$, $p < 0.001$) and Sd ($t = 7.105$, $p < 0.001$) were the greatest contributors followed by M and E, while OM was found with no significant effect. *Figure 3* presents the partial plots of linear relationship between each significant regressor and richness after controlling the effects of the other variables. The plots confirm that RT and Sd have the greatest impacts as each accounted for about 82% of the variance (R^2 Linear) (*Figure 3-b,d*), while E and M explained 51% and 57%, respectively (*Figure 3-a,c*). The model residuals for regression assumptions of homoscedasticity and normality are presented in *Figure 4*. Based on the unstandardized coefficients (B) values, the relationship between model 4 and richness can be expressed by the following MLR equation.

$$\begin{aligned} Richness = 1.832 &- (4.258\ Rock\ type) + (0.033\ Elevation) \\ &+ (0.125\ Slope\ degree) + (0.138\ Moisture) \\ &- (0.300\ Organic\ matter) \end{aligned}$$

Table 2. *MLR between richness as response and a) the single groups of rock type, soil, or environmental factors as predictors (models 1, 2, 3) before combining variables from the three groups, b) their joint effects as predictors (model 4) after combining variables from all three groups (95% CI; N=17)*

Models	R	R^2	Adj. R^2	F-ratio	P	Predictors	B	t	p
1	.411	.169	.113	3.044	.101	Constant	10.682	5.013	.000
						Rock type	-2.591	-1.745	.101
2	.376	.141	-.145	.494	.741	Constant	6.546	.573	.578
						Soil depth	-.017	-.524	.610
						Moisture	.129	1.071	.305
						PH	-.196	-.128	.900
						Organic matter	-.322	-.790	.445
3	.752	.565	.368	2.860	.068	Constant	-2.110	-.169	.869
						Elevation	.057	2.570	.026
						Slope degree	.097	2.414	.034
						Canopy Cover	-.081	-1.701	.117
						Ground Cover	.022	0.621	.547
						Ground Temperature	.082	0.165	.872
4	.956	.914	.875	23.318	.000	Constant	1.832	1.209	.252
						Rock type	-4.258	-7.083	.000
						Elevation	.033	3.367	.006
						Slope degree	.125	7.105	.000
						Moisture	.138	3.816	.003
						Organic Matter	-.300	-2.100	.060

R^2 and adjusted R^2 (adj. R^2) both indicate the proportion of variance in richness explained by the models. The value of adj. R^2 is reported to avoid over-estimation of the success of the models. Adj. R^2 is more restrictive and takes into consideration the number of predictor variables, and also the number of observations the model is based on.

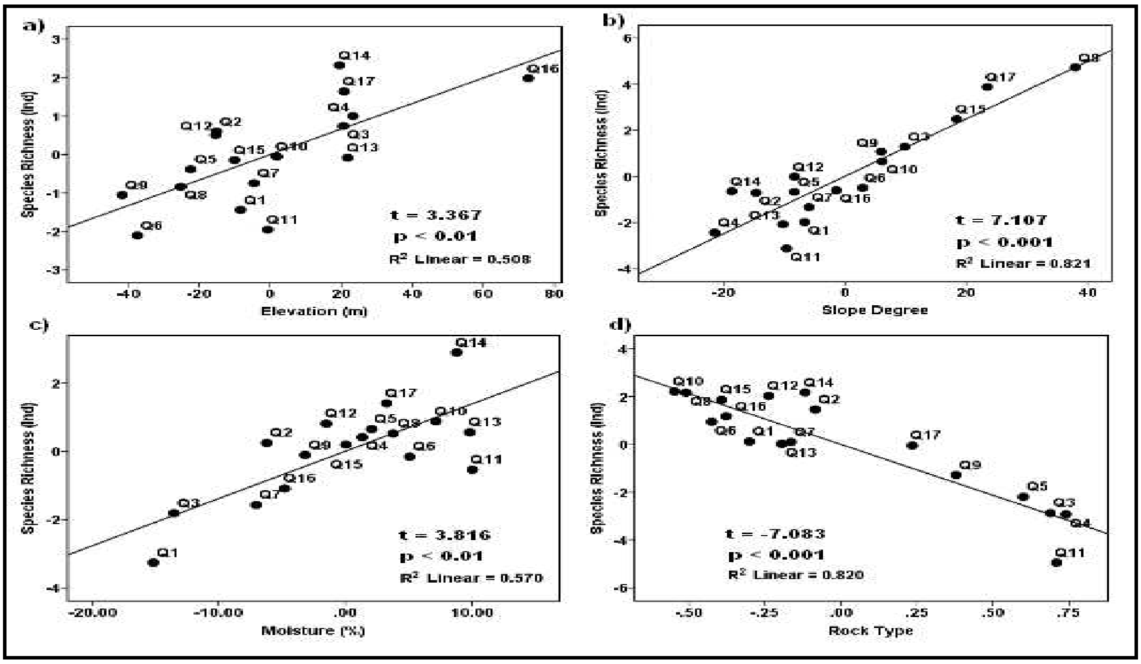

Figure 3. *Partial regression plots displaying linear correlations between richness and the significant factors in MLR analysis. R^2 Linear values indicate the amount of variation explained by each variable while controlling the effects of the other predictors*

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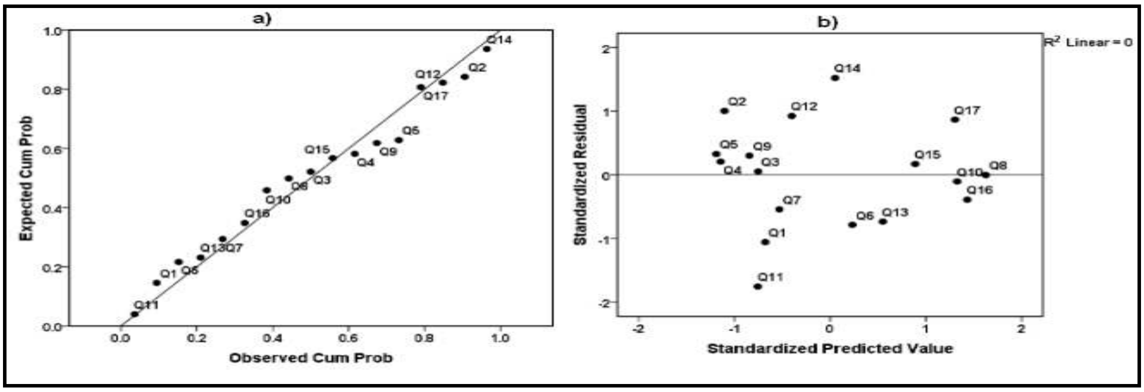

Figure 4. MLR model 4 residuals' plots in predicting richness for regression assumptions of a) Normality: Normal P-P plot of regression standardized residuals vs. standardized predicted values; b) Constant variance: scatterplot of standardized residuals versus standardized predicted values

GzLM Poisson regressions for richness response to the single or combined effects of geological, soil and environmental factors

The conditional variance of richness was a little higher than its mean, but the difference was not significant. In fact, Negative binomial regression (NBR) model was also applied to see if it gives any improvement over the PR. However, the values of the log-likelihood for the NBR were lower than those of PR, indicating that NB does not improve the models and that the Poisson model form was a better fit. Furthermore, the PR analysis presented no indication of model inadequacy, as the GOF statistics (Deviance, Pearson X^2) were found insignificant ($p>0.05$) for all four models (*Table 3*), suggesting that this model form fits the data reasonably well. Nevertheless, the likelihood-ratio chi-square (LR X^2) of the omnibus tests which compares the fitted model against the intercept-only model (null-model) was insignificant for models 1, 2, and 3. This suggests that there was no improvement over the null-model when examining richness as a function of the single groups of soil, geological, or environmental variables.

In contrast to the first three models, model 4 examined richness as a function of the combination of variables selected from the three groups (RT, E, Sd, M, OM), and the LR X^2 suggests that the fitted model outperforms the null model (LR X^2=91.423, $p<0.001$). Compared to the models designed with single groups, the values of deviance, Pearson chi-square, and AIC were smaller, while the log-likelihood was higher indicating that model 4 was a better fit. Additionally, the p-values for the coefficients and Wald X^2 of the tests of model effects and parameter estimates show that each of the main terms significantly contributed to richness (*Table 4*), except OM which significance was marginal ($p=0.071$). There was a positive trend of richness with E, Sd, M, and in dolomite dominated areas, but a negative trend with OM. According to the values of Exp(B), the incidence rate (IR) for dolomite areas was 1.837 times that of limestone areas. The percent change in the IR of richness was an increase of 0.4% for each meter increase in E, 1.6% for a degree increase in slope, 2% for every percent increase in M, but a decrease of about 4% for every percent increase in OM, while holding the other variables at constant. The plot of deviance residuals against the estimated linear predictors for model 4 is presented in *Figure 5*. Thus, based on the estimated coefficients (B) values, species richness as a function of soil, geological, and environmental variables, can be expressed by the following PR equation.

$$\begin{aligned} Log\ (Richness) &= 0.011 + 0.608\ Dolomite + 0.004\ Elevation + 0.016\ Slope\ degree \\ &+ 0.020\ Moisture - 0.044\ Organic\ matter \end{aligned}$$

$$\begin{aligned} Richness = (e)^{0.011} \times (e)^{0.608\ Dolomite} \times (e)^{0.004\ Elevation} \times (e)^{0.016\ Slope\ degree} \\ \times (e)^{0.020\ Moisture} \times (e)^{-0.044\ Organic\ matter} \end{aligned}$$

Table 3. *Poisson GOF tests between richness as response and the single group of geological, environmental or soil factors as predictors (model 1, 2, 3) before combining variables from all groups, and their joint effects as predictors (model 4) after combining variables from all groups (95% CI; N=17)*

Model	Predictors	Tests	Value	Df	P
1	Rock type	Deviance	17.716	15	0.278
		Pearson X^2	18.986	15	0.214
		Log Likelihood	-40.683	-	-
		AIC	85.367	-	-
		LR X^2 (Omnibus Test)	2.993	1	0.084
2	Elevation Slope degree Ground cover Ground temperature	Deviance	12.251	12	0.426
		Pearson X^2	11.822	12	0.460
		Log Likelihood	-37.951	-	-
		AIC	85.901	-	-
		LR X^2 (Omnibus Test)	9.394	4	0.052
3	Soil depth Moisture pH Organic matter	Deviance	18.359	12	0.105
		Pearson X^2	17.953	12	0.117
		Log Likelihood	-41.005	-	-
		AIC	92.010	-	-
		LR X^2 (Omnibus Test)	2.102	4	0.717
4	Rock type Elevation Slope degree Moisture Organic matter	Deviance	2.366	11	0.997
		Pearson X^2	2.303	11	0.997
		Log Likelihood	-33.009	-	-
		AIC	78.017	-	-
		LR X^2 (Omnibus Test)	91.423	5	0.000

AIC: Akaike's Information Criterion; LR X^2: Likelihood ratio chi-square; Df: degree of freedom; P: Significance p-values

Table 4. *Poisson regression model 4 tests of model effects and parameter estimates in predicting richness*

Model	Parameters	Coefficients (B)	Wald X^2 (Df=1)	Sig. P	Exp (B)
4	Intercept	0.011	0.002	0.964	1.011
	Rock type = 1	0.608	36.017	0.000	1.837
	Rock type = 2	0	-	-	1
	Elevation	0.004	9.410	0.002	1.004
	Slope degree	0.016	41.005	0.000	1.016
	Moisture	0.020	10.534	0.001	1.020
	Organic matter	-0.044	3.268	0.071	0.957

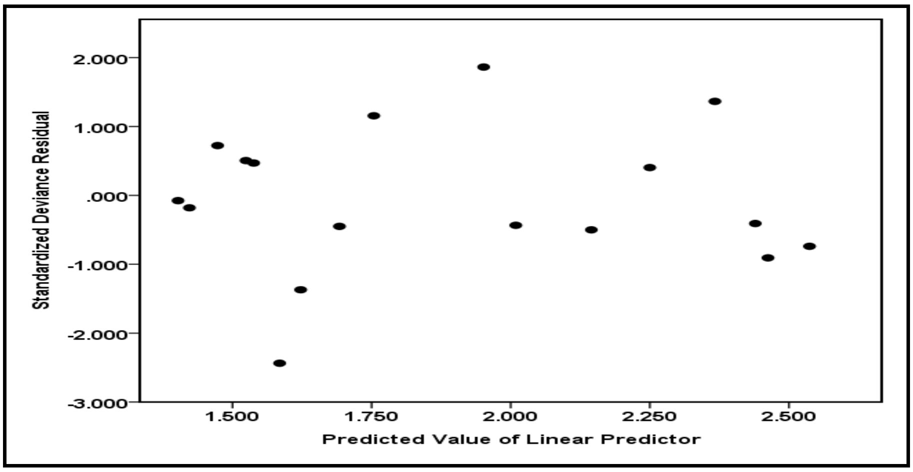

Figure 5. *Poisson regression model 4 plot of standardized deviance residuals versus the predicted values of linear predictors*

Discussion

Analysis of variables related to environmental and geological factors revealed some of their interactions with Longhushan soils upon which plant species depend. With correlation and ANOVA procedures we assumed there was insufficient evidence to establish associations between richness and the predictors. ANCOVA established a relationship between richness and ST confirming the findings of Sala et al., (1997) and that ST, RT, M, and GC have some interactions feedbacks on richness. The association between ST and plant richness may be explained by the fact that soil texture affects soil behavior particularly its retention capacity for nutrients and water (Brown, 2003), which availability have significant feedback on plant composition, distribution, and performance. Water availability is also reported as one of the most important environmental parameters controlling plant richness (Lavers and Field, 2006), and it is said to be even more profound in environments where soil moisture is a major limiting resource like karst areas.

Furthermore, GLM and GzLM methods both failed to prove that the soils, geological, or environmental variables examined separately, have significant effects on richness. In contrast, strong associations were observed when variables from the three groups were combined to test their joint effects. In both methods, the combination of RT, E, Sd, M, and OM was found as the best fit for richness, which increased with M, Sd, E, and in dolomite areas, but decreased with OM content. The results thereby, revealed species' associations with rock type, water and nutrient availability combined with topographic factors, supporting the conclusions of other researches (Pausas and Carreras, 1995; Leathwick et al., 1998; Clark et al., 1998; Hawkins et al., 2003; Cannon and Leighton, 2004; Valencia et al., 2004; Engelbrecht, 2007; Fayolle et al., 2012). They contrasted however, with studies which found a decreasing trend in species richness with altitude (Pausas, 1994; ReyBenayas, 1995; Odland and Birks, 1999). Nonetheless, the length of elevation gradient of our surveyed plots varied from 109 to 243 m, further studies that cover higher elevations perhaps could help confirm the exact trend. Moreover, in both methods, OM (nutrients store for plants) contributed less to the fit of the regression equations. Yet, there was some trend (its effect was found marginally significant)

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and its presence in the models was essential for the predictability of richness, suggesting that OM content in soil should be considered when examining the relationships between environmental factors and plants in karst habitats.

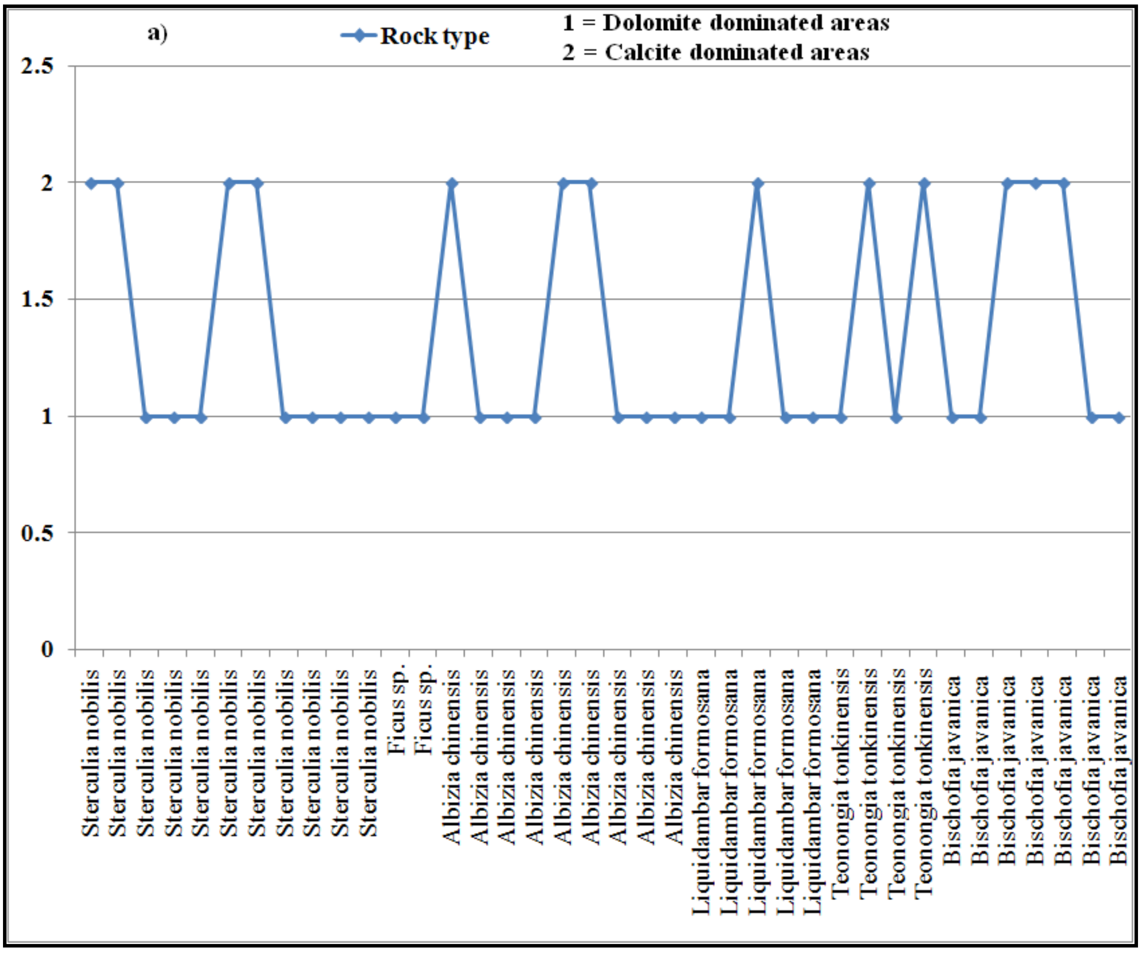
A particularity of these findings is that rock type was found among the most significant environmental factors that explained richness, and this agreed with other studies (Pausas and Carreras, 1995; Fayolle et al., 2012). In this case there was significant difference in richness between dolomite and calcite areas, suggesting that carbonate rock type may be a key factor in karst habitats as it was found with a strong effect, and its inclusion in the models was determinant in predicting richness. There was positive trend of richness in high dolomite percentage areas but the inverse trend in calcite dominated areas. We may postulate for instance that since the predictable variation in species richness is important in determining areas of conservation, this geological factor is an indicator of high species richness areas in the Longhushan Karst Mountains, which could be used for assigning priority sites for conservation or restoration.

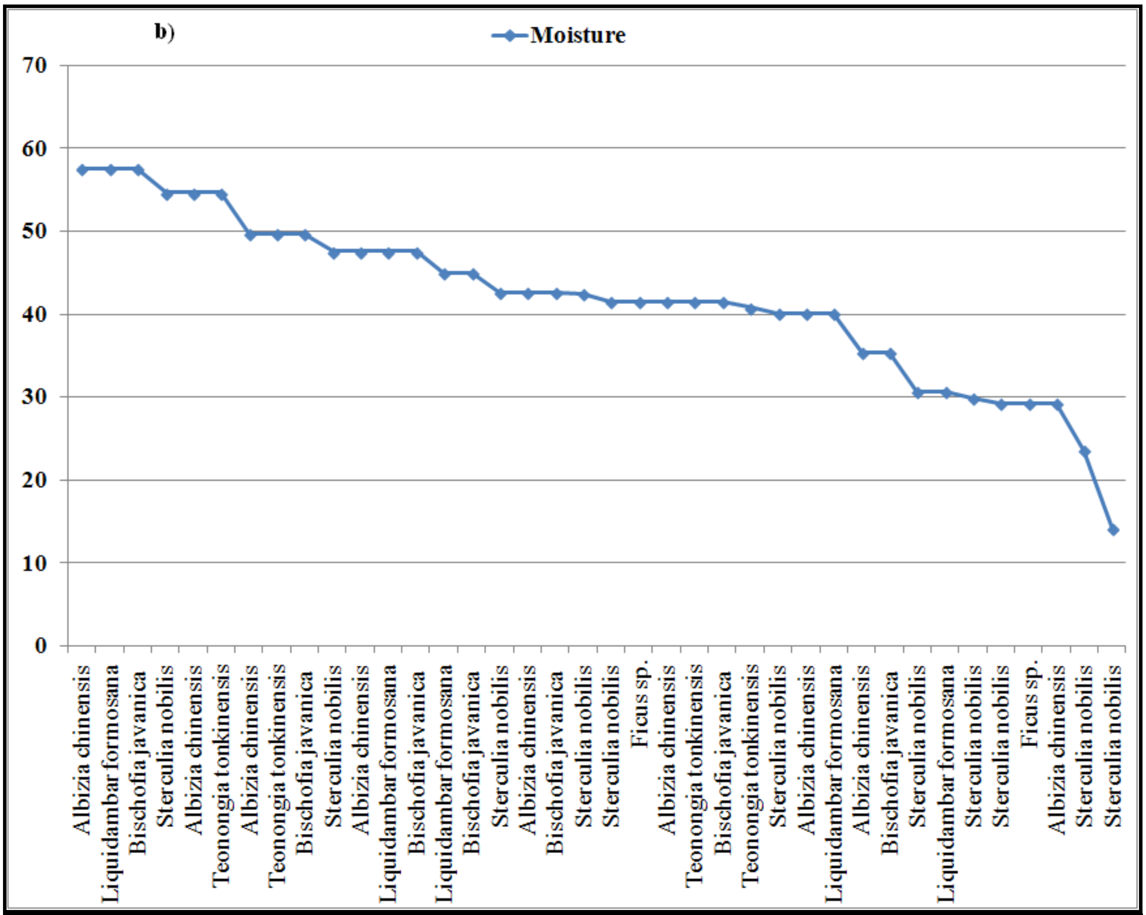
Another particularity is that OM content was negatively associated with plant richness. The negative trend could be related to a slow decomposition of OM resulting in its accumulation and the tie up of nutrients that are held in it. As reported by Foth, (2006), high organic matter contents in soils are the result of slow decomposition rates rather than high rates of organic matter addition. The ability of species to perform at different resource availability levels was discussed by Grime, (1973, 1977) who reported that few species can tolerate the stress characteristic of low-resource habitats. As productivity increases, more resources become available, thereby relaxing the abiotic stress and allowing the establishment and growth of more species.

The slow decomposition of OM could be due to several factors, as the rate of decomposition is mainly dependent on the abundance of soil microbes (e.g. bacteria, fungi), the substrate quality (nutrient content: C/N ratios, OM composition, etc), and soil environmental conditions (pH, moisture, texture, temperature, etc). The analysis showed that over 88% of the sampled plots have insufficient available moisture, while soil biological activity requires sufficient air and moisture. In fact, it is said that optimal microbial activity occurs at near “field capacity”, which is equivalent to 60% water-filled pore space (Linn and Doran, 1984). In addition to the insufficient moisture content, the results also showed that about 75% of the sampled plots was dominated by fine and medium textured soil indicating high clay content, while clay particles are believed to protect some of the more easily decomposable organic compounds from rapid microbial breakdown through encrustation and entrapment (Paul and vanVeen, 1978; Anderson, 1979; Tisdall and Oades, 1982). Therefore, although the two factors (moisture and texture) may not be totally enough to explain the negative association between plant species and OM content, but the lack of adequate soil moisture combined with the dominance of finer textured soil may have contributed to limited microbial metabolisms, resulting in the accumulation of organic matter and the tied up of nutrients needed by the plant species to perform. This conclusion supports those of Woods and Raison, (1983) who suggested that moisture was a major factor in controlling OM decomposition, and Killham et al., (1993) reported that substrate utilization by microbes in soil is strongly affected by its location, both in terms of pore size and the matric water potential under which turnover takes place. Nevertheless, it should be noted that further studies in the reserve might help to further explain the different factors that affect OM decomposition in karst areas and their impact on plants. For instance, the microbial biomass, the substrate quality (OM composition, C:N ratios), and the anthropogenic disturbance on different ecological processes could represent interesting subjects for future studies to better understand the complexity of processes OM undergoes in order to establish suitable management strategies.

Distribution of dominant species along significant environmental gradients

Key species or function groups are known to play an important role in maintaining the whole karst ecosystem (Wang et al., 2002; Ackerly, 2004). *Figure 6* analyzes how the significant factors influenced the dominant species distribution in the reserve. The dominant species responded differently to the significant factors and a number of those species were found typically well adapted to the special karst environment. Among them *Ficus sp.* was only adapted in high dolomite percentage areas, while the remaining species were all apt to survive in both habitats (dolomite or calcite) (*Figure 6a*), confirming the fact that some limestone species conform well to a calcium rich environment (Mao and Zhang, 1987; Xu, 1993). Most dominant species were also suitable for habitats with sufficient and insufficient moisture conditions, except *Ficus sp.* recorded only in low moisture habitats (*Figure 6b*), suggesting that this species was adapted to extreme drought situation. *Sterculia nobilis, Teonongia tonkinensis,* and *Albizia chinensis* were susceptible to live at both slight and steep slopes (*Figure 6c*), and also at all elevations (*Figure 6d*).




b)
Moisture
70
60
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40
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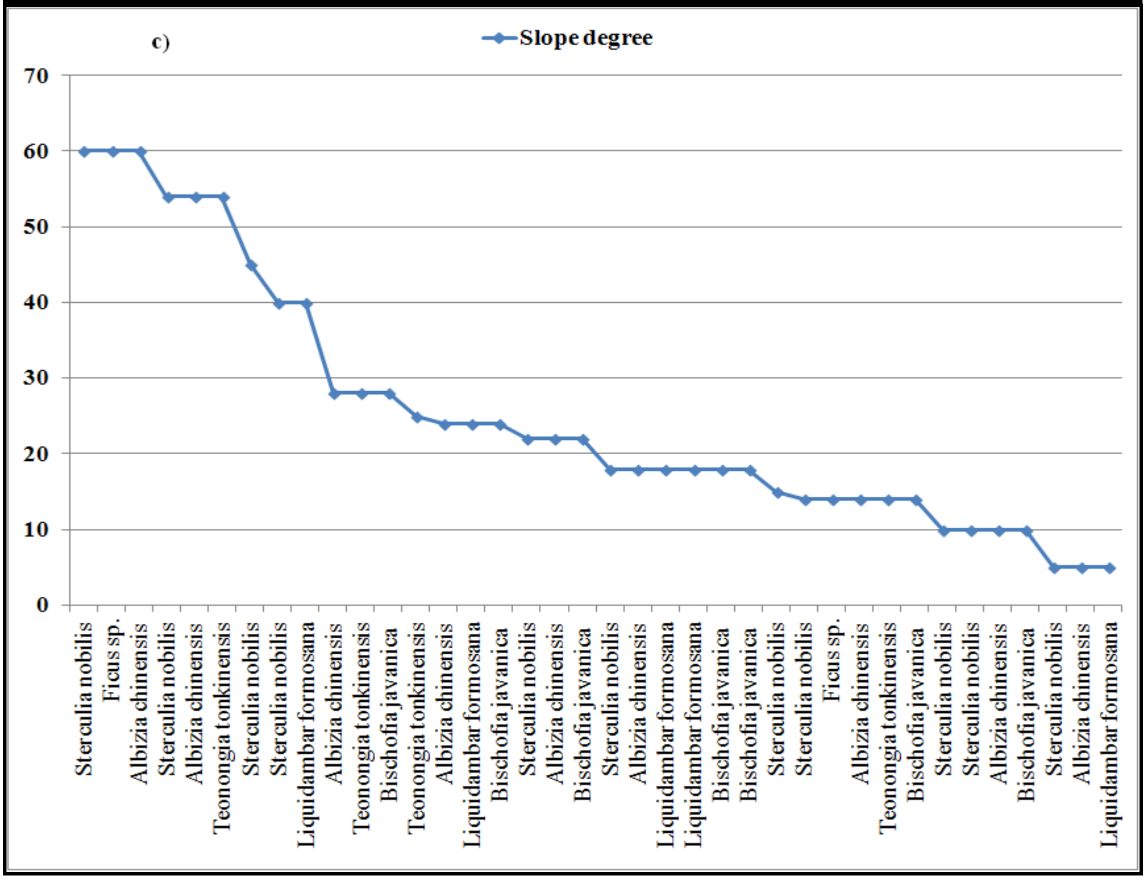
20
10
0
Albizia chinensis
Liquidambar formosana
Bischofia javanica
Sterculia nobilis
Albizia chinensis
Teonongia tonkinensis
Albizia chinensis
Teonongia tonkinensis
Bischofia javanica
Sterculia nobilis
Albizia chinensis
Liquidambar formosana
Bischofia javanica
Liquidambar formosana
Bischofia javanica
Sterculia nobilis
Albizia chinensis
Bischofia javanica
Sterculia nobilis
Sterculia nobilis
Ficus sp.
Albizia chinensis
Teonongia tonkinensis
Bischofia javanica
Teonongia tonkinensis
Sterculia nobilis
Albizia chinensis
Liquidambar formosana
Albizia chinensis
Bischofia javanica
Sterculia nobilis
Liquidambar formosana
Sterculia nobilis
Sterculia nobilis
Ficus sp.
Albizia chinensis
Sterculia nobilis
Sterculia nobilis


c)
Slope degree
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Sterculia nobilis
Ficus sp.
Albizia chinensis
Sterculia nobilis
Albizia chinensis
Teonongia tonkinensis
Sterculia nobilis
Sterculia nobilis
Liquidambar formosana
Albizia chinensis
Teonongia tonkinensis
Bischofia javanica
Teonongia tonkinensis
Albizia chinensis
Liquidambar formosana
Bischofia javanica
Sterculia nobilis
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Albizia chinensis
Liquidambar formosana
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Sterculia nobilis
Ficus sp.
Albizia chinensis
Teonongia tonkinensis
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Liquidambar formosana

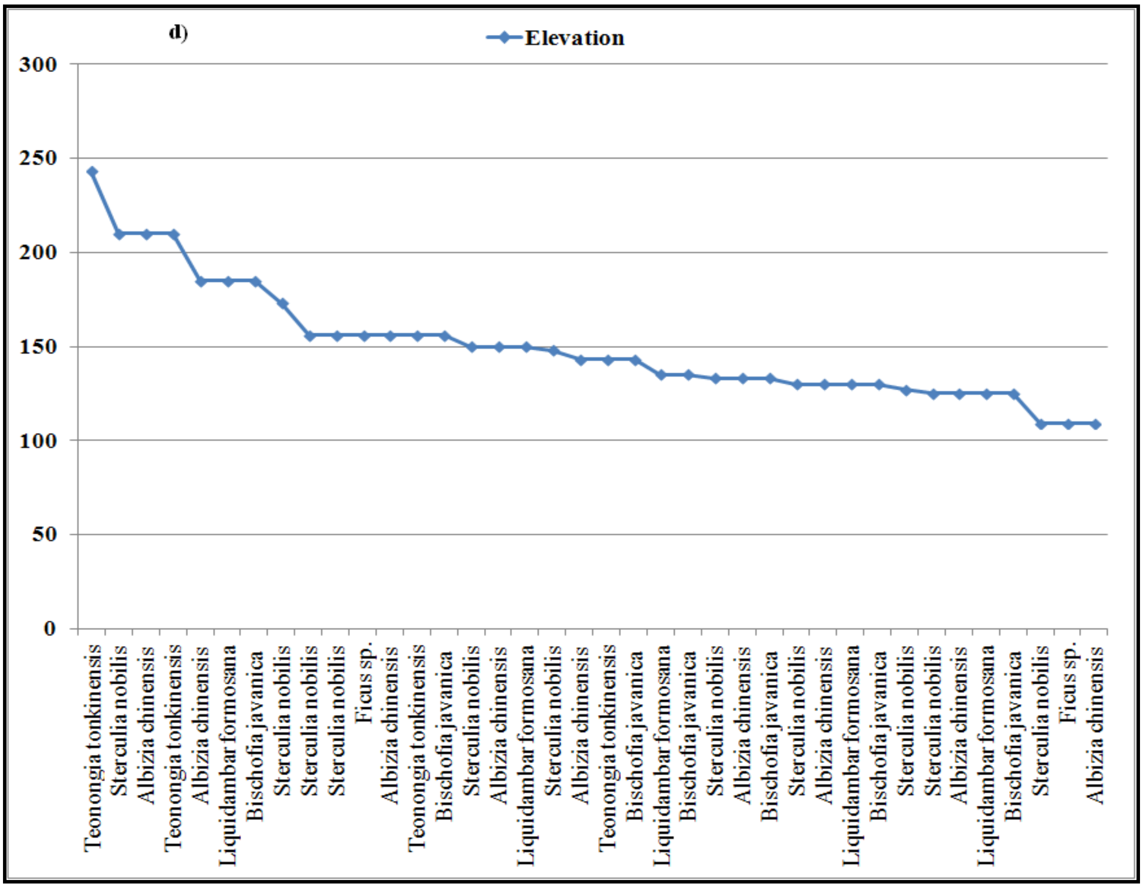

Figure 6. *Dominant species distribution along a) rock type, b) moisture, c) slope degree, and d) elevation, as significant environmental factors in LNR*

Ficus sp., *Liquidambar formosana*, and *Bischofia javanica* were suitable to live at low and middle elevations, while *Bischofia javanica* grew only at slight slopes. Each of these species performed well in their habitats suggesting they could be appropriate for the restoration of degraded parts of the mountains with similar conditions. For instance, the fragmented areas by tourist roads network, the denuded ground by primate damage, or the abandoned agricultural lands which are all exposed at risk of erosion. Based on the dominant species response to the significant environmental factors, *Sterculia nobilis* and *Albizia chinensis* appears to be the most appropriate species for restoration and forest amelioration measures, since they were suitable to all karst environmental habitats conditions.

Moreover, although found with the lowest IVs, several other species including Allophylus caudatus, Alphonsea mollis, Bridelia balansae, Canthium dicoccum, Eurya groffii, Hartia sinensis, Litsea variabilis, Murraya kwangsiensis, Cinnamomum saxatile, Diospyros eriantha, Ficus glaberrima, and Hainania trichosperma were recorded only at high elevations. These species might be endangered as most of them were found just once in the reserve or in one quadrat, thus need special protection for the conservation of biological diversity of the area. In fact, among them Canthium dicoccum is included in the UICN Red List for endangered species and Hainania trichosperma is an endemic species.

Conclusion

In LNR, variations in plant richness were dependent on complex relationships between soil, environmental, and geological factors of karst habitats. Within this complexity of relationships it appeared that the geological factor played an important role in the spatial patterns of plant species in the area. Patterns in richness were related to rock type, soil moisture, texture, and nutrient status combined with topographic factors. The strong association between tree species richness and rock type, on which soils develop with different properties and consequently different water and nutrient availability, suggests that soil resources availability is important for these patterns.

The results clearly demonstrated that the evidence of significant variation in richness was provided after combining variables from soil, geological, and environmental factors, suggesting their interactions influence on plants. Evidence supports these interactions and no single context explained the different associations between richness and any group of factors. The findings have implications for the understanding of these interactions and suggest that not only plant species can be affected by it, but also rock type may be an important parameter influencing the overall relationships between plant species and other environmental habitat factors in karst areas. Therefore, effective and efficient management of karst forest ecosystems requires an elaborate data set and understanding of all the components and physical features, as well as the complex links and interactions between them and plant communities, if species and their habitats are to be managed in a way that can sustain their diversity. This information can provide a reference for assigning priority sites for biodiversity conservation, prevention of rock desertification, and development of sustainable management, conservation, and restoration strategies of karst ecosystems.

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PLANT DIVERSITY AND STRUCTURAL DYNAMICS OF MANGROVES IN THE SOUTH WEST COAST OF KERALA, INDIA - APPENDIX (IMAGES)

SHAKTIKULANGARA






Ayiramthengu






AZHIKKAL

MUNROE ISLAND

ASRAAMAM

CHERIKADAVU

PLANT DIVERSITY AND STRUCTURAL DYNAMICS OF MANGROVES IN THE SOUTHWEST COAST OF KERALA, INDIA

VIJAYAN, V.* – RAHEES, N. –VIDYASAGARAN, K.

Department Of Forest Management and Utilization, College of Forestry,
Kerala Agricultural University,
Vellanikkara, Kerala – 680656, India

**Corresponding author*
e-mail: vishal00v@gmail.com

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Abstract. Mangroves are the plants adapted to strident environment with unvarying inundation of the soil and extremely diverse salinities, habitual in river banks, sand flats and coastlines of the tropics and subtropics of the world. Mangrove vegetation in six geographical regions of Kollam on the Southwest coast of India was selected to study the phytosociological characteristics and diversity indices. We analysed the pattern of mangrove species diversity, density and basal area and similarity parameters. Totally, 12 species of mangroves belonging to 8 genera and 6 families were identified; Rhizophoraceae and Avicenniaceae were the most dominant group represented. Highest density was recorded for *Avicennia officinalis* (3760stems/ha), followed by *Avicennia marina* (3721stems/ha). *Avicennia officinalis* is the most common mangrove counted with the highest Important value index (IVI) and Relative IVI; 64.19 and 21.40 respectively. Shannon- Weiner Index of diversity ranged from 2.002 to 3.11; species richness from 0.429 to 1.033 and the similarity in floral composition varied from 0.33 to 0.66. The sites selected were distinct from one another and the species diversity varied from one place to another; influenced by the climatic, biotic, anthropogenic stresses.

Keywords: *mangroves, floral diversity, Importance Value Index, Similarity Index, density*

Introduction

Mangrove ecosystems are found all over the world in tropical and subtropical regions along the land-sea interface, bays, estuaries, lagoons, backwaters and in rivers reaching upstream up where the water still remain saline (Qasim, 1998). Biodiversity is prevalent in the tropical estuaries, particularly in the intertidal dense vegetation known as mangroves (Mooney et al., 1995).The development and structure of mangrove forests result from an interaction of many physical factors and environmental variables (Hogarth, 2007; Lacerda et al., 2002). Mangrove plantations are one among the most productive ecosystem on this planet and serve as a nursery for marine and neptunian livelihood, they serve as guardian of their juvenile stock and form most valuable biomass (Odum, 1971).They all have usual assortment of adaptations that facilitate them to cope with regular tidal flooding, strong winds, waves and water currents, muddy soils, high levels of salts in the water, and oxygen deficient soils. In addition, they have an astonishing capability of carbon sequestration; suffice ability to serve as provenance and store sediments and nutrients for further near coastal marine habitats including coral reefs and seagrass beds (Polidoro et al., 2010). They are found between the Tropic of Cancer and the Tropic of Capricorn on all continents covering an estimated 75percent of the tropical coastline worldwide. The total area of terrestrial ecosystem in the world is occupied by only 0.2 percent mangrove lids. However, mangrove ecosystems hold a wide diversity of aquatic and terrestrial species of different taxonomic groups. When all species are considered, mangrove ecosystem rivals many

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other tropical habitats in alpha diversity (Peter, 1999). India has a total area of 4461 sq.km of mangroves; India is the fourth largest mangrove area in the world with 0.14% of country’s total geographical area (Naskar and Mandal, 1999).The extent of mangrove of Kerala is 2502 ha out of which 1189 ha belongs to the state and 1313 ha is under private ownership, but various studies showed that the vegetation of mangroves in Kerala covers only 1095 ha (Kurien, 1984), Early study reported that out of the total 1671 ha of mangroves in Kerala, 1470 ha are with private ownership (Basha, 1991). Increase in population density of the Kerala coast has resulted stupendous pressure on the natural ecosystem, partially on the mangroves.

Mangrove vegetation in Kollam occur along the banks of estuarine water bodies and adjacent to the back water channels, in the form of narrow continuous belt or patches. Total of 15 pure mangroves and 33 semi mangroves had been recorded from entire costal area of Kerala, Kollam district contribute maximum percentage of private forest (73.3%) and only 26.7% under state ownership (Vidyasagaran and Madhusoodanan, 2014). When compared to other districts, Kollam has the second highest mangrove diversity (11 out of 15 pure species). Mangrove ecology are discoursed with undivided attention globally, since because as the result of their economic values in boosting profitable fisheries alone (Jayadev, 2012). Mangroves of south coastal region of Kerala have indigenous characteristics compare to the mangrove ecosystem of other region. Therefore, this study was carried out to identify the present status of mangroves forest in Kollam through investigating the plant species composition and diversity. Nonetheless no literature exists on mangroves of Kollam in any aspects. Hence, this is an attempt made to unlock the peach in mangrove ecosystems of Kollam through scrutinizing structural dynamics and diversity, which might help to provide guidance for conservation forethoughts and scientific management of the locations.

Materials and Methods

Study Area

Kollam district encompasses with highest extent of mangroves among southern Kerala (Vidyasagaran and Madhusoodanan, 2014). To assess the location of structures that might influence the occurrence of mangroves and to recognize the extent of area covered by mangroves, each site was assessed and mapped using the Global Positioning System. All through the year humidity is higher during morning hours and during monsoon periods, Kollam receives an annual average rainfall of 2,555 millimeters and average temperature ranges 25° Celsius to 35° Celsius (Jayadev, 2012). The investigation was carried out among six stations: Ayiramthengu (N 09° 06’ 59.8” E 76° 28’ 52.5”), Azhikkal (N 08° 07’ 36.4” E 76° 28’ 14”), Asraamam (N 08° 53’ 41.4” E 76° 35’ 8.6”), Shaktikulangara (N 08° 56’ 23.44” E 76° 33’ 9.88’’), Cherikadavu (N 08° 59’ 47.2” E 76° 35’ 4.4”), Munroe Island (N 08° 58’ 57.5” E 76° 36’ 59.2’’) (*Fig. 1*). All these sites had a mangrove distributional area of two hectares and above. The sites were widely separated, had dissimilar sediment characteristics and were influenced highly by monsoon (Cintron et al., 1980).

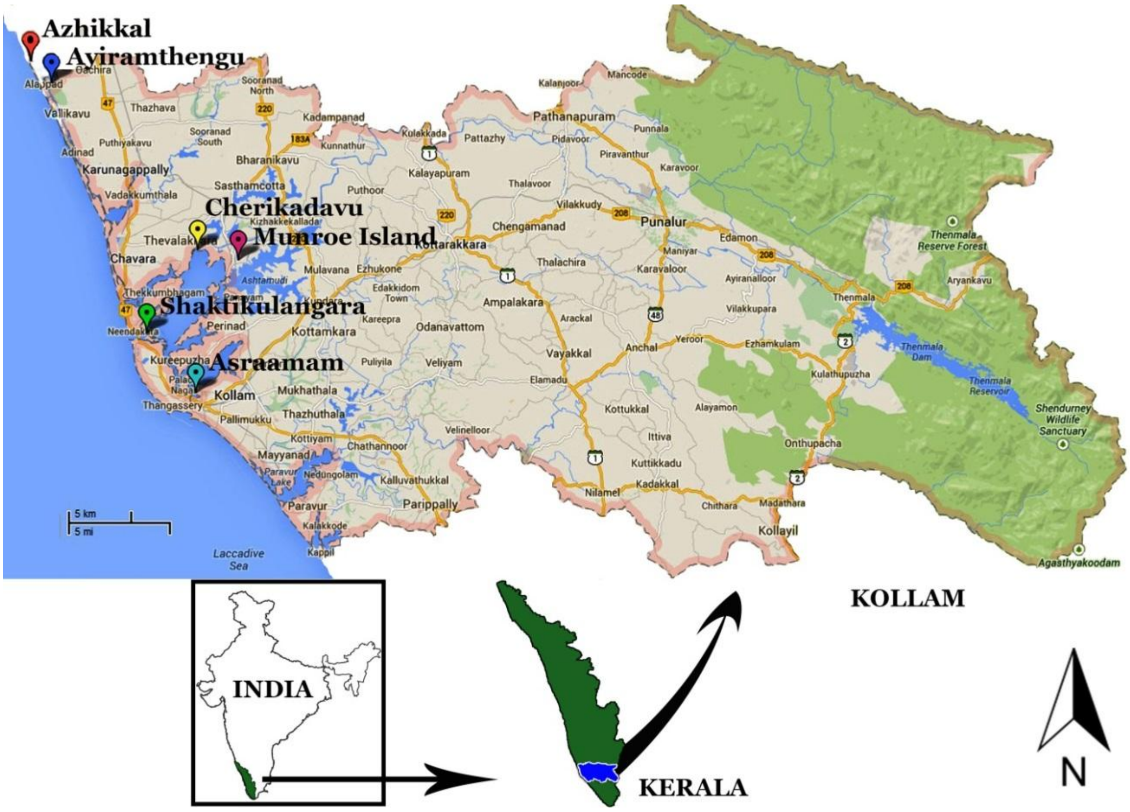

Figure 1. *Map of Kollam district showing different study locations*

Ecological data analysis

The distribution patterns of mangroves were studied using species area method and quadrat analysis with in each locations (Michael, 1998) fifteen quadrats each of 5×5m size were taken on the basis of data obtained. Density, frequency, basal area and their relative values and importance value index (IVI) of mangrove species were calculated by following standard Phytosociological methods (Curtis and Mclntosh, 1951). The IVI for the species was determined as the sum of the relative frequency, relative density and relative dominance (Curtis, 1959). Girth of trees exceeding 10cm diameter at breast height (dbh, at 1.37m above the ground) was measured. In order to determine the quantitative relationship between the plant species, the subsequent parameters were determined.

$$\text{Density} = \text{Number of individuals}/_{\text{ha}} \qquad \text{(Eq. 1)}$$

$$\text{Frequency} = \frac{\text{No.of quadrates in which a species occurs}}{\text{Total no.of quadrates}} \times 100 \qquad \text{(Eq. 2)}$$

$$\text{Abundance} = \frac{\text{Total no.of individuals of a species in all quadrates}}{\text{Total no.of quadrates in which the species occur}} \qquad \text{(Eq. 3)}$$

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$$\text{Basal area} = \frac{G^2}{4\pi} \qquad \text{(Eq. 4)}$$

G = girth at breast height (1.37m)

$$\text{Relative Basal Area} = \frac{\text{Basal area}}{\text{Total Basal area}} \times 100 \qquad \text{(Eq. 5)}$$

$$\text{Relative density} = \frac{\text{Total number of individuals of a species}}{\text{Total number of individuals of all species}} \times 100 \qquad \text{(Eq. 6)}$$

$$\text{Relative Frequency} = \frac{\text{No.of occurrence of a species}}{\text{No.of occurrence of all species}} \times 100 \qquad \text{(Eq. 7)}$$

$$\text{IVI} = \text{R. Basal Area} + \text{R. Density} + \text{R. Frequency} \qquad \text{(Eq. 8)}$$

$$\text{RIVI} = \frac{\text{IVI}}{3} \qquad \text{(Eq. 9)}$$

Species Richness

Species richness is describing the overall range of species in an area, on a macroscalic level, a distinct relationship between an areas locations and its number of species is recognizable. The species richness was calculated using Margalef's Index (Margalef, 1958):

$$\text{Margalef Richness Index (d)} = \frac{S-1}{In\ N} \qquad \text{(Eq. 10)}$$

Where, S = total number of species, N = basal area of species ($m^2.ha^{-1}$).

α Diversity analysis

Measure of diversity is frequently seen as indicators of the well-being of any ecosystem, the following indices were worked out to assess and compare the range and distribution of plant species in different locations.

a) The species diversity was calculated using Shannon- Wiener Index (Shannon and Weaver, 1963).

Shannon Index was calculated as,

$$H' = 3.3219[log_{10}N - (\sum Nilog_{10}Ni/N)] \qquad \text{(Eq. 11)}$$

Where, ni was the total number of species i and N was the total number of all the species. The factor 3.3219 was used to convert the index value to log2.

b) Concentration of dominance was measured by Simpson Index (Simpson, 1949).

$$Cd = 1 - \Sigma \left(\frac{Ni}{N}\right)^2 \qquad \text{(Eq. 12)}$$

Where, N_i and N were the same as explained above.

c) Equitability (e) was calculated following (Pielou, 1966):

$$e = H'/H_{max} \qquad \text{(Eq. 13)}$$

Where, H_{max} = 3.3219$\log_{10}$S, S = Total no. of species and H' = Shannon index.

β Diversity

β diversity estimates the species turnover or the similarity index. Jaccard's index was used to quantify the extent to which family and species compositions overlapped between sample sites (Jaccard, 1908). The Jaccards cluster diagram or dendrogram was fabricated with similarity matrix by simple coefficient of distance working on Biodiversity professional (version 2) (McAleece et al., 1997), using unweighted pair group method with arithmetic Mean (UPGMA) (Sneath, 1973).

$$J_{ab} = 1 - [c/(a + b - c)] \qquad \text{(Eq. 14)}$$

Where, J_{ab} = Jaccard's Index, a = number of species present in one location, b = number of species present in another location, c = number of species that are common to both locations.

Result and Discussions

Species Composition

Floristic study of different sites in Kollam revealed the occurrence of 12 species belonging to six families (*Table 1*). Among the families, *Rhizophoraceae* was the most frequent genera having five species, followed by *Avicenniaceae* and *Sonneratiaceae* with two species each. Among the location Shaktikulangara recorded the highest number of species (11species) abided by Ayiramthengu (9 species) and the least was recorded in Cherikadavu (5 Species). Distribution of mangrove at different site indicates that *Avicenna marina, Rhizophora mucronata, Excoecaria agallocha* were noticed in the entire study site. *Avicennia officinalis* occur found in five sites; these species were found as dominant species across the study sites. *Bruguiera cylindrica, Aegiceras corniculatum, Rhizophora apiculata* were found in four sites whereas *Bruguiera gymnorrhiza and Sonneratia caseolaris* were noticed in three sites. *Luminitzera racemosa, Ceriops tagal*, *Sonneratia alba* were rare species, which was confined only in single sites, In wetlands an increase in the number of the plant species is indicative of ecosystem health, but in case of mangroves, their distribution of species is determined by salinity, competition and other physical factor (Hogarth, 2007). The species composition and the agent causing maximum destruction are related to the difference in localities (Rao, 1986). Apart from the noted sites, Kollam have many other small mangrove patches which are under serious threats of degradation. Extensive land filling has affected the mangrove vegetation of some area in a drastic manner. The existing mangrove patches in Kerala with high diversity are mangroves of Kumarakom, Kannamali, Mangalavanam, Chettuva, Nadakkavu, Edakkad, Pappinisseri and Kunjimangalam, Veli, which have been singled out for conservation and rehabilitation (Suma, 1995).

Table 1. *Occurrence of different mangrove species at different sites*

True Mangrove species	Family	Ayiram thengu	Azhik-kal	Asraa-mam	Shaktikul-angara	Cherik-adavu	Munroe island
Aegiceras corniculatum	Myrsinaceae	+	+	−	+	+	−
Avicennia marina	Avicenniaceae	+	+	+	+	+	+
Avicennia officinalis	Avicenniaceae	+	−	+	+	+	+
Bruguiera cylindrica	Rhizophoraceae	+	+	−	+	−	+
Bruguiera gymnorrhiza	Rhizophoraceae	−	−	+	+	−	+
Ceriops tagal	Rhizophoraceae	−	−	−	+	−	−
Excoecaria agallocha	Euphorbiacea	+	+	+	+	+	+
Lumnitzera racemosa	Combretaceae	+	−	−	−	−	−
Rhizophora apiculata	Rhizophoraceae	+	+	−	+	−	+
Rhizophora mucronata	Rhizophoraceae	+	+	+	+	+	+
Sonneratia alba	Sonneratiaceae	−	−	−	+	−	−
Sonneratia caseolaris	Sonneratiaceae	+	−	+	+	−	−

Vegetation Structure and Importance Value

Vegetative composition is important in defining a wetland because vegetation affects its hydrology (Brinson, 1993). Phytosociology is the study of characteristics, classification, relationship, and distribution of plant communities, via different measures the dynamics of each species as well as their relation among each other in a community can be analyzed with a sufficient number of samples as database. This study is important for understanding the functioning of community, implies knowledge of structure and composition of the component species. Various parameters on the basis of which the present Phytosociological investigation has been done, the parameters include frequency (*Fig. 2*), relative frequency, density, relative density, abundance, basal cover and important value index (Cottom and Curtis, 1956). The density values (*Table 2*) shows that, *Avicenna officinalis* has the highest stem density (3760 stems/ha) among the

12 true mangrove species, followed by *Avicenna marina* (3721 stems/ha). *Rhizophora mucronata* ranked third based on the stems density (1938 stems/ha), the lowest density was recorded for *Sonneratia caseolaris* (267 stems/ha). Relative density of the species ranged from 1.46% to 20.57% (*Table 2*). *Avicenna officinalis* has the highest basal area (29.70 m^2ha^{-1}) followed by *Avicennia marina* (19.64 m^2ha^{-1}) and *Rhizophora mucronata* (14.11 m^2ha^{-1}), it is seen that the mangroves on sea fronts generally have high basal area. The frequencies of different species revealed the degree of dispersion of individual species in an area and are expressed in terms of percentage occurrence (*Table 2*). Relative frequency was highest for *Avicenna marina* (21.35%) whereas, *Avicennia officinalis* (16.29%) even it has higher density and basal area. Analysis of the abundance of species shows that *Lumnitzera racemosa* has the least (4.83%) followed by *Sonneratia caseolaris* (5%) and *Bruguiera cylindrica* (5.91%). *Avicenna officinalis* shows highest abundance and relative abundance (12.16%) and (14.51%).

Species dominance is calculated based on the Important Value Index and was calculated every species found in each study sites. It is usually practiced in ecological scrutinies; IVI indicates the ecological importance of a species in a specified ecosystem which can be used for computing species conservation and management through which species having low IVI value require soar protection preference (Kacholi, 2013). The IVI for a species was calculated as the sum of its relative basal area, relative frequency, relative density and often to describe and compare the species dominance of the sites (*Fig. 3*). In Kollam, the highest IVI and RIVI value were recorded for *Avicennia officinalis* (64.19 and 21.40) and was found to be the dominant species owing to high values of Relative density and Relative frequency, followed by *Avicenna marina* (59.78 and 19.93), *Rhizophora mucronata* and *Sonneratia alba* (*Table 2*). *Rhizophora mucronata* was the foremost dominant mangrove based on the species important value index in Kannur, Kerala (Vidyasagaran et al., 2011). The results indicate that *Sonneratia caseolaris, Lumnitzera racemosa* and *Ceriops tagal* are the least dominant mangrove species of Kollam region, the lowest IVI and RIVI were recorded for *Sonneratia caseolaris* (4.60 and 1.53) revealing the rarity and sporadic distribution of those species.

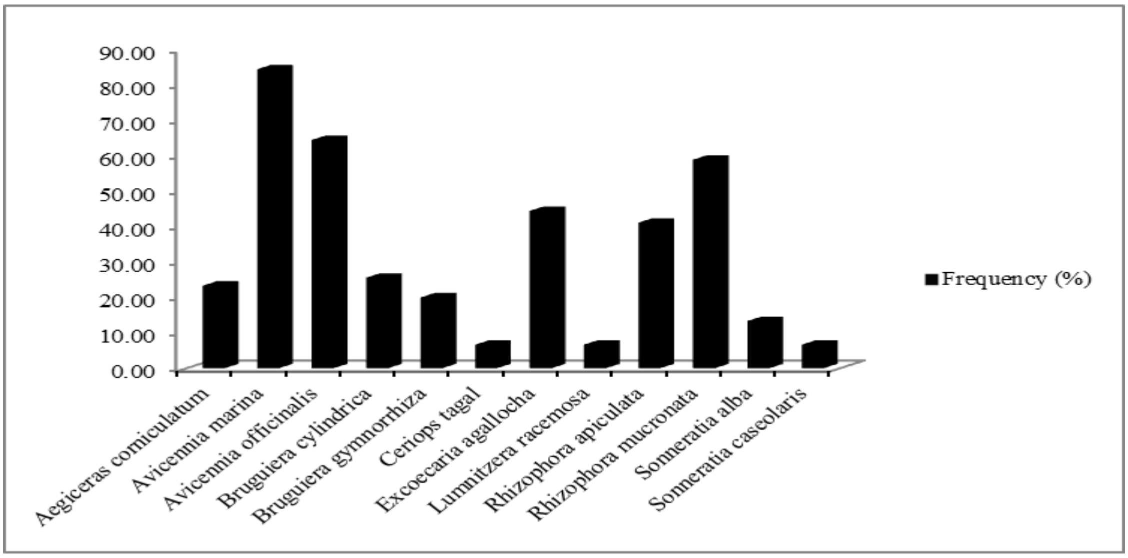

Figure 2. *Frequency distribution of mangrove species recorded in the present study*

Table 2. *Phytosociological parameters of mangroves in Kollam*

Name of the species	Frequency (%)	Density (Stems/ha)	Basal Area (m^2/ha)	Abundance	Rel. Frequency	Rel. Density	Rel. Basal Area	Rel. Abundance	IVI	Rel. IVI
Aegiceras corniculatum	23.33	1007	4.39	7.19	5.90	5.51	4.04	8.58	15.44	5.15
Avicennia marina	84.44	3721	19.64	11.01	21.35	20.36	18.07	13.13	59.78	19.93
Avicennia officinalis	64.44	3760	29.70	12.16	16.29	20.57	27.33	14.51	64.19	21.40
Bruguiera cylindrica	25.56	907	3.81	5.91	6.46	4.96	3.51	7.05	14.93	4.98
Bruguiera gymnorrhiza	20.00	1183	4.76	7.39	5.06	6.47	4.38	8.82	15.92	5.31
Ceriops tagal	6.67	987	4.60	6.17	1.69	5.40	4.23	7.36	11.32	3.77
Excoecaria agallocha	44.44	1294	7.13	7.28	11.23	7.08	6.56	8.68	24.87	8.29
Lumnitzera racemosa	6.67	560	1.59	4.83	1.69	3.06	1.46	5.76	6.22	2.07
Rhizophora apiculata	41.11	1640	10.30	6.70	10.39	8.97	9.48	7.99	28.84	9.61
Rhizophora mucronata	58.89	1938	14.11	7.02	14.89	10.60	12.98	8.37	38.48	12.83
Sonneratia alba	13.33	1013	7.07	3.17	2.37	5.54	6.51	3.78	15.42	5.14
Sonneratia caseolaris	6.67	267	1.58	5.00	1.69	1.46	1.45	5.96	4.60	1.53
TOTAL	395.55	18277	108.67	83.83						

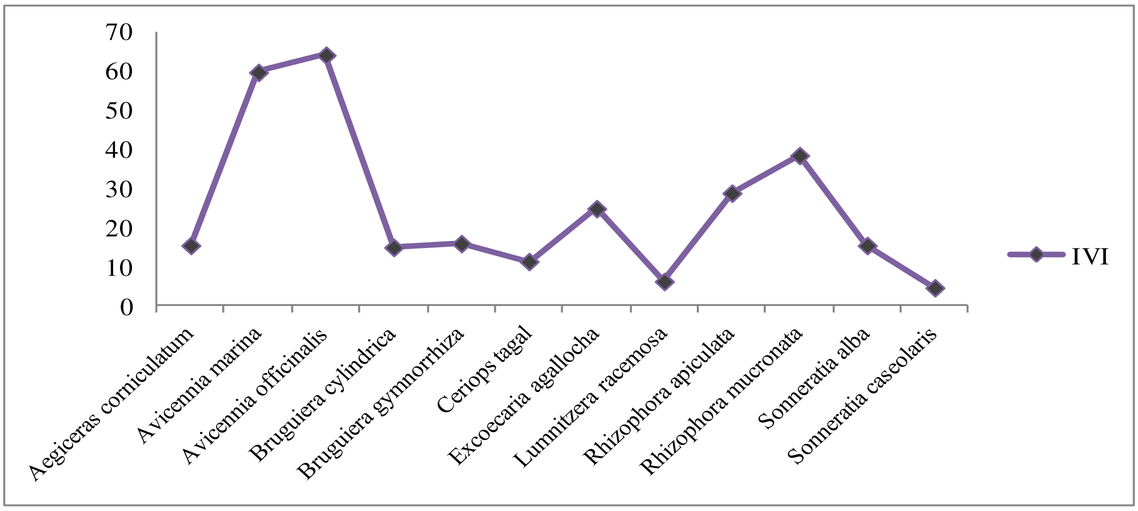

Figure 3. *Importance Value Index of different mangrove species at Kollam*

Diversity Indices and Species Richness

Diversity indices can be used to characterize the species abundance relationship in a community. Shannon's diversity index is a simple measure to seek out species diversity. The species diversity indices revealed that Kollam had a high species diversity and better evenness figure. In the present study, disclosed that Kollam had total Shannon index as 3.266 (*Table 3*), it rewarded highest value in Shaktikulangara (3.112) and lowest in Cherikadavu (2.002). It indicates that sites with higher Shannon – Weiner had comparatively maximum number of species. An ecosystem with H' value greater than 2 has been treated as medium to high diverse in terms of species (Barbour et al., 1999). Thus Kollam has rationally high species diversity. The present study indicates, Simpson index of diversity of mangroves for whole Kollam as 0.879, Simpson value of species was found to be higher (0.858) at Shaktikulangara abide by Ayiramthengu (0.825) and least at Azhikkal (0.674) followed by Cherikadavu (0.796). The species evenness (Pielou's evenness) or equitability ranged from 0.902 to 0.791 (*Table 4*). The intact species richness worth of mangroves at Kollam revealed as 3.276, the species richness of various locations fluctuated between 1.022 and 0.429. Relatively higher value was recorded in Shaktikulangara and lower value in Cherikadavu (0.429) followed by Azhikkal (0.536) and Asraamam (0.539), the estimated species richness values of different site are bestowed in *Table 4*.

Table 3. *Diversity indices of different mangrove sites of Kollam*

Name of the locality	Shannon Index(H')	H max	Equitability(e)	Simpson´s Index(Cd)	Species richness(d)
Ayiramthengu	2.763	3.170	0.872	0.825	0.819
Azhikkal	2.044	2.585	0.791	0.674	0.536
Asraamam	2.200	2.585	0.851	0.741	0.539
Shaktikulangara	3.112	3.461	0.899	0.858	1.033
Cherikadavu	2.002	2.323	0.862	0.706	0.429
Munroe Island	2.460	2.808	0.876	0.778	0.623
Entire Kollam District	3.266	3.585	0.911	0.875	1.121

Similarity Indices

Compared to α-diversity, however, knowledge of β-diversity in the marine ecosystem context is more limited (Gray, 2000). This index depends on the number of species shared by two assemblages and the number of species unique to each of them. The results presents in Table 4 revealed that the highest value of β-diversity was registered between Azhikkal- Asraamam (0.66) abided by Shaktikulangara-Cherikadavu (0.55) which is comparatively higher than Cherikadavu- Munroe Island (0.50). The reduced level of similarity (0.33) was recorded between Ayiramthengu-Azhikkal and Ayiramthengu- Shaktikulangara (0.33). The higher similarities for mangroves in internal and central sites are due to the better organic carbon content of soil while, fringe areas uphold low plant growth diversity due to less organic carbon content of soil and high variations (Brahmaji, 1998). (*Fig. 4*) Dendrogram results the hierarchical clustering using the UPGMA algorithm (Odum, 1969). The resulting dendrogram display the level of similarity in percentage among different sites of Kollam and to obtain the natural grouping of different species along different sites. Locations in same groups shows much similarity than locations in disimilar groups, dendrogram also revealed that various grouped locations indicate distinction on species composition and abundance. It shows Cherikadavu- Asraamam, Munroe-Shaktikulangara and Azhikkal-Ayiramthengu are similar and are naturally grouped.

Table 4. *Similarity Index in species composition among study sites*

Locations	Ayiramthengu	Azhikkal	Asraamam	Shaktikulangara	Cherikadavu	Munroe Island
Ayiramthengu	—	0.33	0.50	0.33	0.44	0.40
Azhikkal		—	0.66	0.45	0.43	0.38
Asraamam			—	0.45	0.43	0.38
Shaktikulangara				—	0.55	0.36
Cherikadavu					—	0.50
Munroe Island						—

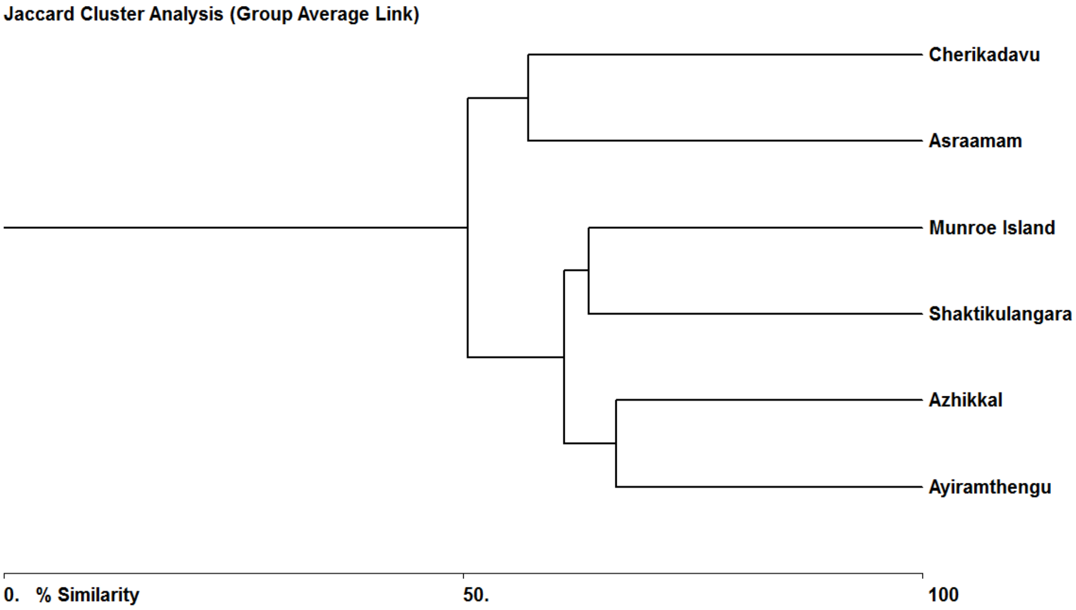

Figure 4. *Dendrogram of Kollam mangroves showing grouping of stations sampled during the study period*

Conclusion

This study concentrates Mixed Mangrove forest in Kollam, a total of 12 species of mangroves belonging to six families were identified. The result shows that family Rhizophoracae is the largest family in Kollam region followed by Avicenniaceae and Sonneratiaceae. The sequences of distribution of each station were erratic and vegetation was diverse. *Avicennia marina, Excoecaria agallocha* and *Rhizophora apiculata* were found distributed in all six stations followed by *Avicennia officinalis* in five stations. Almost all stations were dominated by *Avicennia officinalis* followed by *A. marina*, *Lumnitzera racemosa and Ceriops tagal* are least diverse species in Kollam. The diversity of *Ceriops tagal* is proscribed to Shaktikulangara region in Kerala, no other studies shows the diversity of the species in Kerala. High species diversity indicates the maturity of an ecosystem (Odum, 1969).The Simpson index diversity of mangroves of Kollam (0.875) is comparatively similar to the values of diversity in Kannur, Kerala (0.821 and 0.854) (Jose, 2003). Existence of variation in species richness in mangroves of different locations was reported by several workers, the species richness always influenced by variation in rainfall, temperature, anthropogenic pressure and edaphic factors (Parthasarathy and Sethi, 2001). Almost every mangrove species in Kollam are of conservation and importance value. Extensive land fillings has affected the mangrove vegetation in a drastic manner, human population have major impact on the diversity, structure of forest ecosystems (Kacholi, 2013), In our study we also noted that mangroves are negatively compact by human development, they are mainly ditched to several industrial developments, housing etc. Early studies results that mangrove forests are under immense pressure from clear cutting, land use change, hydrological alternation, chemical spill and climate change (Blasco, 2001) The similarity indices also revealed the similar trend like that of evenness index, Highest similarity indices between the sites in the present study are attributed to the formation of common species into dense thickets; similar results are observed earlier (Banerjee and Gosh,1998). Seen a widespread overlap and similarity between all the sites through ordination visualization and direct comparison, thus comparisons of mangrove factors to richness, diversity and similarity indices was performed for Kollam as a whole. Trees basal area is another physical measurement that can be used to compare and characterize trees (Cris, 1999). As a recommendation, by considering the importance of mangroves and its associates in coastal ecosystem, the respective authority should enforce the regulation in order to protect and conserve the mangrove patches in Kollam.

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APPENDIX

Electronic Appendix 1: Basic Research Data

Electronic Appendix 2: Images

DIVERSITY AND PREDATION POTENTIAL OF MAJOR APHIDOPHAGOUS PREDATORS IN MAIZE

SWAMINATHAN, R.* – MEENA, A. – MEENA, B. M.

Department of Entomology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur-313001, Rajasthan, INDIA

** Corresponding author*
e-mail: udaiswami57@gmail.com; udaiswamirussia@yahoo.co.uk

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Abstract. Investigation on, "*Diversity and predation potential of major aphidophagous predators in maize*" was carried out at the Instructional Farm, Rajasthan College of Agriculture, Udaipur during summer (April to July, 2013) and monsoon (July to October, 2013) seasons with the objectives to study the diversity of aphids feeding natural enemies and evaluate the predation potential of major insect groups of the aphidophagous guild in the field and laboratory. The aphid pest recorded on maize was *Rhopalosiphum maidis* (Fitch). Among the aphidophagous predatory guild, the major insect groups included coccinellids (*Coccinella septempunctata* Linnaeus, *Cheilomenes sexmaculatus* (Fabricius), *Brumoides suturalis* (Fabricius) and *Illeis cincta* (Fabricius): Coccinellidae, Coleoptera); lygaeid bug (*Geocoris* sp.: Lygaeidae, Hemiptera,); rove beetle [*Paederus fuscipes* Curtis: Staphylindae, Coleoptera] and syrphid flies (*Ischiodon* sp.: Syrphidae, Diptera; being dominant). The seasonal mean population of aphids was higher during monsoon season crop (369.09/plant) than during the summer season (291.26/plant). Likewise, the seasonal mean population of the aphidophagous predators per plant was relatively more during monsoon season being 8.56 (coccinellids), 3.76 (*Geocoris* sp.), 3.14 (*P. fuscipes*) and 2.41 (syrphid flies); whereas, in summer season the corresponding values were 7.78 (coccinellids), 3.60 (*Geocoris* sp.), 2.87 (*P. fuscipes*) and 1.89 (syrphid flies). Exclusion of the ground dwelling aphidophagous predators resulted into significantly more aphid predation (99.75%) than when aerial aphidophagous predators were excluded from access to aphid prey (89.38%). In the no exclusion treatment, where both types of predators (aerial and ground dwelling) had equal access to aphid prey, the decrease in aphid numbers was the maximum (100%). All the three coccinellid grubs and adult beetles consumed relatively more aphids at lower aphid densities (25, 50 and 75), significantly being the maximum at a prey density of 75. At higher aphid densities (100, 125 and 150) the consumption rates declined. The feeding behavior of coccinellids showed a sharp decline in percentage feeding with an increase in prey density for both adults and grubs.

Key words: *maize aphid, aerial, ground dwelling aphidophagous predators*

Introduction

Agro-ecosystems under intensive agriculture often present unfavourable environments for natural enemies due to high levels of anthropological disturbance, particularly through intensive agriculture. Habitat management that aims at utilizing practices to favour natural enemies, especially, predators and parasitoids towards achieving conservation biological control has been often advocated. A higher natural enemy abundance (Östman et al., 2001) or diversity (Snyder et al., 2006) may not often lead to improved biological control, because prey other than the pest species may be preferred. The corn leaf aphid, *Rhopalosiphum maidis* (Fitch) is one of the important pests of maize with worldwide distribution (Carena and Glogoza, 2004; Plewa and Pankanin-Franczyk, 1989). It was observed that infestation from 10-leaf stage to tasseling caused 28.14 per cent yield losses (average aphid density 818 aphids/plant); while, infestation

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through ripening stages caused 16.28 per cent yield losses (average aphid density 1038 aphids per plant). Yield losses of corn due to aphid infestation from 10-leaf stage to ripening stage were 14.66, 22.9, 35.28 and 36.03 per cent at average aphid densities of 100, 1000, 2000 and 3000 aphids per plant. This clearly indicates that plant lice, i.e. aphids can cause considerable losses to the crop and thus must be managed through eco-safe methods (Al-Eryan and El-Tabbakh, 2004).

The management of sap sucking insect pests through bio-intensive methods renders it important to record the diversity of natural enemies of aphids, both generalist and specific, commonly occurring in any crop ecosystem to exploit them in favour. The aphidophagous arthropod guild can be divided broadly into specialists that include Braconidae and Aphidiinae parasitoids; predatory coccinellids, lacewings and hoverflies (Müller and Godfray, 1999) or generalists that include euryphagous predators like ground beetles and spiders (Lang, 2003). Intra-guild competition is often reported among aphidophagous natural enemies due to their foraging activity when they frequently encounter hetero-specific aphid predators, which may disrupt biological control efforts against aphids where more than one predator species is present; hence, this necessitates carefully choosing a combination of predators for success in biological control of aphids (Hindayana et al., 2001). The present investigation thus envisages working out the diversity of aphidophagous arthropod natural enemies in maize cultivated during summer and in the monsoon season, and their predation potential.

Materials and Methods

a) Experimental site

The experimental site was located at the Instructional Farm, Rajasthan College of Agriculture, Udaipur, situated at 75.4^0 E Latitude and 23.4^0 N Longitude at an elevation of 582.17 MSL in the sub-humid southern region of Rajasthan, India. The zone has a typical sub-tropical climatic condition characterized by moderate winter and hot summer associated with high humidity especially during months of August and September. The average rainfall of this tract ranges between 450-650mm, contributed by South-West monsoon from July to September with occasional rains during the winter season. During summers, the atmospheric temperature may go as high as 45.5^oC, while in winters, it may fall as low as 3.5^oC occasionally.

b) Field preparation and sowing of maize crop

One deep ploughing and two cross harrowing were done to improve the field condition. Sowing of summer crop was done on 3th April, 2013 and maintained with timely irrigations; while that of the monsoon crop on 9th August, 2013 and was cultivated rainfed. Phosphatic fertilizer was applied as basal dose of 40 kg/ha, while N was applied at 80 kg/ha in two split doses. The other agronomic practices such as thinning, hoeing and weeding were performed as and when needed following the package of practices for cultivation of maize.

c) Natural enemy collections

The aphidophagous predators were collected along with their aphid prey from maize (Variety: *Pratap makka*) fields during summer and monsoon seasons in

2013. Collections were made twice a week during the aphid infestation period beginning at tassel initiation stage. The aphidophagous natural enemy diversity was also recorded and collected from the same maize fields.

d) Arthropod monitoring

a) Pest aphids count

The population count was made from 5 randomly-selected maize plants from each replicate of the 4 treatments (mentioned later), selecting the top 20cm meristematic region where aphids usually congregate and feed. The number of winged and wingless adults, nymphs and mummies (mummified aphids hosting parasitoids) for each aphid species was accounted for during observation.

b) Aphidophagous natural enemy count

i. Parasitoid count

The aphid parasitoids were estimated from the count of aphid mummies per plant as per methodology mentioned above.

ii. Specific and generalist predator count

The predator abundance was recorded as per stage of development:

a) First, larvae of lacewings, coccinellids and hoverflies were counted on the same shoots used to count the aphids.

b) Second, adult aerial predators, mainly coccinellids, were recorded visually by walking in between the central rows of maize in each replicate and expressing the population on a per plant basis from the top 20cm shoot.

c) Third, the ground-dwelling predators were collected using pitfall traps. Three pitfall traps (*ca* 500ml glass jar with ethylene glycol) were positioned diagonally within each replicate. The traps were dug down to ground level. Transparent plastic covers were placed above the traps to prevent flooding by rain during the monsoon season.

d) Aphids and aerial predator transects were inspected between 7:00 and 8:00 a. m.

e) Ambient atmospheric temperature (^{o}C) and relative humidity (%) was measured prior to sampling. Sampling was carried out as soon as aphid infestation began.

f) The aphidophagous predator community was determined to species, wherever possible, in addition, the predators were classified into specific or generalist predators.

e) Efficacy of aphidophagous natural enemy guild

A field cage experiment to test the effect of aphidophagous predator guild on aphid population was carried out at the Instructional Agronomy Farm, RCA Udaipur on summer and monsoon season maize. In each selected field the following treatments were taken that were replicated 6 times:

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(1) An open control (O)

(2) Ground-dwelling predator exclusion (G)

(3) Aerial predator and parasitoid exclusion (F) and

(4) Total exclusion (T), which was the combination of treatments (Asin and Pons, 1998) and (Carena and Glogoza, 2004), *i.e.*, G and F.

Metallic barriers to exclude ground-dwelling predators (e.g., spiders, carabids, staphylinids) were placed. The barriers were circular, 0.5 m in diameter, dug into the soil. Ground-dwelling predators captured in these traps were counted and removed. Aerial aphid predators and parasitoids were excluded by setting wire cages over the plants. The cages were of a mesh size of 8mm allowing airflow to avoid changes in microclimate conditions (Schmidt et al., 2003) and were covered with sticky glue to intercept or hinder aerial predators and parasitoids from entering. The bottom edge of the cages was left without glue to permit ground-dwelling predators access. With a view to maintain a uniform initial population, at initiation of tassel in the summer crop of maize, aphid abundance was recorded on 20cm length of top shoot and 1-day before caging, ca 500 aphids were left on the shoot removing excess aphids, wherever present, with the help of a camel hair brush.

Culture of coccinellids in the laboratory and predation potential trial

a) The adult coccinellids were field collected from the untreated crop fields of the Instructional Farm of the College and brought to the laboratory to maintain a stock culture under ambient conditions of temperature and humidity. The mean atmospheric temperature ranged from 23 to 34^{o} C and at 51 to 74 per cent relative humidity. Mating pairs were kept in glass jars (500ml capacity) covered with muslin cloth that was fastened with rubber bands. The eggs laid on maize leaves (Soares et al., 2003) were placed into fresh glass jars till the first instar grubs emergence, which were then provisioned with fresh maize aphids daily as food.

b) To evaluate the predation potential of coccinellid grubs at different aphid densities (25, 50, 75, 100, 125 and 150), four healthy second instar grubs were separated with the help of a camel hair brush after the first moult and transferred into individual glass jars of 500ml capacity covered with a muslin cloth held together by rubber bands, considered as 4 replicates. Observations on the consumption of aphids were recorded in each replicate under the different treatments after 24h. The experiment was continued for 5 days and left over aphids and dried maize shoots were replaced daily with fresh ones to avoid contamination and consequent mortality.

c) To evaluate the predation potential of adult beetles, *Coccinella septempunctata* (Linneaus)*, Cheilomenes sexmaculata* (Fabricius) and *Illeis cincta* (Fabricius) were field collected from the untreated maize crop and reared on the maize aphid (dominant being *R. maidis*) as prey. Having starved the adult coccinellids for 6h they were individually transferred to glass jars with different aphid prey densities on fresh maize leaves replicated 4 times. Observations on consumption of aphids were recorded in each replicate under the different treatments after 24h. The evaluation was continued for 5 days replacing left over aphids and dried maize shoots daily with fresh ones to avoid contamination and consequent mortality.

Mathematical and statistical analyses

The following mathematical analysis was made towards estimating the species richness of aphidophagous natural enemies and their diversity indices:

Mean density (MD)

$$\text{Mean density (MD \%)} = \frac{Xi}{N} \times 100$$

Where, Xi = Numbers of insects; N = Total numbers of plants sampled.

Relative density (RD)

$$RD\% = \frac{\text{Number of individual of one species}}{\text{Total number of individual of all species}} \times 100$$

Shannon –Weiner diversity index (H')

$$\text{Shannon – Weiner diversity index (H')} = -\Sigma\, pi \ln pi$$

Where, Pi = the decimal fraction of individuals belonging to ith species

Predation Efficiency

The predation efficiency of dominant aphidophaous coccinellids was evaluated through the determination of the voracity following the model of Soares et al. (2003):

$$Vo = (A - a\,24)\, ra\,24$$

Where, Vo = number of aphids eaten; A = number of aphids available
a 24 = number of aphids alive after 24 h and
ra 24 = ratio of aphids found alive after 24 h in the absence of predators.

The data obtained were subjected to Analysis of Variance and other suitable statistical procedures to analyze the results of the investigations conducted.

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Results

Aphid incidence and the diversity of their natural enemies

The aphids collected from maize were *Rhopalosiphum maidis* (Fitch) [Aphididae: Hemiptera]. The associated natural enemies of aphids on maize included coccinellids [*Coccinella septempunctata* Linneaus, *Cheilomenes sexmaculata* (Fabricius), *Brumoides suturalis* (Fabricius), and *Illeis cincta* (Fabricius) Coccinellidae: Coleoptera]; the big-eyed bug [*Geocoris* sp., Lygaeidae: Hemiptera]; the rove beetle [*Paederus fuscipes* Curtis, Staphylinidae: Coleoptera]; and syrphid flies [major being *Ischiodon* sp., Syrphidae: Diptera].

The seasonal incidence of the maize aphid during the summer and monsoon seasons, as presented in *Tables 1* and *2* respectively, indicate that the seasonal mean aphid abundance on maize during monsoon was relatively more than that during summer season. The mean population ranged from 237.00 aphids per plant to 373.05 aphids per plant with the seasonal mean being 291.26 aphids per plant on the summer crop (*Table 1*).

Table 1. *Population dynamics of maize aphid and its natural enemies during summer, 2013*

Dates of observation	Mean Atm. Temp. (°C)	Mean R. H. (%)	Mean Population per Plant				
			Aphids	Coccinellids	*Geocoris sp.*	Rove beetles	Syrphids
20-05-2013	34.50	24.50	271.01	5.43	3.55	2.98	1.69
23-05-2013	34.65	14.95	272.85	5.51	3.48	2.91	1.71
26-05-2013	33.10	34.00	274.54	5.70	3.39	2.86	1.78
29-05-2013	33.35	37.00	276.06	6.28	3.60	3.03	1.82
01-06-2013	32.15	42.50	275.28	6.91	3.42	2.88	1.79
04-06-2013	32.70	57.00	278.85	7.69	3.53	2.84	1.84
07-06-2013	30.10	59.50	300.34	7.65	3.66	3.09	1.86
10-06-2013	31.10	54.50	345.62	9.16	3.68	3.17	1.91
13-06-2013	28.30	84.00	347.58	11.77	4.01	3.27	2.40
16-06-2013	30.20	58.50	373.05	11.71	4.50	3.32	2.56
19-06-2013	30.60	62.00	302.07	9.34	3.43	2.95	2.15
22-06-2013	28.95	66.50	277.71	9.08	3.41	2.93	2.05
25-06-2013	31.20	60.50	245.68	7.61	3.68	2.29	1.76
28-06-2013	28.55	74.50	237.00	5.14	3.05	1.64	1.11
Seasonal Mean	**31.39**	**52.14**	**291.26**	**7.78**	**3.60**	**2.87**	**1.89**
CC: r-values for population and mean atm. temp.			-0.30	-0.61*	-0.16	0.17	-0.25
CC: r-values for population and mean R .H.			0.28	0.63*	0.17	-0.18	0.26
CC: r-values between populations of aphids and natural enemies				0.84*	0.82*	0.76*	0.84*

CC = Coefficient of correlation; * Values significant at 5% level; Population data are means of 20 plants (5 plants x 4 replications)

Likewise, the mean population ranged from 269.85 aphids per plant to 520.12 aphids per plant with the seasonal mean being 369.09 aphids per plant on the monsoon season crop (*Table 2*). During summer season, the mean atmospheric temperature had a mild negative correlation with the aphid population, while the mean relative humidity evinced a mild positive correlation with the aphid population though the r-values were non-significant. During monsoon season, the mean atmospheric temperature had a significant positive correlation with the aphid population (r = 0.65); whereas, the mean relative humidity showed a mild positive influence on aphid population. It could be inferred that an increase in the mean atmospheric temperature during summer caused a reduction in aphid numbers, while during monsoon it caused a significant increase in aphid numbers.

Table 2. *Population dynamics of maize aphid and its natural enemies during monsoon, 2013*

Dates of observation	Mean Atm. Temp. (°C)	Mean R. H. (%)	Mean Population per Plant				
			Aphids	**Coccinellids**	***Geocoris* sp.**	**Rove beetles**	**Syrphids**
02-09-2013	26.70	71.00	327.43	8.42	3.61	3.13	2.11
05-09-2013	26.45	51.00	334.53	8.91	3.75	3.07	2.18
08-09-2013	26.85	61.00	403.02	9.56	3.70	3.23	2.28
11-09-2013	28.75	68.50	425.75	9.46	3.83	3.09	2.35
14-09-2013	28.00	62.50	502.12	10.58	4.83	4.45	3.23
17-09-2013	29.80	79.50	520.12	10.41	4.70	3.66	4.07
20-09-2013	26.50	86.00	423.69	9.70	4.03	3.05	2.67
23-09-2013	25.25	84.00	384.75	9.10	4.15	3.13	2.35
26-09-2013	25.25	79.50	355.63	7.85	3.71	2.97	2.26
29-09-2013	25.90	77.00	337.43	8.23	3.53	3.00	2.18
02-10-2013	27.95	80.50	302.42	7.43	3.29	2.84	2.16
05-10-2013	26.75	67.00	299.95	7.23	3.32	2.86	2.05
08-10-2013	24.85	68.50	280.63	6.61	3.22	2.74	1.96
11-10-2013	25.90	60.00	269.85	6.40	2.96	2.72	1.91
Seasonal Mean	**26.78**	**71.14**	**369.09**	**8.56**	**3.76**	**3.14**	**2.41**
CC: r-values for population and mean atm. temp.			0.65*	0.59*	0.52*	0.53*	0.70*
CC: r-values for population and mean R.H.			0.19	0.10	0.20	-0.10	0.25
Coefficient of correlation between aphids and natural enemies				0.94*	0.95*	0.83*	0.89*

CC = Coefficient of correlation; * Values significant at 5% level; Population data are means of 20 plants (5 plants x 4 replications)

In accordance to the aphid numerical abundance and being density-dependent, the associated aphidophagous natural enemies were relatively a little more during the monsoon season as compared to that during the summer season. The mean adult coccinellid population (inclusive of 4 species) ranged from 5.14 to 11.77 per plant with the seasonal mean of 7.78 beetles per plant on the summer crop; while the corresponding values for monsoon crop were 6.40 to 10.58 per plant with the seasonal mean of 8.56 beetles per plant. The lygaeid bug, *Geocoris* was in significant numbers with the seasonal mean of 3.60 and 3.76 bugs per plant during summer and monsoon seasons, respectively. The staphylinids had a seasonal mean population of 2.87 and 3.14 per plant, while the syrphids were 1.89 and 2.41 per plant during the summer and monsoon seasons, respectively. It could be observed the populations of the aphidophagous natural enemies were more during monsoon than in summer, possibly being favoured by the humid conditions. The population of coccinellids showed much variation, while that of *Geocoris*, staphylinids and syrphids did not show much variation within the seasons (*Tables 1* and *2*). The population trend of the maize aphid and the associated natural enemies has been depicted in the *Figures 1* and *2*, based on log population of both during summer and monsoon seasons, respectively.

Among the aphidophagous natural enemy guild recorded during both the summer and monsoon seasons, coccinellids dominated with 48.23 and 47.91 per cent relative density, respectively; followed by the big-eyed bug, *Geocoris* with a RD value of 22.30 and 21.03 per cent (*Table 3*). However, the Shanon diversity index values did not differ for the two seasons (1.25 and 1.24 for summer and monsoon, respectively).

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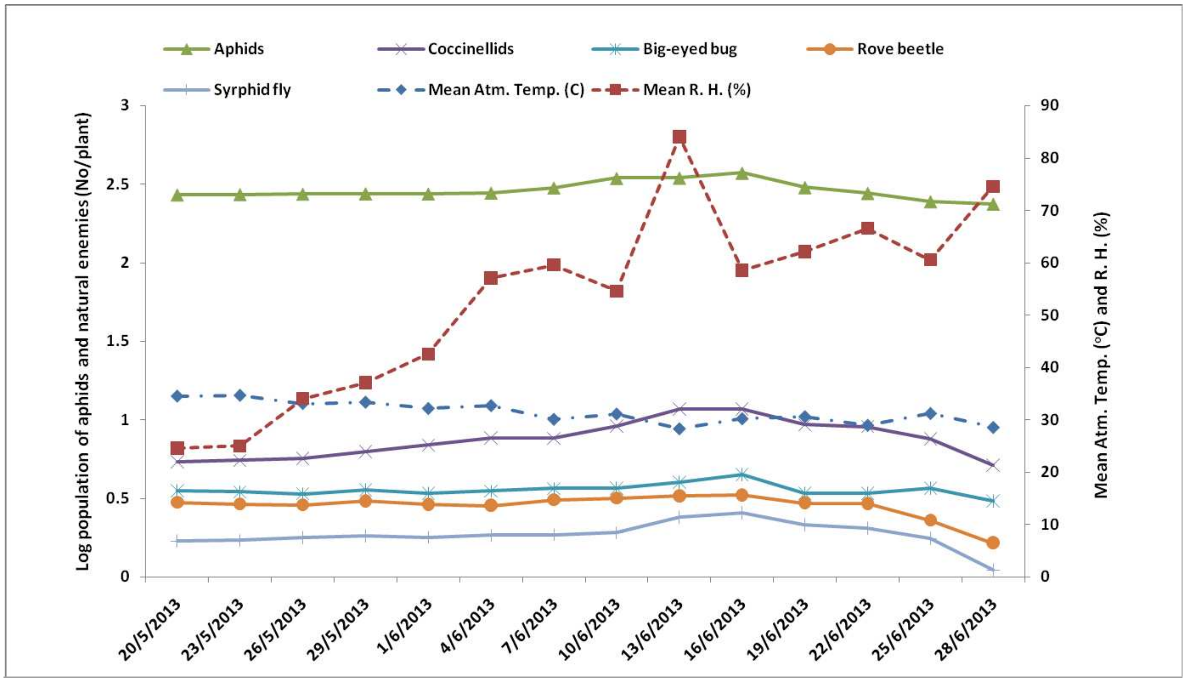

Figure 1. *Population trend of maize aphids and associated natural enemies during summer, 2013*

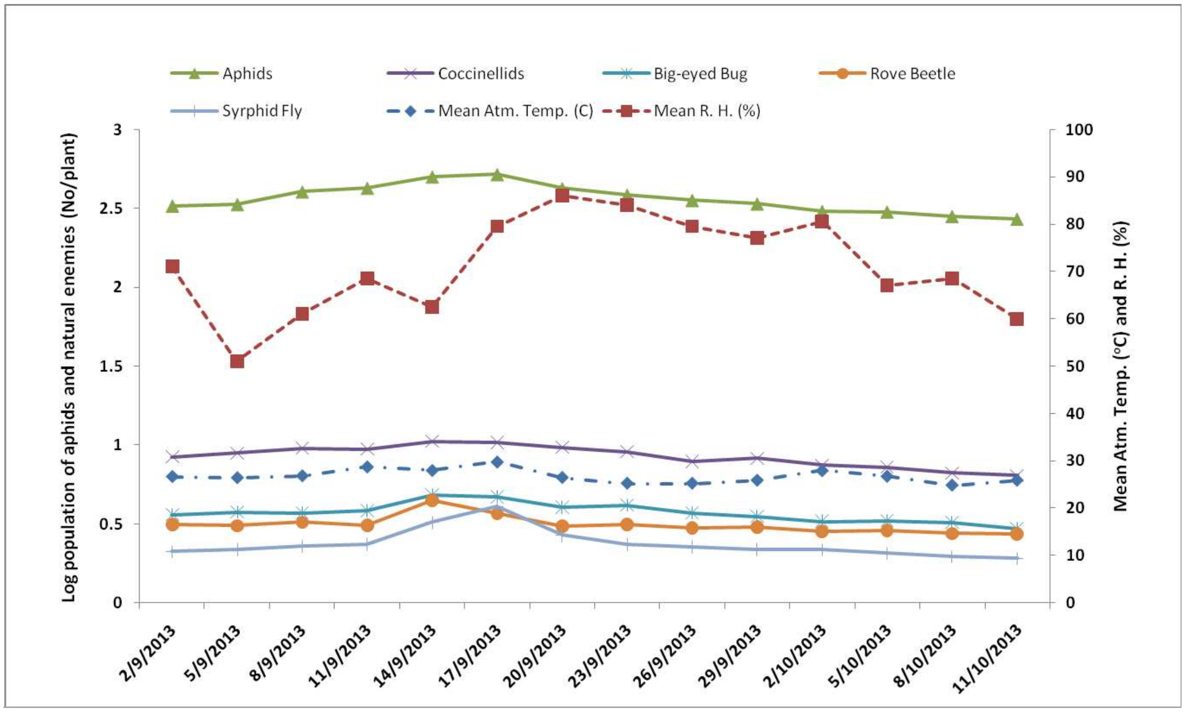

Figure 2. *Population trend of maize aphids and associated natural enemies during monsoon, 2013*

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Table 3. *Dominant predators of maize aphid during two seasons in 2013*

Aphidophagous predators	**Summer Crop**		**Monsoon Crop**	
	Seasonal Mean*	**Relative Density (%)**	**Seasonal Mean***	**Relative Density (%)**
Coccinellids	7.78	48.23	8.56	47.91
***Geocoris* spp.**	3.60	22.30	3.76	21.03
Rove beetles	2.87	17.77	3.14	17.56
Syrphid fly	1.89	11.70	2.41	13.49
Shanon D I	**1.24**		**1.25**	

* Seasonal mean population expressed as numbers per plant

Efficacy of the aphidophagous guild

The efficacy of the aphidophagous guild was evaluated in a field experiment wherein known numbers of aphids were caged or excluded from predation by aerial predators, ground dwelling predators, both and an uncaged or open control. It was notable that excluding aerial predators enabled ground dwelling predators to access the aphids, while exclusion of ground dwelling predators enabled the aerial predators to access the apids. Likewise, exclusion of both types of predators resulted in exponential population growth of the aphids. From the *Table 4* and *Figure 3* it becomes clear that aerial predators contributed significantly more towards aphid predation than the ground dwelling predators, as shown by the decrease in aphid numbers from 500 to 4.17 at five days after treatment; whereas, ground dwelling predators caused a decrease in aphid numbers from 500 to 56.67 at five day after treatment. However, in the no exclusion treatment, where access to aphid prey was provided to both types of predators (aerial and ground dwelling) the decrease in aphid numbers was the maximum being 3.00 at four days after treatment and zero at five days after treatment. It is therefore conspicuous that the aphidophagous predators play a major role in maintaining the aphid populations below those causing economic damage. Nevertheless, one can observe these predators actively foraging except on rainy days while in the field.

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Table 4. *Prey consumption by aphidophagous predator-guild in different exclusion treatments*

Exclusion Treatment for Aphidophagous Predators	PTP of Aphids (No./plant)	Left-over Aphid Population (№:s/plant)					Consequent Prey Consumption by
		1-DAT	2-DAT	3-DAT	4-DAT	5-DAT	
Aerial	500	406.67	331.67	240.00	136.67	56.67	Ground dwelling predators
Ground dwelling	500	283.33	137.50	55.00	21.67	4.17	Aerial predators
Both aerial and ground dwelling (Total Exclusion)	500	670.00 (34.00)	820.83 (47.89)	936.67 (53.20)	1055.00 (59.25)	1163.33 (62.87)	No predators
Control (No exclusion)	500	154.17	48.33	12.83	3.00	0.00	Both aerial and ground dwelling predators
S. Em. ±	--	30.27	33.54	35.69	40.50	37.11	
C.D. (5%)	--	91.22	101.08	107.54	122.03	111.82	

Note: Population count of aphids per plant on 20 cm top shoot in each treatment; DAT = Days after Treatment
Values in parentheses indicate the cumulative percent increase in aphid population in the total exclusion treatment

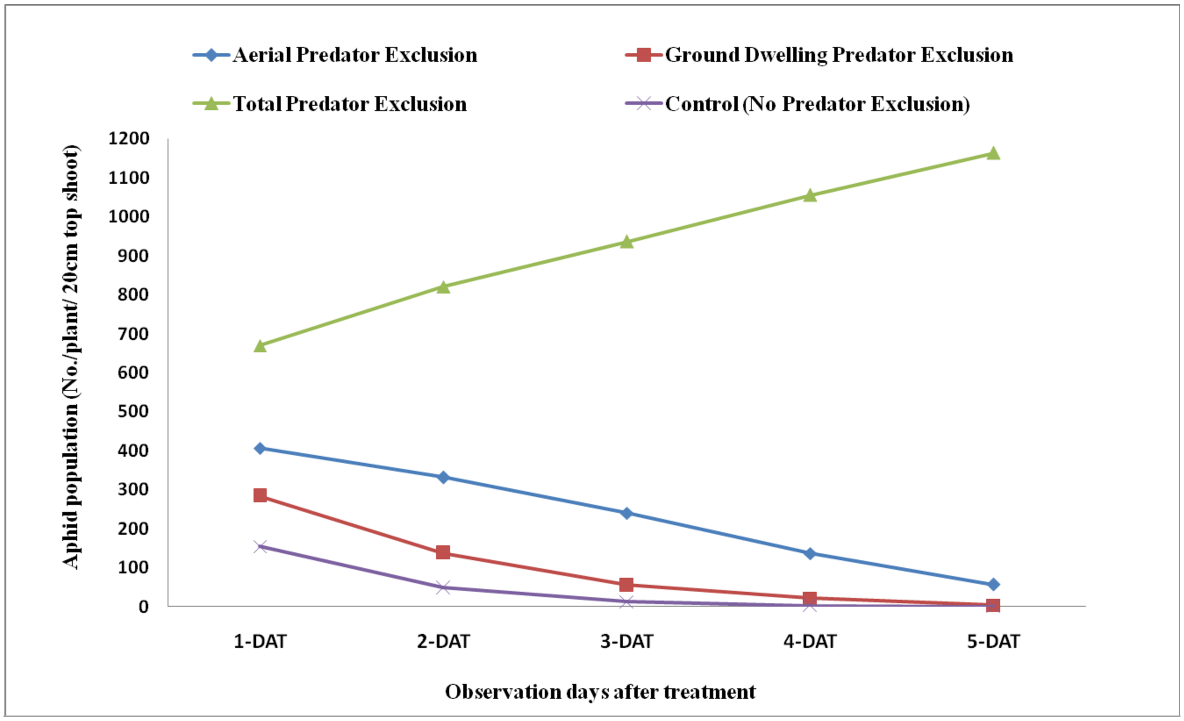

Figure 3. *Influence of aphidophagous predator exclusion treatments on prey population*

A perusal of the *Table 5* explains the feeding propensity of these aphidophagous predators under different exclusion treatments. Aphid consumption (%) was maximum (100 per cent) under no exclusion treatment (control) closely followed by when ground dwelling predators were excluded (99.75%); whereas, it was 89.38 per cent when aerial predators

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were excluded. The treatment of total exclusion (excluding both aerial and ground dwelling predators), in contrast, showed a cumulative increase in the aphid population over the initial population of 500 aphids per plant depicting an increase from 34.00 to 62.87 per cent (*Table 4*) (*Fig. 3*).

Table 5. *Feeding propensity of aphidophagous predators under different exclusion treatments*

Exclusion Treatment for Aphidophagous Predators	**Aphid Consumption (%)**					**Consequent Prey Consumption by**
	1-DAT	**2-DAT**	**3-DAT**	**4-DAT**	**5-DAT**	
Aerial	25.37^{a} (18.36)	35.27^{a} (33.34)	46.18^{a} (52.05)	58.99^{a} (73.46)	70.98^{a} (89.38)	**Ground dwelling predators**
Ground dwelling	41.00^{b} (43.04)	58.93^{b} (73.36)	71.87^{b} (90.31)	80.31^{b} (97.17)	87.12^{b} (99.75)	**Aerial predators**
Control (No exclusion)	56.42^{c} (69.41)	72.31^{c} (90.76)	81.04^{c} (97.58)	86.88^{b} (99.70)	90.00^{b} (100.00)	**Both aerial and ground dwelling predators**
S. Em. ±	2.68	3.03	2.30	2.84	1.43	
C.D. (5%)	8.44	9.56	7.24	8.94	4.51	

DAT = Days after Treatment; Figures in parentheses are retransformed percent values; Values with similar letters are statistically at par

Predation potential of the major aphidophagous guild

The predation potential of the dominant aphidophagous guild comprising the adult and grubs of coccinellids of three species was evaluated by their feeding behavior at different aphid prey densities (25, 50, 75, 100, 125 and 150 per beetle). From the *Table 6* it can be inferred that the grubs of *Coccinella septempunctata* could feed significantly more aphids at the different densities evaluated followed by *Illeis cincta* and *Cheilomenes sexmaculata*.

Table 6. *Feeding behaviour of aphidophagous coccinellid grubs at different prey densities*

Aphid Prey Provided (№:s/ 24h)	***Coccinella septempunctata***		***Illeis cincta***		***Cheilomenes sexmaculata***	
	Prey Consumed (№:s/ 24h)	**Feeding (%)**	**Prey Consumed (№:s/ 24h)**	**Feeding (%)**	**Prey Consumed (№:s/ 24h)**	**Feeding (%)**
25	20.55^{a}	82.20	19.70	78.80	19.00	76.00
50	22.95^{b}	45.90	21.65	43.30	20.55	41.10
75	24.70^{c}	32.93	23.00	30.66	21.15	28.20
100	22.10^{ab}	22.10	20.75	20.75	19.80	19.80
125	22.30^{ab}	17.84	21.72	17.37	20.05	16.04
150	21.40^{ab}	14.26	20.00	13.33	18.95	12.63
S. Em.±	**0.73**		**0.49**		**0.34**	
C.D. (5%)	**2.20**		**1.47**		**1.03**	

Prey = Maize aphid; values with similar letters are statistically at par.

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All the three coccinellid grubs increasingly and significantly consumed more aphids at aphid densities of 25, 50 and 75, but at higher aphid densities (100, 125 and 150) the consumption rate declined. The prey consumption on a number basis differed significantly at different densities with *C. septempunctata* feeding 20.55 to 24.70; *I. cincta* feeding 19.70 to 23.00 and *Ch. sexmaculata* feeding 18.95 to 21.15 aphids in 24 hours with the maximum being when 75 aphids as prey were provided. From the *Figure 4* on feeding behavior it becomes clear that an increase in prey density sharply decreased the per cent feeding by the grubs. Notwithstanding the fact that aphidophagous coccinellids prefer to feed on aphids, they are specific predators of aphids at low densities between 25 and 50. However, this gives an indication that field releases of coccinellid grubs might prove useful at higher aphid densities considering the feeding ability of a single coccinellid grub.

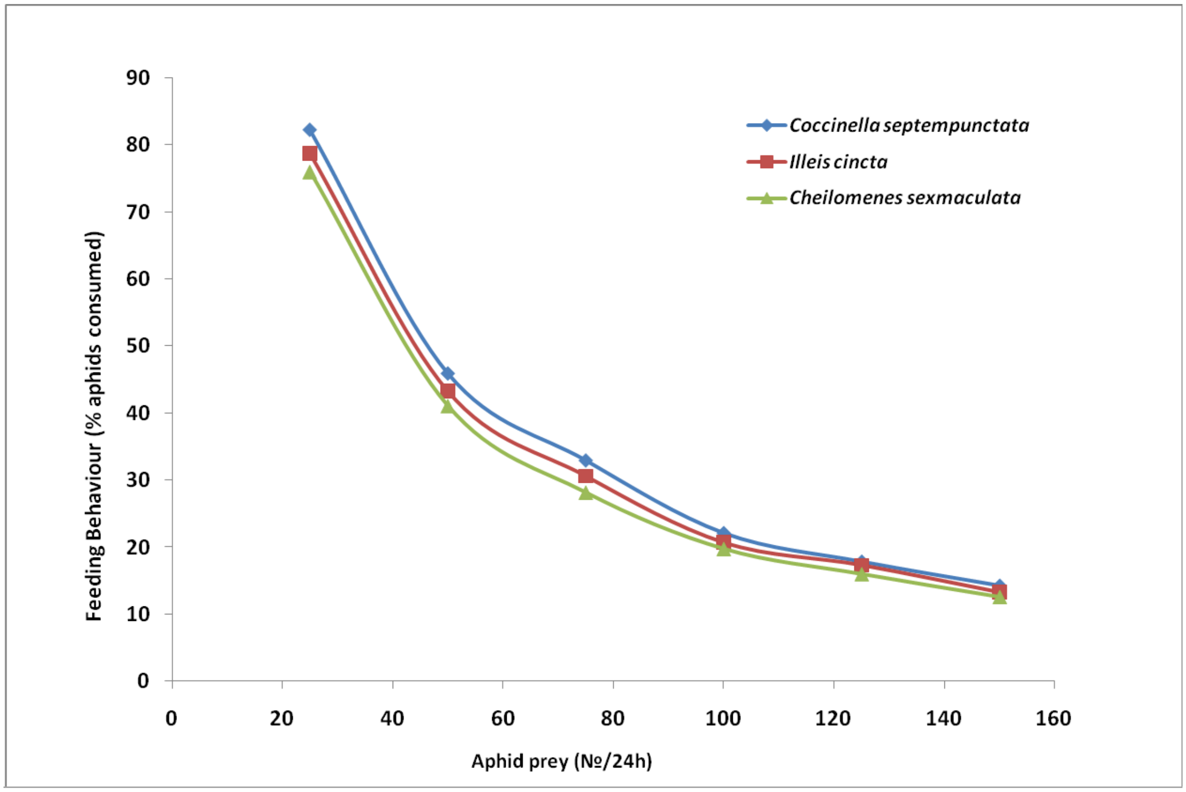

Figure 4. *Response of maize aphid density on coccinellid grub feeding behaviour during summer, 2013*

A perusal of the *Table 7* indicates that the adult coccinellids showed little difference in their feeding potential. Similar to the behavior of grubs, all the three adult coccinellids consumed more aphids with increase in the prey density up to 75 aphids per adult; thereafter their feeding capacity declined. The prey consumption on a number basis did not differ much at different densities with *C. septempunctata* feeding 18.70 to 22.55; *I. cincta* feeding 17.80 to 20.95 and *Ch. sexmaculata* feeding 15.95 to 19.35 aphids in 24 hours with the maximum being when 75 aphids as prey were provided. Similar to the feeding behavior

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of grubs, from the *Figure 5* it can be observed that an increase in prey density sharply decreased the per cent feeding.

Table 7. *Feeding behaviour of aphidophagous adult coccinellids at different prey densities*

Aphid Prey Provided (№:s/ 24h)	***Coccinella septempunctata***		***Illeis cincta***		***Cheilomenes sexmaculata***	
	Prey Consumed (№:s/ 24h)	**Feeding (%)**	**Prey Consumed (№:s/ 24h)**	**Feeding (%)**	**Prey Consumed (№:s/ 24h)**	**Feeding (%)**
25	18.70^{a}	74.80	17.80^{a}	71.20	16.80^{a}	67.20
50	20.40^{b}	40.80	19.60^{ab}	39.20	17.50^{ab}	35.00
75	22.55^{c}	30.06	20.95^{c}	27.93	19.35^{b}	25.80
100	20.10^{b}	20.10	18.95^{a}	18.95	17.20^{a}	17.20
125	20.20^{b}	16.16	19.20^{ab}	15.36	17.35^{ab}	13.88
150	19.55^{ab}	13.03	17.95^{a}	11.96	15.95^{a}	10.63
S. Em. ±	**0.35**		**0.39**		**0.46**	
C.D. (5%)	**1.05**		**1.18**		**1.39**	

Prey = Maize aphid; values with similar letters are statistically at par

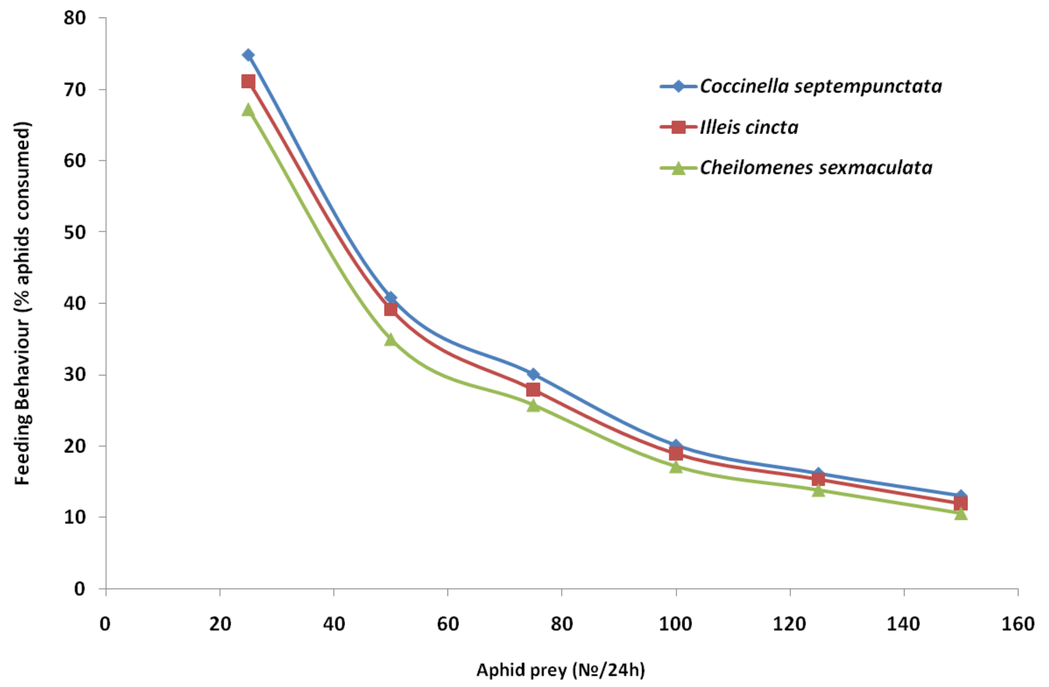

Fig. 5: Response of maize aphid density on adult coccinellid feeding behaviour during monsoon, 2013

Figure 5*. Response of maize aphid density on adult coccinellid feeding behaviour during monsoon, 2013*

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Discussion

Earlier workers have also reported *Rhopalosiphum maidis* (Fitch) as the major aphid pest on maize (Razmjou and Golizadeh, 2010) ; however, *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum* were also recorded in addition (Coderre and Tourneur, 1988; Coderre et al., 1989; Plewa and Pankanin-Franczyk, 1989). During 1988 and 1989, *Schizaphis graminum* and *Rhopalosiphum maidis* were reported to infest maize (Darwish and Ali, 2001). The aphid, *Rhopalosiphum maidis* was recorded on maize from 3 regions of Romania to be controlled by 23 species of predatory insects, including 2 anthocorids, 3 nabids, 2 chrysopids, a cantharid, 2 malachiids, 10 coccinellids and 3 syrphids (Voicu, 1989). Coccinellids (4 species) and chrysopids (3 species) were the most abundant predators, followed by syrphids and nabids, and 6 species of aphidiid [Braconidae] parasitoids (Plewa and Pankanin-Franczyk, 1989). Among aphid predators, the coccinellids, *Coleomegilla maculata lengi* and *Hippodamia tredecimpunctata* were significantly more abundant in the monoculture than in the maize intercropped with beans, but *Coccinella septempunctata* and spiders were not (Coderre et al., 1989). Though coccinellids (*Coccinella septempunctata*, *Adonia variegata*, *Propylaea quatuordecimpunctata*) were the most common aphid-specific predators, polyphagous predators were more abundant, mainly anthocorids (*Orius* spp.), carabids (*Demetrias atricapillus*, *Harpalus rufipes*, *Bembidion* spp., *Poecilus cupreus*, *Agonum dorsale*), dermapterans (*Labidura riparia*, *Forficula auricularia*), nabids (*Nabis provencalis*) and spiders (Asin and Pons, 1998). The relatively more common aphidophagous predators on maize belonged to the insect families Chrysopidae, Coccinellidae, Nabidae and Syrphidae; types of soil, forerunner crops and fertilizers affected the dynamics of the aphid-predator system in Romania (Paulian, 1999). Similar to our observation, among coccinellids, *C. septempunctata*, *C. transversalis*, *Brumoides suturalis* and *Cheilomenes sexmaculata* are the most widespread (Singh and Brar, 2004). Thus, it can be inferred that coccinellids happen to be the most dominant aphidophagous predator of maize aphids as recorded during the present investigation; however, the coccinellid, *Illeis cincta* was equally abundant in our trials that earlier workers have not reported, which could be specific to our climatic conditions.

The aphid, *Rhopalosiphum* (two species) showed a bimodal seasonal distribution, with a decrease in abundance at the end of July. Climatic factors and emigration could not explain this decrease, but was probably related to diminished plant nutritional quality and an increase in predation (Coderre and Tourneur, 1988). Aphids occurred at the beginning of August, and reached their maximum abundance (406 and 518 per plant) in the 4th week of August, when the plants were in their reproductive stage. Aphid populations began to decrease from the 3rd week of September, to reach their lowest levels in mid-October, when the plants were mature (Darwish and Ali, 2001).

The mean daily consumption of the aphid, *R. maidis* by adults and larvae of *C. septempunctata* to be 30.4 and 27.7 at 18.6^o C and 71 per cent relative humidity that increased with an increase in the daily temperature (Harjit and Deol, 1999). Contrary to our findings that coccinellids were more efficient at lower aphid densities, earlier it was reported that the number of attacks by coccinellid adults increased significantly and linearly with increasing prey density. Adult consumption rates were 82-85 per cent in 1997 and 76-

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97 per cent in 1998. Larvae consumption also increased significantly and linearly with prey density and the consumption rates were 80-93 per cent in 1997 and 85-94 per cent in 1998 (Dicko, 2000). Adults of *C. septempunctata* consumed a significantly higher number of aphids than larvae of *Syrphus* sp., but less than adults of *M. sexmaculata*. Adults consumed a significantly higher number of maize aphids than immature stages. Grubs of *C. septempunctata* were more active than maggots of *Syrphus* (Singh and Marwaha, 2002).

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PREDICTING ASSAM TEA DISTRIBUTION IN UPPER NORTHERN THAILAND USING SPECIES DISTRIBUTION MODELS

NANGLAE, S.[1] – NILTHONG, R.[1*]

[1]*School of Science, Mae Fah Luang University*
333 moo 1, Thasud, Muang, Chiang Rai 57100 THAILAND
(phone: +66-5-391-6775; fax: +66-5-391-6776)

**Corresponding author*
e-mail: rungrote@mfu.ac.th

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Abstract. The aim of this study is to build species distribution models and find factors affecting Assam tea distribution in upper Northern Thailand. The data were analyzed by the logistic regression of 185 records of Assam tea and generated pseudo-absence data using the two-step method. The re-sampling technique used the bootstrapping method to interpolate small sample size problems, and the k-fold cross-validation technique was used to find the best testing data set. Furthermore, statistical values (average value, standard deviation and coefficient of variation) were used to find the best final model. From the effective predictor variables at a significance level of 0.05: aspect, annual mean temperature, mean temperature of the wettest quarter, annual precipitation, precipitation of the wettest month and precipitation of the driest quarter all had positive effects upon model. Conversely: distance from the river, minimum temperature of the warmest month, precipitation of the driest month and precipitation of the wettest quarter all had negative effects upon model. Furthermore, the most suitable areas were found to be predominantly in the north-northwest region of upper Northern Thailand.

Keywords: *species distribution models, logistic regression, Assam tea, resampling technique*

Introduction

Assam tea (*Camillia sinensis* ver. *assamica*) or wild tea is the most commonly used plant in tea production. It originates in India and naturally distributes through highland forests where the climate is rainy and moderately sunny. Assam tea has been continuously maintained in the forests and is recognized as an agroforestry system. The Assam tea forests cover approximately 170,000 km^2 in Thailand, and 45% of these regions consist of highland areas (Pagella et al, 2002). Interestingly, many biodiversified features are found in the Assam tea forests, especially herbal plants. Preechapanya (1996) found that there were 149 plant species from 71 families in the Assam tea forest area of Phi Pan Nam Mountain. Currently, village expansion and farming in and around the Assam tea forests is on the increase, which is resulting in a rapid decrease of numbers of Assam tea forests (Kowsuvon, 2008). A survey of Assam tea distribution is difficult and high cost. Therefore, alternative approaches such as mathematical or statistical models could be tools used to predict the most suitable areas when considering environmental factors. One approach that can be used to identify most suitable areas is ‘Species Distribution Models’ (SDMs).

Species Distribution Models (SDMs) are important tools for predicting how species distribute across all studied areas. They have been used in many fields of biology, such as conservation planning, surveying, evolution studies or assessing the impacts of climate change (Thuiller et al., 2005; Guisan and Thuiller, 2005; Engler et al.,

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2004; Marini et al., 2009). In general, the main objectives for building species distribution models are to indicate appropriate environmental factors, including topography, climate or geology, and to identify areas which generate the most suitable environmental factors (Pearson, 2007). SDMs can be divided into two groups of techniques: The first group is profile techniques, such as environmental envelope (BIOCLIM) or environmental distance (DOMAIN) (Franklin, 2010). These techniques require presence-only data which are based upon the ranges, means, or other values of environmental variables for locations where a species of interest has been in existance. The second group is group discrimination techniques, such as generalized linear models (GLMs), generalized additive models (GAMs), boosted regression trees (BRTs) etc. which need both presence and absence data (Stokland et al., 2011) in order to generate statistical functions or discriminative rules. Generally, group discrimination techniques can produce much better results than profile techniques (Mateo et al., 2010), primarily because these techniques acquire absence data or background data used to improve the relationships between species occurrence and environmental variables (Brotons et al., 2004). However, such group discrimination techniques lack true absence data, and therefore pseudo-absence data or random samples of the background data need to be generated for the inclusive models. Pseudo-absence data are based upon statistical theory, while random samples of background data are based upon machine-learning; e.g. maximum entropy (Maxent) (Mateo et al., 2010). Contemporary statistical analysis has been used extensively, including SDMs. From 1998-2007, approximately 70-80% of articles published in the *Journal of Wildlife Management* and *Landscape Ecology Journal* used regression-based models in predictive modeling (Drew et al., 2011). These methods also need absence or pseudo-absence data in their models. Most pseudo-absence data can be divided into two groups (Lobo and Tognelli, 2011). The first group chooses pseudo-absence data by simple and random selection in the study area (Barbet- Massi et al., 2012; Lobo and Tognelli, 2011). The second group chooses pseudo-absence data using the following two-step techniques (Barbet-Massin et al., 2012; Lobo and Tognelli, 2011; Wisz and Guisan, 2009): First, build suitable areas using profiling techniques, and then randomly select pseudo-absence data outside of those suitable areas. The models with two-step pseudo-absence data produce better results than those with random pseudo-absence. However, the results of these models could lead to weaker predictive power because they lead to over fitting (Wisz and Guisan, 2009).

The objectives of this study were to find the factors affecting the distribution of Assam tea in upper Northern Thailand, and to identify suitable environmental areas for Assam tea production using logistic regression analysis (GLMs technique). The selection of pseudo-absence data using the two step technique and the bootstrap technique were applied to this model, and a resampling technique (bootstrap) was used to remedy the sample size limitations.

Methods

Study area and occurance data

The upper Northern part of Thailand covers nine provinces, namely: Chiang Mai, Chiang Rai, Mae Hong Son, Nan, Phayao, Phrae, Uttaradit, Lampang and Lamphun (shown in *Figure 1*). The area is situated between latitudes 17° 6’ 39” - 20° 29’ 50” N and longitudes 97° 16’ 36” - 101° 27’ 55” E, and covers an area of 96,025 km^2. Most areas are hilly and mountainous, and approximately 64% is covered by forest (Forest Land Management Bureau, 2013).

The data used in this study were obtained from the Tea Institute of Mae Fah Luang University, Thailand. One hundred and eighty five records of Assam tea data (presence data) in the form of geographic coordinates were collected. These data were part of a collection of tea varieties grown a in Northern Thailand project (Sampanvejsobha et al., 2009).

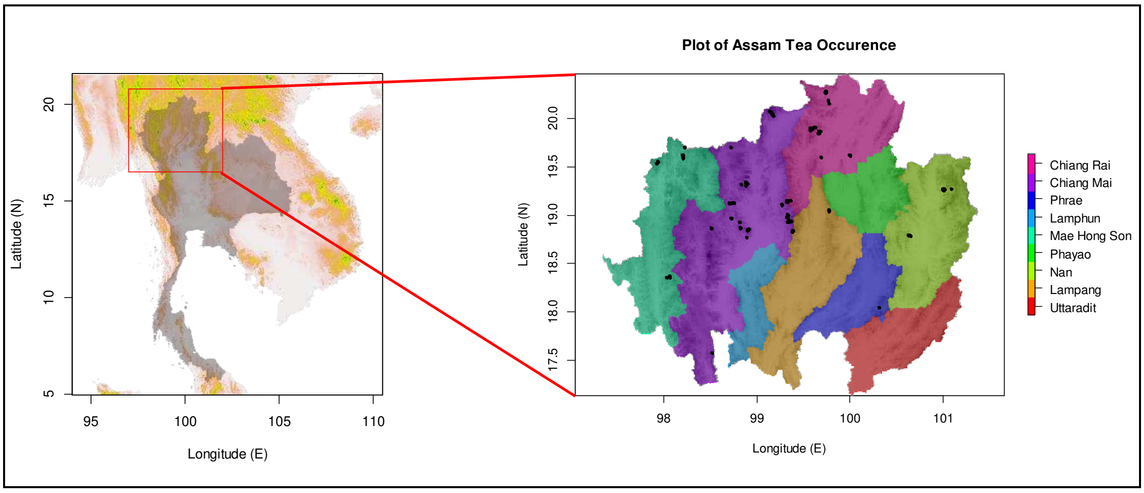


Figure 1. *The demarcation of study areas including 9 provinces in Northern Thailand. The black dots indicate 185 records of Assam tea data.*

Environmental data

Environmental data were separated in two groups: topography factors and climate factors (*Table 1.*). Topography factors were composed of elevation (DEM), slope aspect (Aspect), distance from the river (Distance) and modified groups of rock type with slope (Class1-Class8). The climate factors were composed of relative humidity (Humidity) and bioclimatic variables (Bio1-Bio19). DEM data were obtained from the Land Development Department, Thailand. The distance was calculated from river data that had been obtained from DIVA-GIS (http://www.diva-gis.org). The aspect was calculated from the elevation data, and relative humidity was obtained from the Marc Souris's page (http://www.rsgis.ait.ac.th/~souris/thailand.htm). Bioclimatic variables were obtained from the WorldClim database, which offered approximately 1 km^2 of spatial resolution (Hijmans et al., 2005; http://www.worldclim.org). The WorldClim data were derived from measurements of altitudes, temperatures and rainfall from weather stations across the globe (Period 1950-2000). Finally, nineteen bioclimatic variables from the WorldClim datasets were used to assess current climatic conditions.

Generating pseudo-absence points

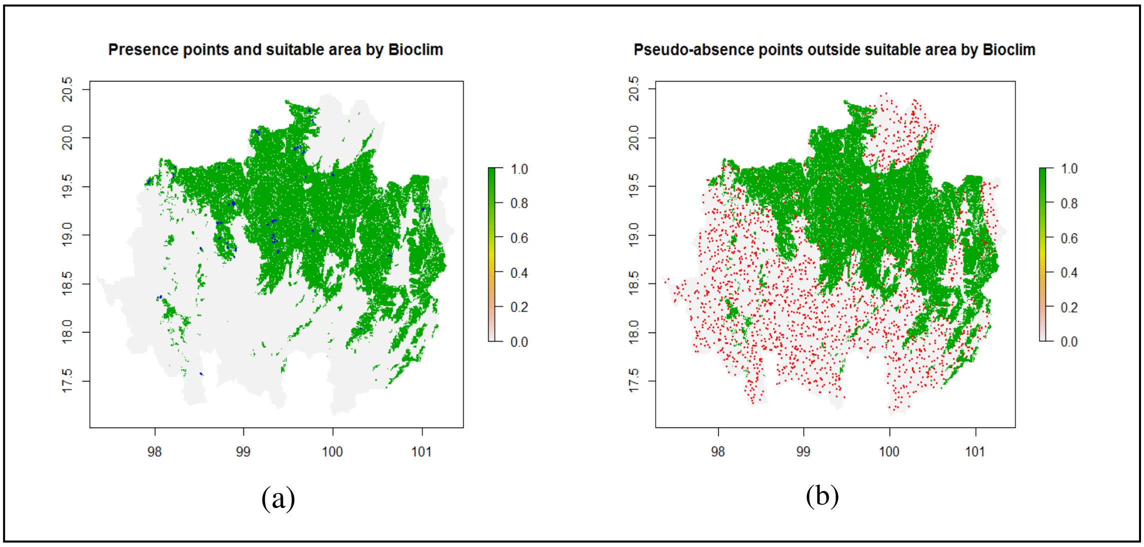
Pseudo-absence points were generated using the two-step method (Barbet-Massin et al., 2012; Lobo and Tognelli, 2011; Wisz and Guisan, 2009): First, a suitable area was built using profiling techniques. In this work, the Bioclim method was used to build a suitable area using the *bioclim* function on dismo package in R programming.

Second, pseudo-absence points were randomly chosen outside of the suitable area (as shown in *Figure 2*).

Table 1. *The variables used in the species distribution models*

Variable	Unit	Description	Variable	Unit	Description
DEM	m	Elevation	Bio5	°C × 10	Max Temperature of Warmest Month
Distance	km	Distance from the river	Bio6	°C × 10	Min Temperature of Coldest Month
Aspect	degree	Direction of the slope facing (0-360 degree)	Bio7	°C × 10	Temperature Annual Range
Class1	-	Sedimentary and Metamorphic rocks, slope 0-15 degree	Bio8	°C × 10	Mean Temperature of Wettest Quarter
Class2	-	Sedimentary and Metamorphic rocks, slope 15-30 degree	Bio9	°C × 10	Mean Temperature of Driest Quarter
Class3	-	Sedimentary and Metamorphic rocks, slope 30-45 degree	Bio10	°C × 10	Mean Temperature of Warmest Quarter
Class4	-	Sedimentary and Metamorphic rocks, slope >45 degree	Bio11	°C × 10	Mean Temperature of Coldest Quarter
Class5	-	Igneous rocks, slope 0-15 degree	Bio12	mm/year	Annual Precipitation
Class6	-	Igneous rocks, slope 15-30 degree	Bio13	mm/ month	Precipitation of Wettest Month
Class7	-	Igneous rocks, slope 30-45 degree	Bio14	mm/ month	Precipitation of Driest Month
Class8	-	Igneous rocks, slope >45 degree	Bio15	%	Precipitation Seasonality
Humidity	%	Relative humidity	Bio16	mm/ quarter	Precipitation of Wettest Quarter
Bio1	°C × 10	Annual Mean Temperature	Bio17	mm/ quarter	Precipitation of Driest Quarter
Bio2	°C × 10	Mean Diurnal Range	Bio18	mm/ quarter	Precipitation of Warmest Quarter
Bio3	%	Isothermality	Bio19	mm/ quarter	Precipitation of Coldest Quarter
Bio4	%	Temperature Seasonality			

DEM was obtained from the Land Development Department, Thailand.
Distance was calculated from river data downloaded from http://www.diva-gis.org.
Aspect was calculated from Elevation.
Class1to Class8 were dummy variables.
Humidity was downloaded from http://www.rsgis.ait.ac.th/~souris/thailand.htm.
Bio1to Bio19 were downloaded from http://www.worldclim.org.



(a) (b)

Figure 2. *(a) Suitable areas (green areas) made using the Bioclim method and presence points (blue points); (b) Pseudo-absence points (red points) selected outside the suitable area.*

Logistic regression

Logistic regression is one type of generalized linear model (GLM) that was introduced by Nelder and Wedderburn in 1972. It is suitable for analysis when response data are binary. In this work, the species distribution model was specified by data occurrence (presence data as 1, and absence data as 0). This model used logit link (or logit transformation) to describe the relationships between the response probabilities and the 31 predictor variables (*Table 1*). The logit link is modeled as a linear function:

$$\text{logit}(p) = b_0 + \sum_{i=1}^{n} b_i x_i \qquad \text{(Eq.1)}$$

Where, p is response probabilities to be modeled, n is the number of predictor variables, (xi) and $b_0, b_1, \ldots, b_n$ are the regression constants. The model was fitted using the maximum likelihood method. The prediction of the model was formed as an exponential function:

$$P(Y) = \frac{e^{\text{logit}(p)}}{1 + e^{\text{logit}(p)}} \qquad \text{(Eq.2)}$$

Where, *P(Y)* is the probability of interested event (probability of presence area). This was transformed into a continuous probability Y ranging from 0 to 1.

In this study, the *glm* function in R programming was used for logistic regression and the *logit* on *glm* function was chosen as the link function.

Model processing

In *Figure 3*, data including presence points, pseudo-absence points and environmental data were divided into 5 groups labeled with random numbers 1 to 5. Then, group 1 was assigned as testing data and the remaining groups (group 2, 3, 4 and 5) were assigned as training data. The testing data were used to evaluate the models and the training data were used to build the models.

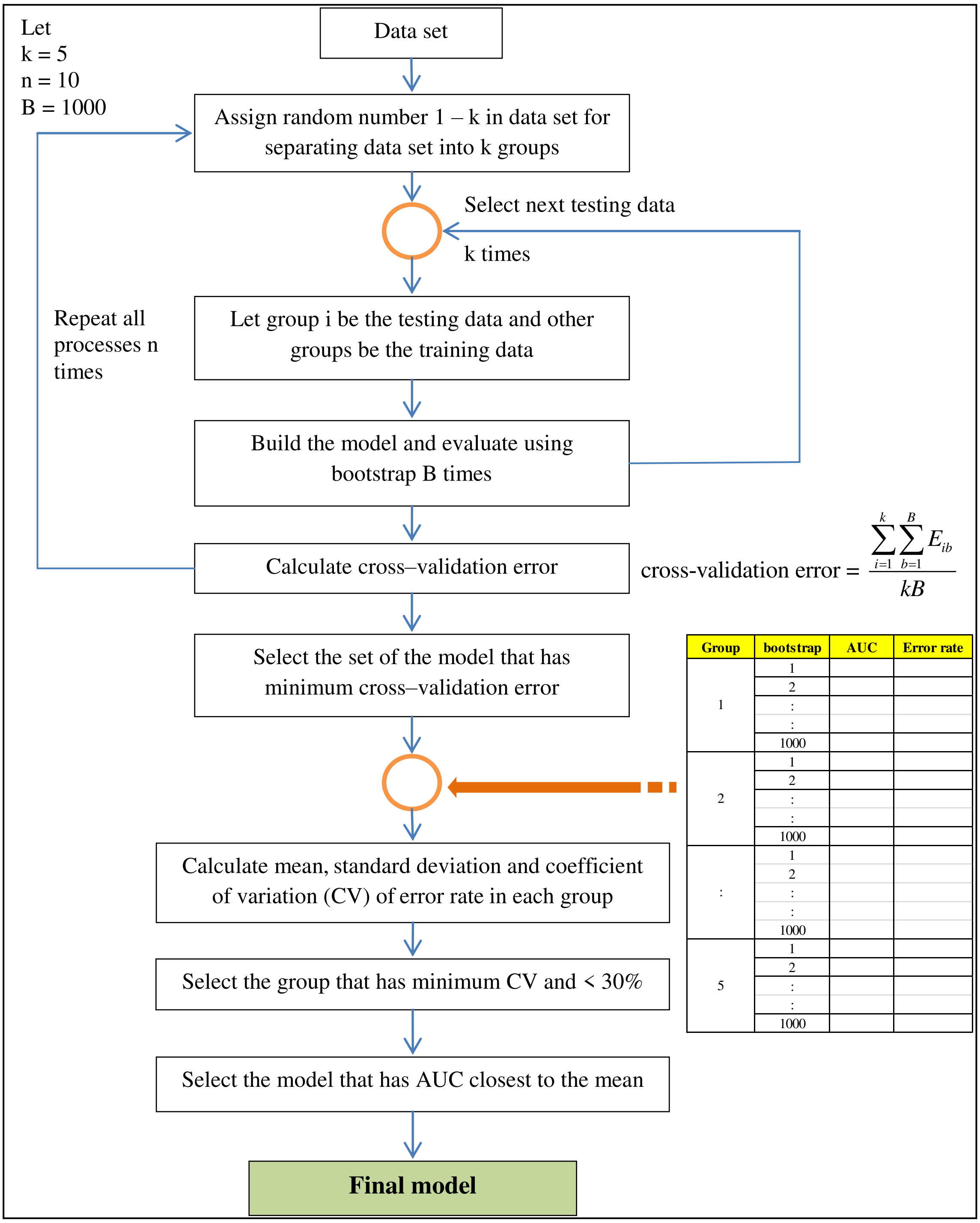


Figure 3. *The model process for selecting the final model for one set of pseudo-absence points with 5 (k)-fold cross-validations, 1000 (B) bootstraps and 10 (n) iterations of all processes*

We evaluated the models using the evaluate function on dismo package in R programming. Bootstrapping with 1000 replications was needed for the training data to build 1000 models in logistic regression. Next, each of the 1000 models was evaluated using the testing data to provide areas under the receiver operating characteristic curve (AUC) and error rate. After that, the next group (group 2) was used as new testing data and the remaining groups would be new training data. The process was repeated until completing all testing data (5 data groups) and obtaining 5000 models with AUC and error rate values. Cross-validation error was then calculated from the average value of 5000 error rates, and then we reassigned random numbers 1-5 to the data set and repeated all processes 10 times, in order to provide 10 cross-validation error values. The best iteration would be selected from the minimum cross-validation error. Mean, standard deviation and coefficient of variation (CV) of error rates would be calculated within each group that had 5000 models. Next, the group that had a minimum CV at less than 30% would be chosen. CV is normally used to measure dispersion and a CV of less than 30% indicates a normal distribution (Forkman, 2005). Finally, the mean of AUC was calculated and the final model would be selected from the model that had AUC closest to the mean AUC. The final model represents the best logistic regression from one set of pseudo-absence points.

In this study, 5 suitable area maps were generated using the Bioclim method (*Figure 4*). In each map, 20 sets of pseudo-absence points for each suitable area map were generated.

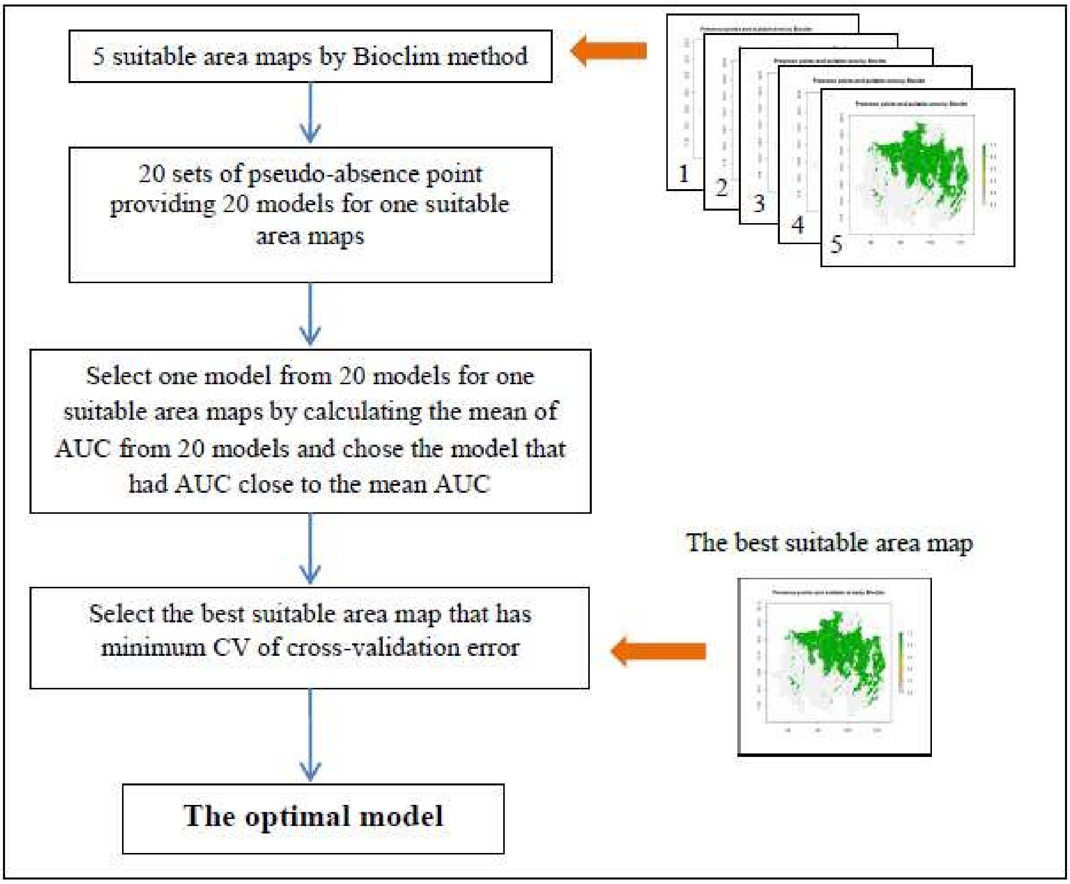


Figure 4. *The process for selecting the optimal model from 5 suitable area maps using the Bioclim method*

To select the best pseudo-absence set among the 20 sets in each suitable area map, the mean value of AUC was calculated and the model that had AUC closest to the mean was chosen. Next, the best suitable area map was selected for its minimum value of coefficient of variation of cross-validation error. Finally, the optimal model would be processed using the stepwise method to search for effective predictor variables using the step function in R programming along with Akaike's information criterion (AIC). The most suitable model would give the lowest AIC value.

Results and Discussion

Selecting the optimal model

From *Table 2.*, the means of AUC at each suitable area map were similar. Values of 0.93-0.94 were considered most outstanding discrimination (Hosmer and Lemeshow, 2000) and highly successful for an accurate predictive model.

The suitable area map with the minimum coefficient of variation (CV) of cross-validation error was selected in order to reduce bias in the iteration process. Therefore, the group that had minimum CV would have minimum dispersion. Eventually, the most suitable area (map 1) and optimal model in pseudo-absence (set 1) was selected, as seen in the dotted box in *Table 2* below:

Table 2. *The average of AUC and coefficient of variation of cross-validation error at each suitable area map. (The dotted box shows the optimal model from suitable area map set 1.)*

Suitable area map (Bioclim)	Mean of AUC	The best pseudo-absence set	AUC	Coefficient of variation of cross-validation error
1	0.94840	1	0.94838	0.00151
2	0.94027	13	0.93994	0.00307
3	0.93958	16	0.94025	0.00551
4	0.93754	3	0.93768	0.00308
5	0.93978	17	0.93974	0.00326

Factors affecting the model

The stepwise method was used to choose the variables affecting the model, using Akaike information criterion (AIC) after the optimal model had been selected. From *Table 3.*, 18 variables were selected and 10 variables were at a significance level of 0.05. Although some variables (Humidity, Bio2, Bio3, Class1, Class2, Class5, Class6 and Class7) in the model were not apparently significant, they were used in the model due to their presumed involvement, and they were selected in the model using the stepwise method. The most significant variables were topography variables (Aspect and Distance), temperature variables (Bio1, Bio6 and Bio8), and precipitation variables (Bio12, Bio13, Bio14, Bio16 and Bio17), respectively.

Considering topography variables, Aspect, Class1, Class2, Class5 and Class6 had positive effects, while Distance and Class7 had negative effects. Thus, the most suitable area for Assam tea would need more solar radiation because the northwest slope is sunnier and drier than the northeastern slope (Fekedulegn et al., 2003). When considering other topography variables, the most suitable area for Assam tea would need a slope of not more than 30 degree and not be far away from the river or water source.

Table 3. *Factors were selected in GLMs using the stepwise method.*

Factor	Estimate	Sig.	Factor	Estimate	Sig.
Aspect	0.003	*	Bio13	0.064	*
Distance	-0.390	*	Bio14	-0.627	*
Humidity	-0.110		Bio16	-0.107	*
Bio1	0.314	*	Bio17	0.156	*
Bio2	0.260		Class1	17.360	
Bio3	0.538		Class2	17.920	
Bio6	-0.917	*	Class5	17.680	
Bio8	0.532	*	Class6	17.300	
Bio12	0.042	*	Class7	-70.300	
Intercept = -176.800					

“ * ” 0.05 significance level

Considering temperature variables, Bio1, Bio2, Bio3 and Bio8 had positive effects, while only Bio6 had negative effects. The average temperature of Bio1 implicates that the annual average temperature should not be too low for the most suitable area for Assam tea production. Moreover, the fluctuation of temperature between day and night (Bio2) and mean temperature in the wettest quarter (Bio8) should be high, while mean temperature should be sufficiently cold in the coldest month (Bio6). Isothemality (Bio3) is a proportion between the day-to-night temperatures and the summer-to-winter temperatures. Thus, the most suitable area for Assam tea should have high isothemality and/or be far away from the sea.

Considering precipitation variables, Bio12, Bio13 and Bio17 had positive effects, while Bio14 and Bio16 had negative effects. Bio12 indicates that the most suitable area for Assam tea should have sufficient precipitation all year round. It should have high precipitation in the wettest month, but not too high during the whole wettest quarter. Moreover, it should have low precipitation in the driest month, but not too dry during the whole driest quarter. In conclusion, the most suitable area for Assam tea production is possibly in the monsoon regions (Elliott et al, 2006), with two distinct wet seasons and two distinct dry seasons each year.

Predicting map and evaluation

To predict the most suitable area for Assam tea, threshold (or cut-offs) was used to transform the probability map to a presence/absence map by maximizing the sum of the sensitivity and specificity method, and only forest areas were taken into consideration (*Figure 5*). This showed that most Assam tea is greatly distributed in the northwest, towards the North. Most areas are located in Chiang Mai, and in some parts of Chiang Rai and Mae Hong Son provinces.

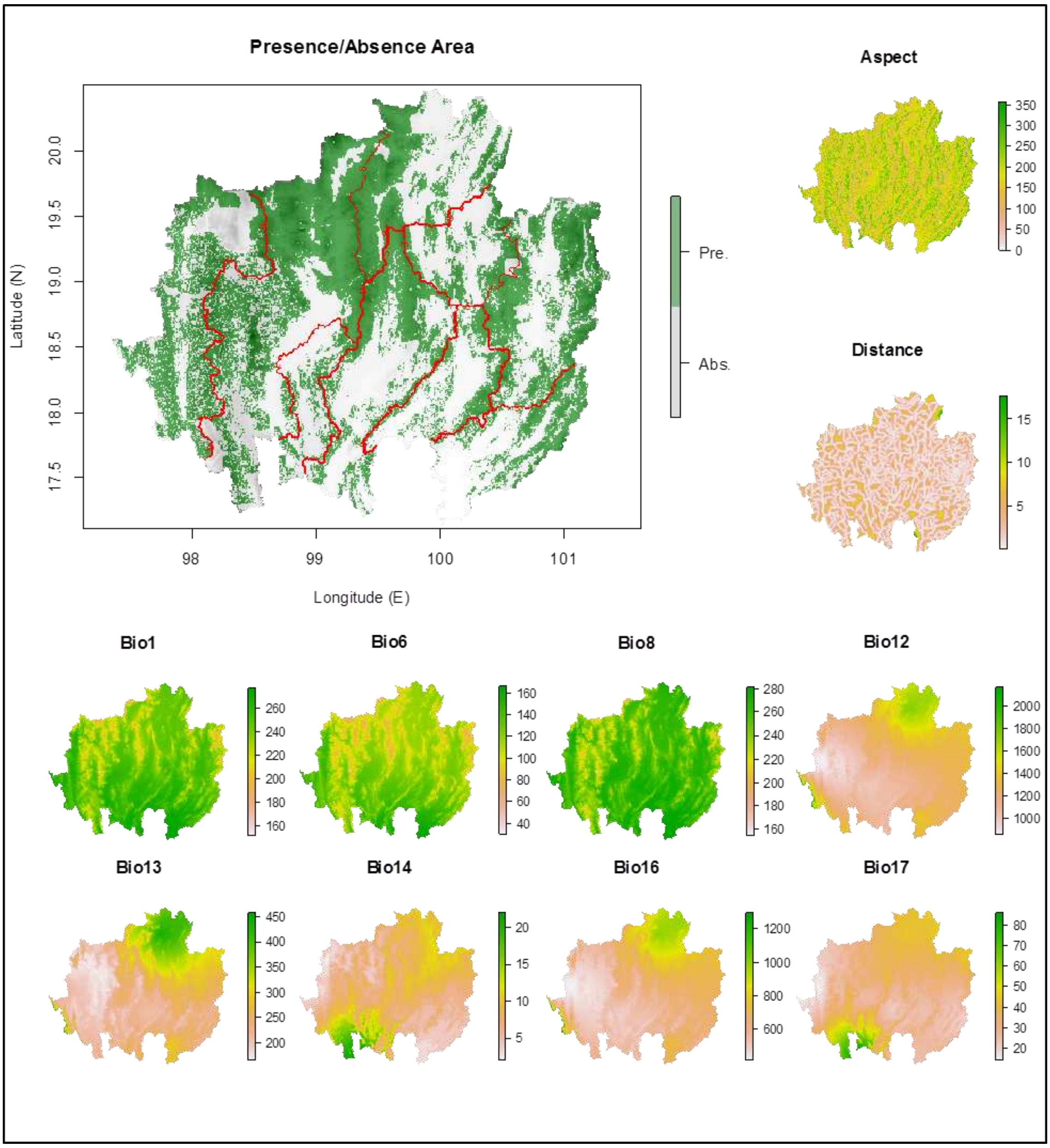


***Figure 5.** The most suitable areas for Assam tea forests and effective predictor variables were at a significance level of 0.05*

Conclusion

GLMs depicting the potential distribution of Assam tea with a high AUC value indicates great success as a predicting model. The potential distribution of Assam tea depends upon topographic and climate variables, with Assam tea being dispersed in a forest that has a slope of not more than 30 degrees and is not too far away from a river source. 'Aspect and Distance' were the two most important topography predictors of Assam tea distribution, while Bio1, Bio6 and Bio8 were the most important temperature predictors, along with Bio12, Bio13, Bio14, Bio16 and Bio17 being the most important precipitation predictors. These data implicate that Assam tea grows well in tropical forests and highland areas because the average temperature in the wettest quarter is high

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and the minimum temperature is low in the coldest month. Thus, potential areas must have sufficient precipitation, but not too much precipitation during the monsoon period. The predictive maps showed taht the most suitable areas were predominantly located in the northwest, towards the North. Climate change is currently a key factor in environmental change. It affects organisms and the biodiversity of the ecological system. Therefore, this study of potential distribution can be beneficial for Assam tea conservation and land management in and around surrounding Assam tea forests. More research is needed to determine whether any available defended areas sufficiently cover the most suitable areas for Assam tea production. The methodological models for Assam tea could be applied to other plant species in other areas of study as well.

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NO EVIDENCE FOR HISTORICAL DECLINES IN POLLINATION SUCCESS IN HUNGARIAN ORCHIDS

MOLNÁR V., A. [1*] – LÖKI, V. [1] – TAKÁCS, A.[1] – SCHMIDT, J.[2] – TÖKÖLYI, J.[2] –
BÓDIS, J.[3] – SRAMKÓ, G.[1, 3]

[1] *Department of Botany, University of Debrecen,*
Egyetem tér 1., H-4032, Debrecen, Hungary,
(phone: +36-52-512-900 / 62648; fax: +36-52-512-943)

[2]*MTA-DE Lendület' Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen,*
Egyetem tér 1., H–4032 Debrecen, Hungary,
(phone: +36-52-512-900 / 62331; fax: +36-52-512-941)

[3]*Department of Plant Sciences and Biotechnology, University of Pannonia, Georgikon Faculty,*
H-8360 Keszthely, Festetics u. 7., Hungary,
(phone: +36-83-545-095; fax: +36-83-545-058);

[4]*MTA-ELTE-MTM Ecology Research Group,*
Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary
(phone: +36-52-512-900 / 62632; fax: +36-52-512-943)

**Corresponding author*
e-mail: mva@science.unideb.hu

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Abstract. Pollination crisis (the decline of pollinator populations) is a global phenomenon which threatens biodiversity, human welfare and economy. The degree to which different plant populations/species are affected by pollination crisis is still unclear. In this study, long-term herbarium dataset was used to quantify the reproduction rate of Hungarian orchids between 1853 and 2008. We quantified fruit-set rate of 663 specimens belonging to 27 species. Data were available from an average of 10.3±9.3 localities, 76.5±43.2 years, and 23.4±25.6 specimens per species. Herbarium data were validated with field-observed data in case of the different pollination strategies. According to our results, the reproductive success of the vast majority of orchid species has not changed during time and pollination crisis is not apparent in Hungary at least until the end of the 20th century.

Keywords: *herbaria, natural history collections, Orchidaceae, pollination crisis, pollination mode*

Introduction

Pollinators provide key ecosystem services in most terrestrial ecosystems (Chee, 2004; Boyd and Banzhaf, 2007; Wallace, 2007; Fisher and Turner, 2008). Their activity is crucially important in the long-term survival of indigenous plant communities (Ashman et al., 2004; Aguilar et al., 2006) and the productivity of cultivated plants (Klein et al., 2007; Ricketts et al., 2008). For most crop plants and wild plants (particularly in the temperate climate zones) the most important pollinators are insects, especially bees, which are declining parallel to insect-pollinated plants at global (Kearns et al., 1998; Potts et al., 2010) and western European (Biesmeijer et al., 2006) scale.

The main causes of the observed global pollination crisis have not been fully identified, but are potentially connected to the fragmentation and destruction of (semi-)

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natural habitats, spread of parasites, the use of chemicals in agriculture and global climate change. From this point of view, the recent and well documented climate change (Parmesan and Yohe, 2003; Root et al., 2003; Schröter et al., 2005; IPCC, 2007) deserves particular attention. Phenological shifts linked to changing climatic conditions were documented in different groups of plants (Fitter and Fitter, 2002; Parmesan, 2006; Post et al., 2008), including orchids (Robbirt et al., 2011; Molnár V. et al., 2012b). So far, little is known about climatic responsiveness of orchid-pollinators, but pollination mode was found to be the most important predictor of phenological response of central European terrestrial orchids (Molnár V. et al. 2012b). Nevertheless, any phenological mismatch between pollinators and flowering plants may substantially reduce the reproductive success of plants through decreased pollination efficiency (Fitter and Fitter, 2002; Hegland et al., 2009; Bartomeus et al., 2011; Rafferty and Ives, 2011). European terrestrial orchids represent an ideal research object for studying temporal changes in pollination efficiency for several reasons. First, since agamospermy (asexual seed production) is very rare in the family (Nygren, 1967; Catling and Catling, 1991), the presence of a fruit is almost always an indication of successful pollination (Neiland and Wilcock, 1995). Second, individual reproductive success of orchids is easy to estimate by counting the flowers and fruits on the shoot (Neiland and Wilcock, 1998). Third, orchids exhibit diverse pollination modes (self-pollinating, nectar-rewarding, food- and sexual deception (Dafni, 1984; Jersáková et al., 2006), consequently, their dependence on the abundance and diversity of pollinating insects differs between species. Fourth, different orchid species are attractive for specific group of insects, such as bees and wasps (Hymenoptera), butterflies (Lepidoptera) and beetles (Coleoptera) (Claessens and Kleynen, 2011).

Our aims in this study were (1) to characterize Hungarian orchid species in terms of reproductive success based on herbarium records, (2) determine the effect of pollination mode on fertilization success and (3) evaluate historical changes in reproductive success in orchids during the past decades.

Our predictions were the following: the reproductive success of autogamous species is temporarly invariable and relatively high (since they do not depend on pollinators), the reproductive success of entomophilous species depends on pollination mode: nectar-rewarding species show higher fructification rate than deceptive taxa, the reproductive success of entomophilous species (both nectar rewarding and deceptive) is decreasing due to the decline of pollinator populations.

Natural history collections contain important and useful information on the reproductive success of orchids in historical context (Farrell, 1985; Pauw and Hawkins, 2011). To test our hypotheses we collected data on fructification rate from all publicly accessible Hungarian herbaria and analyzed these with respect to pollination mode and time of collection.

Materials and methods

Quantifying fruit-set

We used the most widely utilised (and the easiest) method of measuring of reproductive success in orchids: quantifying fruit-set (Neiland and Wilcock, 1998). The Herbarium Database of Hungarian Orchids, compiled recently (Molnár V. et al., 2012a) allowed us to analyse reproductive success of 681 specimens belonging to 27 species based on a historical dataset which spans 155 years (*Table 1*).

Table 1. *List of the species and characterization of the dataset used in this study. Abbreviations: A - autogamous, FD – food deceptive, NR – nectar rewarding.*

Species	**Pollination mode**	**No. of localities**	**Date of collection**		**No. of specimens**
			Median±SD	**Duration (years)**	
Anacamptis coriophora	NR	11	1944±17	55	33
Anacamptis morio	FD	7	1945±26	73	15
Anacamptis palustris	FD	4	1944±52	106	7
Anacamptis pyramidalis	FD	14	1956±40	134	27
Cephalanthera damasonium	A	31	1946±32	132	71
Cephalanthera longifolia	FD	23	1939±26	104	30
Cephalanthera rubra	FD	9	1954.5±12	29	16
Cypripedium calceolus	FD	3	1939.5±13	27	6
Dactylorhiza incarnata	FD	19	1948±29	126	24
Dactylorhiza viridis	NR	7	1947±11	25	17
Epipactis atrorubens	NR	8	1919±21	61	24
Epipactis helleborine	NR	9	1926±19	71	17
Epipactis microphylla	A	22	1937±36	149	71
Epipactis palustris	A	16	1937±31	112	48
Epipactis purpurata	NR	11	1952±22	77	24
Epipactis tallosii	A	3	1948±38	66	6
Goodyera repens	NR	2	1954±1	2	6
Gymnadenia conopsea	NR	5	1922±39	106	7
Gymnadenia odoratissima	NR	2	1904±55	95	8
Limodorum abortivum	A	14	1952±30	93	26
Liparis loeselii	A	2	1969±16	38	10
Neottia ovata	A	12	1949.5±34	134	16
Neottia nidus-avis	A	38	1950±29	121	119
Orchis militaris	FD	13	1951±31	91	59
Orchis purpurea	FD	5	1942±31	77	6
Orchis simia	FD	2	1961±42	60	9
Platanthera bifolia	NR	25	1946±32	137	31

We considered only intact herbarium specimens collected in fruiting stage (*Fig. 1*). The number of flowers (both fertilized and non-fertilized) and the number of fruits were counted on each specimen collected at fruiting stage and identified at species level.

For statistical analyzes we used only species for which at least 3 herbarium records were collected. In the whole dataset the mean±SD numbers of records / species were 23.4±25.6, which were collected from 10.3±9.3 localities, during 76.5±43.2 years.

Life-history characterisation

Species were categorized into three groups according to their pollination mode – autogamy (including obligate and facultative self-pollination), nectar-rewarding entomophily and food deceptive entomophily, following Molnár V. et al. (2012b). A full list of all investigated species, as well as the sequence data used for phylogenetic reconstruction, is given in *Table 1.*

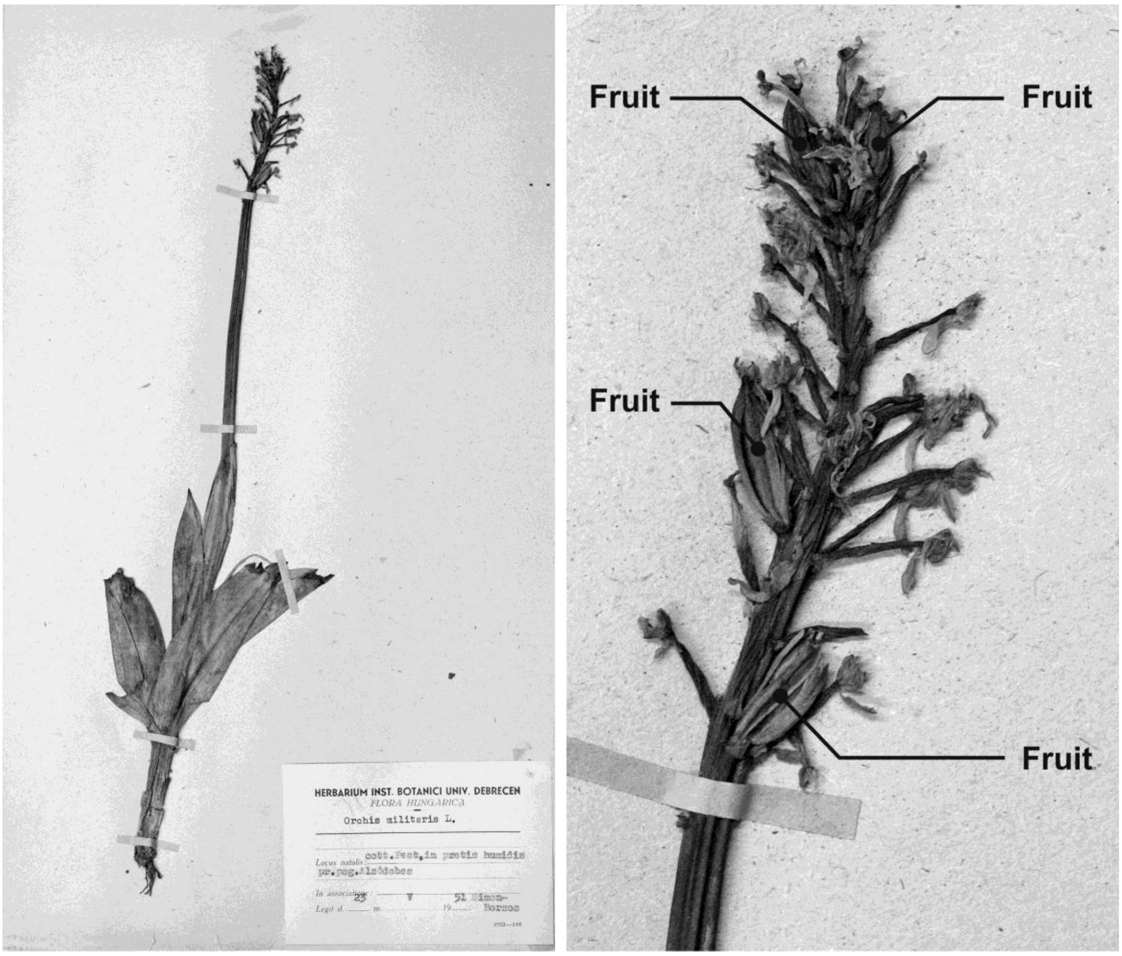

Figure 1. *Orchis militaris sheet from the collection of the Department of Botany at the University of Debrecen (DE), collected near to Dabas on the 23th of May in 1951, by Tibor Simon and Olga Borsos. Its four fruits are well identifiable.*

Statistical analyses

To analyze historical changes in reproductive success and its relationship to pollination mode, we used generalized linear mixed models (GLMMs). Since fruit-set is proportion data following a binomial distribution, we used binomial GLMMs with the number of fruits (successes) and number of unpollinated flowers (failures) as a bivariate response. Binomial GLMMs were performed using the MCMCglmm package (Hadfield, 2010) in the R Statistical Environment (R Core Team, 2014). MCMCglmm implements a Bayesian version of traditional GLMMs and it has the advantage that it can incorporate complex covariance structure in the random effects, such as the covariance arising from the phylogenetic relationship between species; hence, these models can be used to evaluate the relationship between species traits while controlling for the non-independence of data points arising from shared phylogenetic descent.

To prepare a phylogenetic tree that describes phylogenetic distances between the species studied, we used one of the most useful molecular phylogenetic marker, the nuclear ribosomal internal transcribed spacer (nrITS) region (Baldwin et al., 1995; Álvarez and Wendel, 2003). An alignment of the sequences was made by eye in BioEdit v.7.1.3 (Hall, 1999). The resulting matrix was subject to phylogenetic tree reconstruction under the maximum parsimony (MP) criterion in Paup v.4.0b*10 (Swofford, 2003) using a heuristic search with default settings but holding 10 trees at each iteration step and repeating the search 1000-times. As we used a geographically strongly biased sample-set (Hungarian orchids), we had to apply a backbone constraint to overcome false tree-reconstruction due to suboptimal taxon sampling. Thus, the well-established phylogenetic relationship of European orchids (Bateman et al., 2003; Bateman et al., 2005; Bateman, 2009) was fixed as constraint (see *Fig. 2*). The most parsimonious phylogenetic trees compatible with our constraint were saved with branch length corresponding to mutational changes (i.e. as phylograms), then one of these trees was made ultrametric by applying the non-parametric rate smoothing algorithm (Sanderson, 1997) as implemented in r8s v.1.71 (Sanderson, 2003). Statistical robustness of our MP reconstruction was assessed by the non-parametric bootstrap procedure applying 1000 pseudo-replicates in Paup. This above procedure allowed us to assess the genetic distance between each species we studied, and the resulting ultrametric tree (*Fig. 2*) was used as input for analyses using the phylogenetic control.

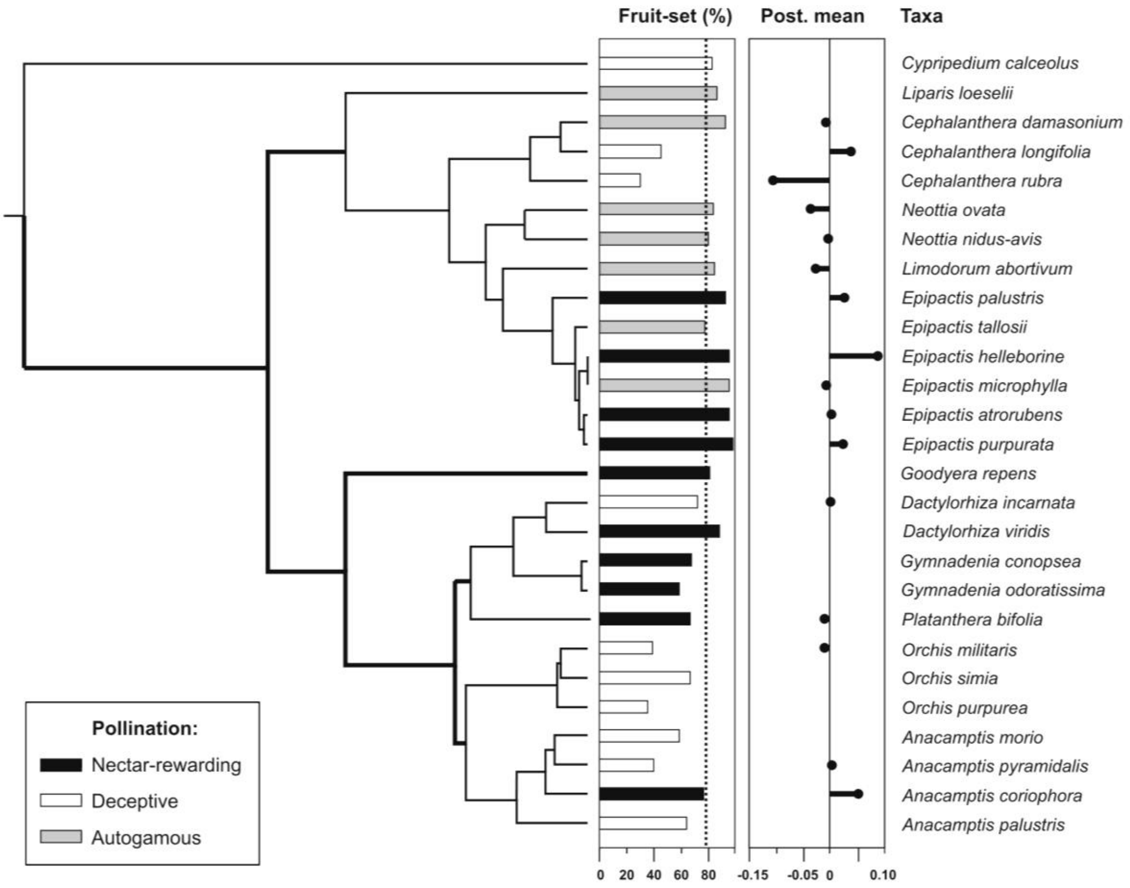

Figure 2. *The favoured phylogenetic tree of four equally most parsimonious trees made ultrametric by non-parametric rate smoothing and used in analysis as phylogenetic control. The backbone constraint applied in the heuristic MP search is indicated by thick branches. Mean fruit-set values calculated (dashed line represents median) for each taxon and changes in fruit-set rates (posterior mean from Bayesian GLMM, see Table 4) calculated for 16 species are given next to the tree.*

We first evaluated the effect of pollination mode on fruit-set; as random factors we included the ID of herbarium records (to control for multiple plant individuals collected at the same time from the same location), species (to control for multiple records per species) and phylogenetic position (to control for shared phylogenetic descent). Next we built a similar model with year as fixed effect, and a third model with the interaction of these two factors.

Lastly, we evaluated temporal changes in fruit-set in species where the number of records per species was >10 (this was the case for N=16 species). These latter models were evidently run without phylogenetic control, but we included the ID of herbarium records as a random factor.

Results

The phylogenetic tree reconstruction found four equally parsimonious trees under the MP criterion that were compatible with the backbone constraint applied. These four trees at step 1320 were found repeatedly during the 1000 random repetitions. The difference between the trees effected the tips; on one part of the trees *Limodorum abortivum* was sister to the genus *Neottia*, while the other part was affected by the exchanged placement of *Orchis militaris* and *O. purpurea*. All these placements are poorly resolved in the currently available studies. The finally favoured tree (*Fig. 2*) is the one fully compatible with those published in the relevant literature (Bateman et al., 2003; Bateman et al., 2005; Bateman, 2009).

Species-specific fruit-set rates in the 27 species ranged between 35 % and 98 %. (*Table 2*). Pollination mode had a significant effect on reproductive success; compared to nectar-rewarding species, deceptive taxa had significantly lower fruit-set (posterior mean: -2.152; lower: -3.380; upper 95% CI: -1.079; p=0.004). Fruit-set of autogamous taxa was not significantly different from nectar-rewarding ones (posterior mean: 0.747; lower: -0.577; upper 95% CI: -1.796; p=0.224) (*Table 3*, *Fig. 3*).

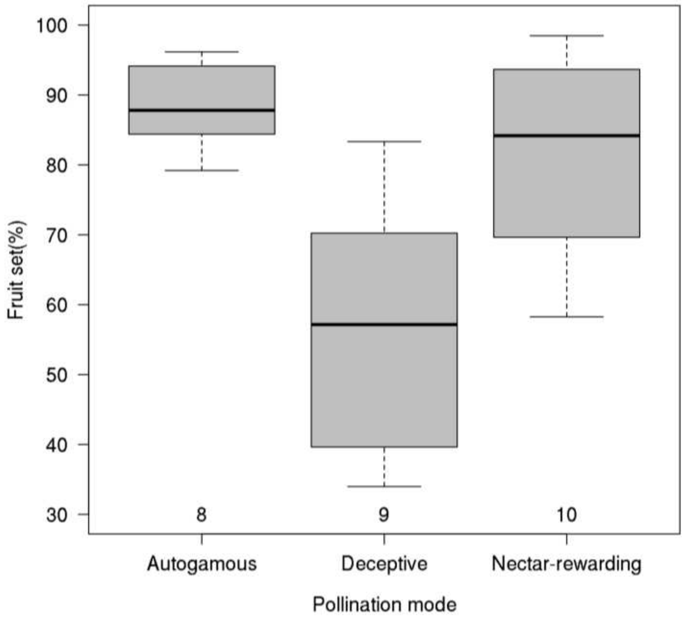

Figure 3. *Reproductive success of orchids with different pollination mode. The number of species with each pollination strategy is indicated below the boxes.*

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***Table 2.** Descriptive statistics on fruit-set data of species examined in this study.*

	Number of flowers		**Number of fruits**		**Fruit-set (%)**
Species	**Total**	**Mean**	**Total**	**Mean**	**Mean ± SD**
Anacamptis coriophora	512	15.5	394	11.9	77 ± 17
Anacamptis morio	147	9.8	86	5.7	59 ± 15
Anacamptis palustris	72	10.3	46	6.6	64 ± 24
Anacamptis pyramidalis	603	22.3	241	8.9	40 ± 25
Cephalanthera damasonium	344	4.9	321	4.5	93 ± 13
Cephalanthera longifolia	236	7.9	106	3.5	45 ± 39
Cephalanthera rubra	164	10.3	50	3.1	30 ± 34
Cypripedium calceolus	6	1.0	5	0.8	83 ± 25
Dactylorhiza incarnata	527	22.0	380	15.8	72 ± 28
Dactylorhiza viridis	217	12.8	190	11.2	88 ± 11
Epipactis atrorubens	362	15.1	347	14.5	96 ± 4
Epipactis helleborine	287	16.9	275	16.2	96 ± 22
Epipactis microphylla	799	11.3	769	10.8	96 ± 4
Epipactis palustris	522	10.9	486	10.1	93 ± 8
Epipactis purpurata	527	22.0	518	21.6	98 ± 3
Epipactis tallosii	85	14.2	66	11.0	78 ± 27
Goodyera repens	96	16.0	78	13.0	81 ± 9
Gymnadenia conopsea	145	20.7	99	14.1	68 ± 31
Gymnadenia odoratissima	175	21.9	104	13.0	59 ± 19
Limodorum abortivum	280	10.8	239	9.2	85 ± 16
Liparis loeselii	38	3.8	33	3.3	87 ± 14
Neottia ovata	556	34.8	467	29.2	84 ± 13
Neottia nidus-avis	2990	25.1	2386	20.1	80 ± 7
Orchis militaris	1243	21.1	483	8.19	39 ± 18
Orchis purpurea	193	32.2	67	11.2	35 ± 14
Orchis simia	146	16.2	98	10.9	67 ± 24
Platanthera bifolia	494	15.9	330	10.7	67 ± 24

***Table 3.** Mean fruit-set rates of the species with different reproductive strategy*

	Number of species	**Number of flowers**			**Number of fruits**		
		Median	**Mean**	**SD**	**Median**	**Mean**	**SD**
Autogamous	9	10.9	13.5	10.2	10.1	11.5	8.3
Food deceptive	12	13	14.9	8.4	6.3	6.9	4.4
Nectar rewarding	10	16.4	17.7	3.3	13.6	14.4	3.4

Fruit-set did not change with time (posterior mean: -0.004; lower: -0.011; upper 95% CI: 0.002; p=0.194, *Fig. 4.*). Furthermore, when year and pollination mode were included in interaction with each other (allowing different temporal changes in the three pollination strategies), none of the model parameters were significant.

Fruit-set decreased significantly with time in *Neottia ovata,* increased with time in *Anacamptis coriophora*, and no statistically significant trend was seen in the remainder of species with at least 10 herbarium records (14 out of 16; *Table 4*).

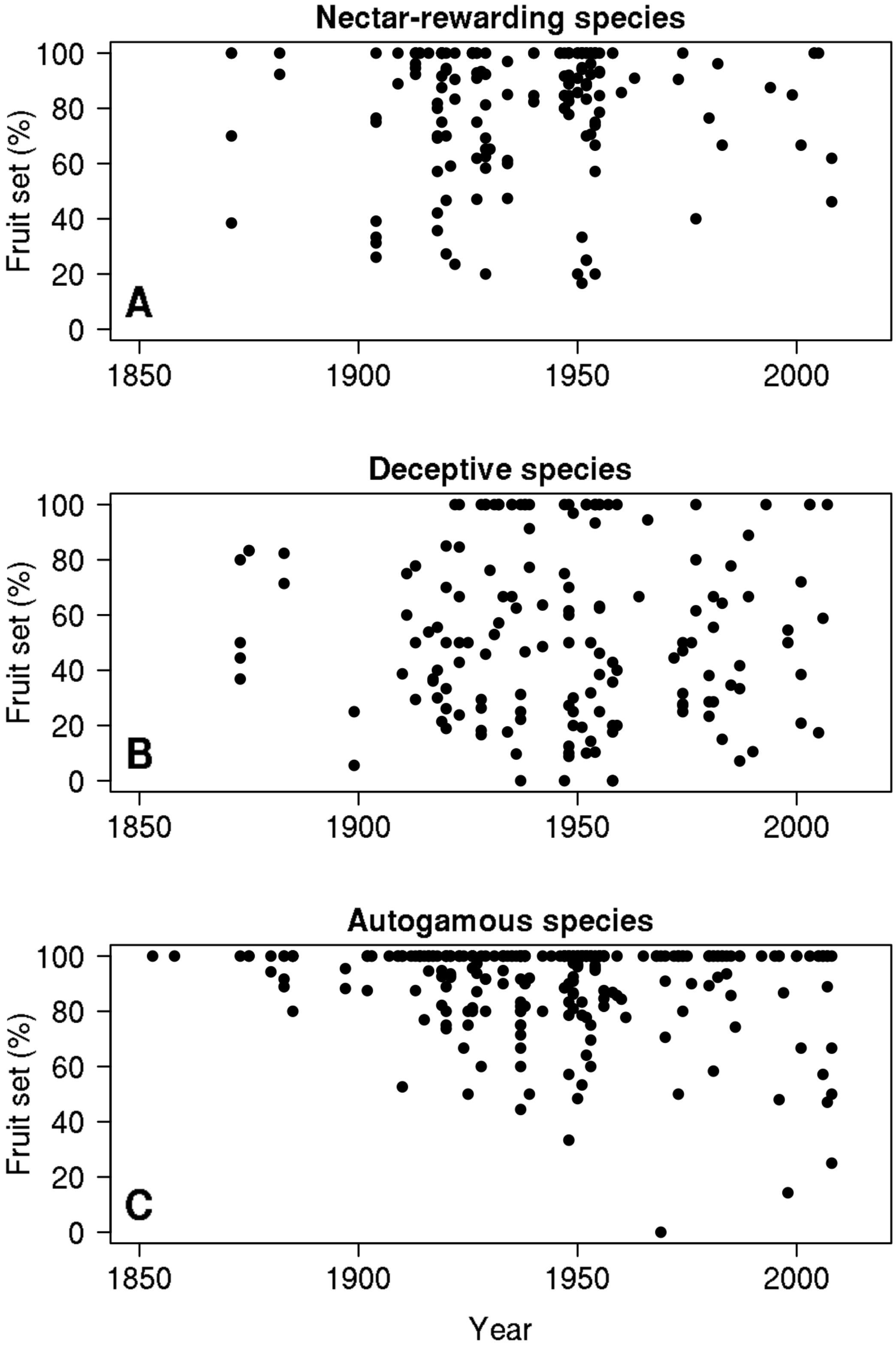

Figure 4. *Fruit-set data of nectar-rewarding (A), deceptive (B) and autogamous (C) taxa in relation to year of collection. Overall, there was no significant temporal changes in fructification rate (see Results)*

***Table 4.** Changes in fruit-set rate during time (results from Bayesian Generalized Linear Mixed Models). Boldface indicates statistically significant change.*

Species	Parameter estimate (posterior mean)	Lower 95% CI	Upper 95% CI	pMCMC
Anacamptis coriophora	**0.052**	**0.009**	**0.094**	**0.020**
Anacamptis pyramidalis	0.004	-0.016	0.023	0.750
Cephalanthera damasonium	-0.007	-0.035	0.024	0.622
Cephalanthera longifolia	0.039	-0.077	0.141	0.422
Cephalanthera rubra	-0.104	-0.277	0.025	0.104
Dactylorhiza incarnata	0.003	-0.032	0.047	0.896
Epipactis atrorubens	0.003	-0.054	0.061	0.996
Epipactis helleborine	0.088	-0.376	0.875	0.734
Epipactis microphylla	-0.006	-0.037	0.019	0.700
Epipactis palustris	0.027	-0.035	0.095	0.360
Epipactis purpurata	0.024	-0.084	0.158	0.694
Limodorum abortivum	-0.026	-0.070	0.025	0.214
Neottia ovata	**-0.035**	**-0.072**	**0.001**	**0.040**
Neottia nidus-avis	-0.003	-0.021	0.012	0.666
Orchis militaris	-0.010	-0.026	0.010	0.266
Platanthera bifolia	-0.010	-0.035	0.014	0.398

Discussion

Long-term data from herbaria were used to quantify the reproductive success of orchids in Hungary. Fruit-set rates of species with different pollination strategies are different; orchids with food deceptive strategy develop significantly less seedpods than autogamous species. This information is in accordance with field experiences (Neiland and Wilcock, 1998).

Our analyses seem to suggest that reproductive success in the vast majority (91%) of Hungarian orchids has not changed temporally, i.e. pollination crisis has not occured in Hungary, at least until the end of the 20th century. Our results are in accordance with a recent paper of E. Vojtkó et al. (2015) who also reported relatively high recent reproduction success of two deceit pollinated *Dactylorhiza* species in comparison to Western European data.

In our dataset only two species showed significant temporal changes in their reproduction success: the Common Twayblade (*Neottia ovata*) showed a significant decrease in fruit set, while in the Bug Orchid (*Anacamptis coriophora*) reproduction success increased significantly. Because our study may have been adversely affected by varying temporal sampling frequency, the significant changes may be results of sampling bias.

There are at least two possible explanations to the detected trends. Firstly, the diversity of the bee communities is much higher in Hungary (Sárospataki et al., 2009), than in some Western European countries (Dauber et al., 2003; Hirsch and Wolters, 2003). Secondly, the very high and continuously increasing honeybee density in Hungary (Tóth, 2013) may contribute to the high recent reproductive success of deceit pollinated orchids (Biró et al., 2015) and also to the temporal invariability of reproduction success of orchids in Hungary. The only species (*Anacamptis coriophora*) with increasing fruit-set is pollinated by many different insects (Hymenoptera, Diptera, Hemiptera, Claessens and Kleynen, 2011) and also honeybee (Dafni and Ivri, 1979; Berger, 2004).

To summarize, our data show that compared to Western European populations, pollination crisis has not affected Hungarian orchids (at least in terms of reproductive success). This conclusion suggests that geographical variation needs to be taken account when studying the consequences of pollination crisis.

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LEAF GAS EXCHANGE CHARACTERISTICS OF DROUGHT STRESSED LINDEN (*TILIA SP.*) TREES

TÓTH, E. GY.[1] – JUHÁSZ, Á.[2] – SÜTÖRINÉ DIÓSZEGI, M.[1] – STEINER, M.[1] – HROTKÓ, K.[1*]

[1]*Corvinus University of Budapest, Department of Floriculture and Dendrology*
1118. Villányi út 29-43., Budapest, Hungary
(phone: +36-1-482-6271)

[2]*Corvinus University of Budapest, Department of Soil Science and Water Management*
1118. Villányi út 29-43., Budapest, Hungary
(phone: +36-1-482-6273)

**Corresponding author*
e-mail: karoly.hrotko@uni-corvinus.hu

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Abstract. The daily course of leaf gas exchange was investigated on six linden cultivars from *Tilia cordata* Mill., *T. platyphyllos* Scop., *T. tomentosa* Moench and *T. americana* L. After the morning peak rapid stomatal closure was detected on leaves of *T. cordata* 'Savaria', 'Greenspire' and *T. platyphyllos* 'Favorit'. The stomatal conductance on leaves of *T. tomentosa* 'Szeleste' after the midday drop was significantly higher, while highest was on *T. americana* 'Redmond' leaves during the whole day. The transpiration resulted significant differences in daily water use (*T. cordata* 'Savaria' and *T. platyphyllos* 'Favorit': 1.0 $kg \cdot m^{-2}$; *T. americana* 'Redmond', 2.3 $kg \cdot m^{-2}$ of leaf area). Due to rapid stomatal closure *T. cordata* and *T. platyphyllos* cultivars realized water saving on the cost of strongly reduced carbon gain. Corresponding to their drought and heat adaptability the performance of leaves of *T. tomentosa* cultivars showed low level stress symptoms, intermediate level of transpiration and carbon gain. *T. americana* 'Redmond' leaves transpired largest water quantity, which resulted in large water loss and relatively intense stress symptoms. The large cultivar differences in the performance of leaf gas exchange should be considered at evaluation of drought stress adaptability and environmental benefits (CO_2 fixation, O_2 and vapor release) of *Tilia* cultivars.

Keywords: *CO_2 assimilation, environmental benefits, transpiration, urban forestry, water use*

Introduction

Linden species (*Tilia sp.*) represent an important genus in Central Europe (Radoglou et al. 2008) and are widespread planted under urban conditions in form of different cultivars. The following species are commonly planted for urban forestry in Hungary: *T. cordata* Mill. and *T. platyphyllos* Scop., (native to Europe forming climax forest); *T. tomentosa* Moench, (native to Southern Europe and Asia); *T. americana* L. (cultivars were introduced recently). Despite of their importance, there is little and inconsistent knowledge on the drought tolerance and leaf gas exchange performance of linden cultivars under urban conditions.

Most of the authors agree on wider adaptability of *T. cordata* Mill. (Hölscher 2004; Hölscher et al. 2005; Radoglou et al. 2008), while data of Köcher et al. (2009) indicate moderate drought sensitivity. Results of Fini et al. (2009) indicate that *T. tomentosa* and *T. cordata* are more drought tolerant during establishment than *T. platyphyllos*. Similarly inconsistent data are available on drought tolerance of *T. americana* (Abrams et al. 1998, Klos et al. 2009, Gustafson and Sturtevant 2012, Gilman and Watson 2012).

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Water stress results in stomatal closure and reduced transpiration rates, decrease in the water potential of plant tissues, and diminish the photosynthesis. Further on higher growth inhibition and accumulation of abscisic acid is caused (Davies and Zhang 1991, Yordanov et al. 2003). Stomatal control of leaf transpiration is considered as short term dynamic adaptation to water stress; the reduced transpiration contributes to avoiding decrease of leaf water potential (Sperry 2000; Bréda et al. 2006). Several investigation showed that stomatal responses are more closely linked to soil moisture content than to leaf water status: stomata are responding to chemical signals (e.g. abscisic acid) produced by dehydrating roots (Davies and Zhang 1991).

High transpiration demand induced by environmental conditions and low soil water content leads to the decrease of the leaf water potential. As a result of this drop, stomatal closure occurs in most tree species, limiting water fluxes at the cost of reduced CO_2 assimilation (Sperry et al. 2002; Bréda et al. 2006). Some drought-tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency. Higher transpiration rates have been reported for *Fraxinus americana* in contrast to *T. cordata*. Linden tends to avoid drought by maintaining low transpiration rates and higher water potential, whereas ash tends to tolerate drought sustaining higher transpiration rate despite decreasing water potential. From the standpoint of the selecting urban use, both water balance strategies are successful (Withlow and Bassuk 1988).

The above leaf gas exchange characteristics influence the drought adaptability and some major environmental benefits (CO_2 fixation, O_2 and vapor release) of urban trees. Since there are little and inconsistent data on drought and heat adaptability of linden cultivars we aimed in this work to evaluate the leaf gas exchange, stomatal performance of leaves on different linden taxa under drought stress conditions in order to gain information on the diurnal course of stomatal conductance, CO_2 fixation and water use.

Materials and methods

Site conditions

The investigations were carried out in Soroksár Station of Experimental Farm of Corvinus University of Budapest. Soroksár station (47°38'LN; 19° 14'LE, 103 m above the sea level) is located in Central Hungary, South-East of Budapest. The yearly average temperature is 11.3 °C, and the total sunshine is 2079 hours. Average annual rainfall is about 550 mm falling mainly in May and June. The soil type is light sandy, lime content is around 2.5 %, soil organic matter is low (0.8 – 0.9 %), pH is 7.7.

An automatic weather system was installed close to the investigated trees (~300 m) to measure meteorological variables at 10 minutes interval, recorded by Campbell CR 100 data loggers. Temperature and relative humidity were observed by Vaisala HMP35 in the research station (*Fig. 1* and *Fig. 2*). Precipitation, wind speed and global radiation were also monitored (*Fig. 2* and *Fig. 3*). 2011 was extremely droughty year in Hungary. Since the beginning of the meteorological observation this year was the driest (Weather Report of the Hungarian Meteorological Society, 2012).

The month August is characterized by increasing air temperature and decreasing air humidity (*Fig. 1*). Further on the low amount of rain measured on the investigated area (total 2.2 mm precipitation in the whole month of August 2011) increased the drought. In the days of 25–26 August there was hot-wave at the site. The daily maximum temperature (T_{max}) reached the 34.6°C, along clear sky on both days (*Fig. 1* and *Fig. 2*).

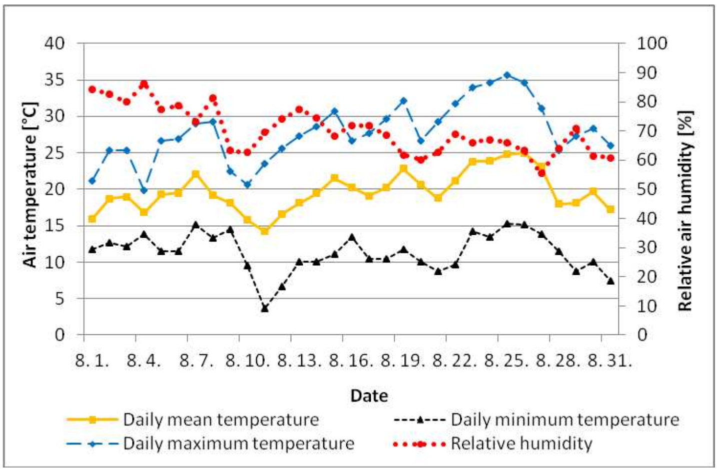

***Figure 1.** Performance of air temperature and relative air humidity at Soroksár research station in August 2011*

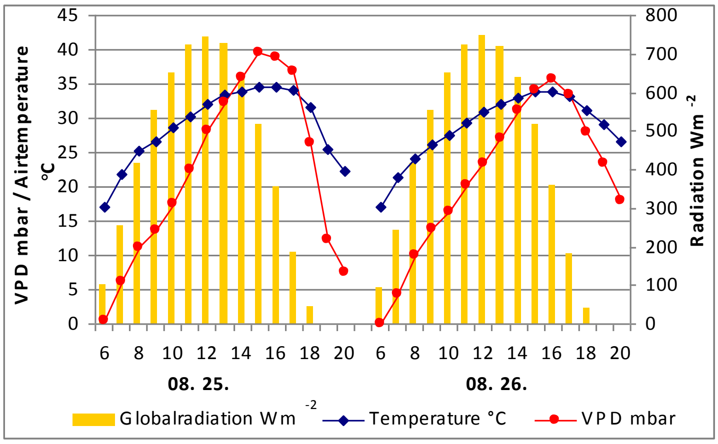

***Figure 2.** Global radiation, air temperature and vapor pressure deficit (VPD) on the days of investigations in 2011*

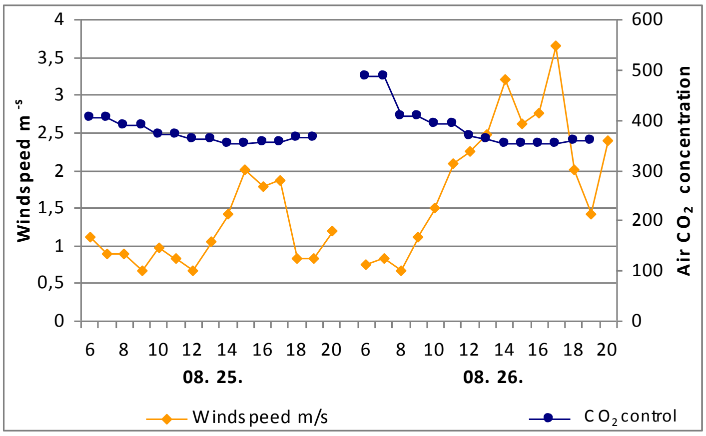

***Figure 3.** Wind speed and ambient air CO2 concentration on the days of investigations in 2011*

The weather circumstances (high vapor pressure deficit (14 mbar) and global radiation (245-247 W m^{-2}) favored the transpiration. Potential evapotranspiration was calculated for 4.0-4.4 mm. The mean relative humidity was 63-66%.

Plant material and methods of leaf gas exchange measurements

Six linden cultivars propagated by budding, were involved in the trial: *Tilia americana* 'Redmond', *Tilia cordata* 'Greenspire', *T. c.* 'Savaria', *Tilia platyphyllos* 'Favorit', *T. tomentosa* 'Szeleste' and *T. t.* 'Zentai Ezüst'. The investigated linden trees were planted in first week of December 2009, with a trunk circumference 120-140 mm. The trees showed a well developing shape in their establishment stage and overall appropriate horticultural performance (Tóth et al. 2012). The canopies reached 1 – 1.4 m diameter, so the leaves were well illuminated.

The visual symptoms of water and heat stress on the leaves were observed and evaluated by rating, as follows. Rating 0: no symptoms, 1: light wilting of leaves, 2: severe wilting, yellowish or dried patches on leaves up to 25%, 3: severe wilting, yellowish and dried patches on leaves up to 50%, 4: severe wilting, yellowish and dried patches on leaves up to 75%, leaf fall.

The parameters of leaf gas exchange were investigated by using portable infrared gas analyzer (LCi, ADC BioScientific Ltd, UK). Healthy and well developed trees were chosen to take the measurements; two trees from each cultivars. We measured the leaf gas exchange on four leaves from each tree, possibly with similar PAR exposition, according to the points of the compass, on each side of trees. The measurements were taken in 2011 august 25 and 26, with very similar meteorological characteristics (*Fig 1* and *2*). The measurements started at 6:30 AM and finished at 7:30 PM. Measurements of one series (four leaves on one tree of the six cultivars) took one hour and were repeated in two hour intervals from 6:30 AM to 7:30 PM. In the first day we measured leaves of one tree of each cultivar, on the other day the same measurements were done on another tree. So in each one-hour-long time interval we collected data from 8 leaves of the same cultivar. Data from eight leaves from the same time intervals of the two days were averaged and statistically analyzed. During the two days, all together 672 leaf data were collected. Further on 2 parameters were calculated; daily (from 6:00 AM to 8:00 PM) accumulated transpiration and CO_2 assimilation as product of time and mean values of the time intervals. In calculation of accumulated transpiration and CO_2 assimilation each mean value from the one-hour-long measurement's interval was applied for two-hours-long time intervals from 6:00 AM to 8:00 PM, assuming that mean value represents the two-hour-long interval. Data were analyzed with SPSS 2.0, Repeated measures ANOVA and One-way ANOVA were used. Data of species and cultivars were compared to *T. tomentosa* 'Szeleste', which is one of the most widespread planted registered cultivar in Hungary.

Results

We observed considerable different visible water and heat stress symptoms on leaves of linden cultivars (*Table 1*). The rating indicates most severe symptoms on *T. cordata* 'Greenspire' while the leaves of *T. tomentosa* 'Szeleste' showed significant less symptoms. Rating of leaves on all the other cultivars resulted intermediate levels of symptoms without any significant differences related to above two cultivars.

***Table 1**. Rating of visible drought stress symptoms on leaves of linden cultivars*

Species and cultivar	severity of visible symptoms	
T. cordata ’Greenspire’	2.58	b
T. americana ’Redmond’	1.92	ab
T. tomentosa ’Zentai ezüst'	1.43	ab
T. platyphyllos ’Favorit’	1.25	ab
T. cordata ’Savaria’	1.21	ab
T. tomentosa ’Szeleste’	0.50	a

The temperature of measured leaves on different linden cultivars showed in each time interval significant differences (*Fig. 4*). There are distinct groups of cultivars, where the leaf temperature showed more or less similar performance. The lowest leaf temperature was measured on *T. americana* cultivars, except for the late afternoon hours (16:00 – 20:00). In the next group of *Tilia cordata* cultivars there are significant differences within the group in the early hours; between 12:00 – 14:00 all the *T. cordata* cultivars showed similarly mesic leaf temperature. The highest leaf temperature was measured in each time interval on *T. tomentosa* cultivars, while *T. platyphyllos* ’Favorit’ showed leaf temperature between *T. cordata* and *T. tomentosa* cultivars.

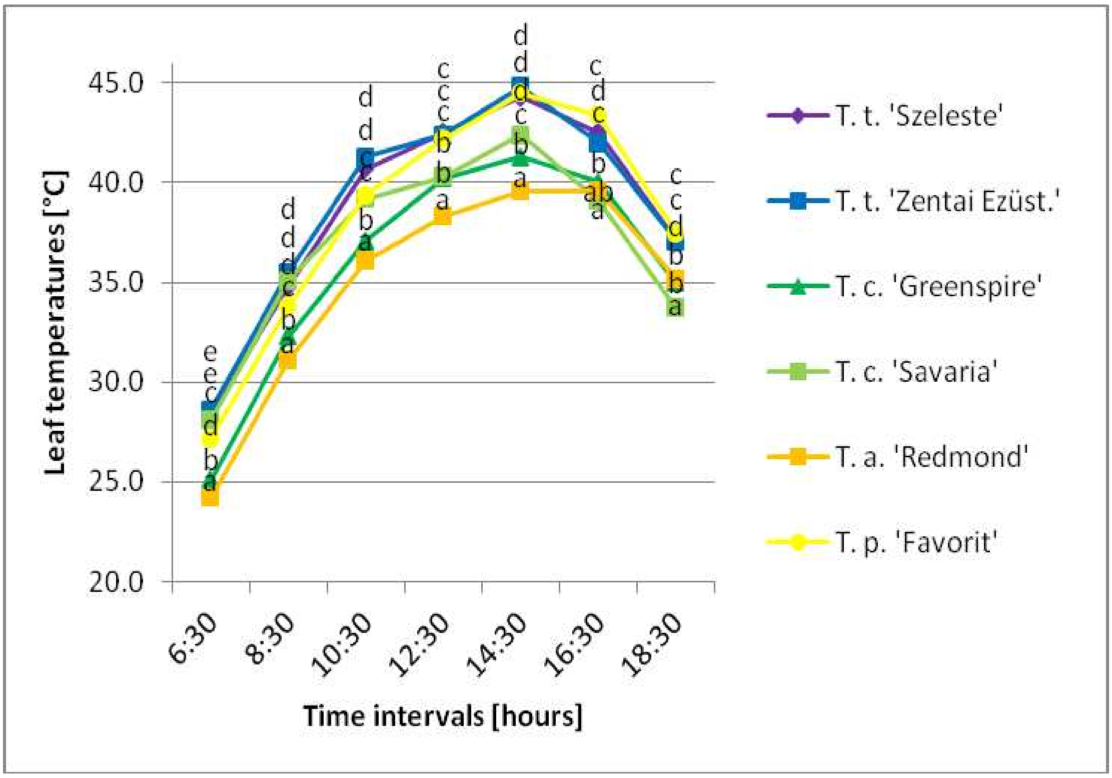

***Figure 4.** Performance of leaf temperature on linden cultivars*

The leaves of linden cultivars showed significant differences in daily cumulated transpiration expressed in g m^{-2} of leaf surface (*Fig. 5*). Leaves of *T. americana* ’Redmond’ (2.3 kg m^{-2}) transpired significant more water than the control, while the

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transpiration of leaves on *T. tomentosa* ’Zentai Ezüst’, *T. cordata* ’Greenspire’, ’Savaria’, and *T. platyphyllos* ’Favorit’ was significant lower than that of control. With the lowest transpiration the *T. cordata* ’Savaria’ and *T. platyphyllos* ’Favorit’ formed a group with low water use, their cumulated transpiration was about 1.0 kg m^{-2}.

The same trend is found on the daily cumulated CO_2 assimilation (*Fig. 6*). The highest assimilation was measured on *T. americana* cultivar, while the lowest on *T. cordata* ’Savaria’ and *T. platyphyllos* ’Favorit’ cultivars.

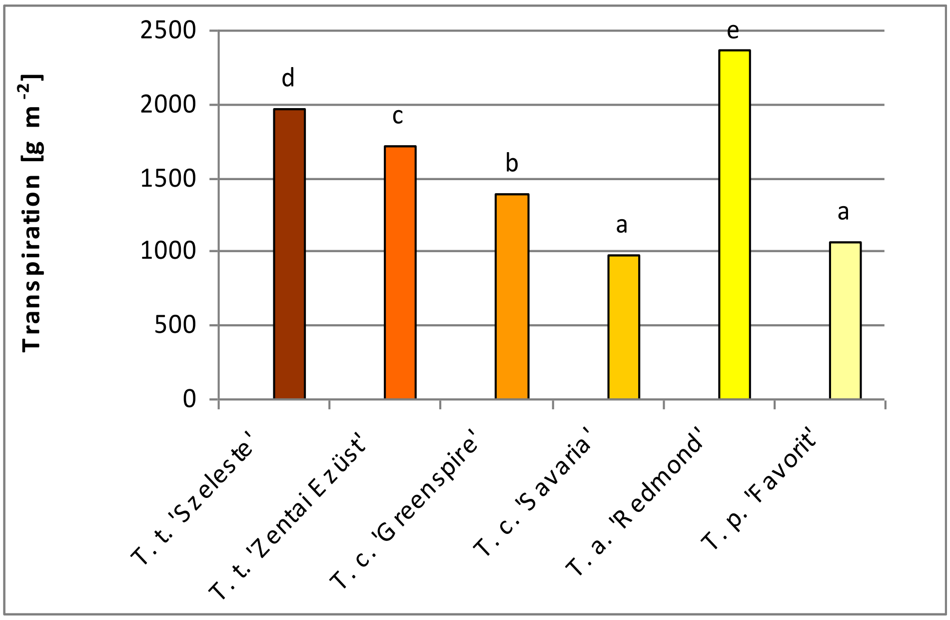

Figure 5. *Daily cumulated transpiration of linden leaves (g·m-2)*

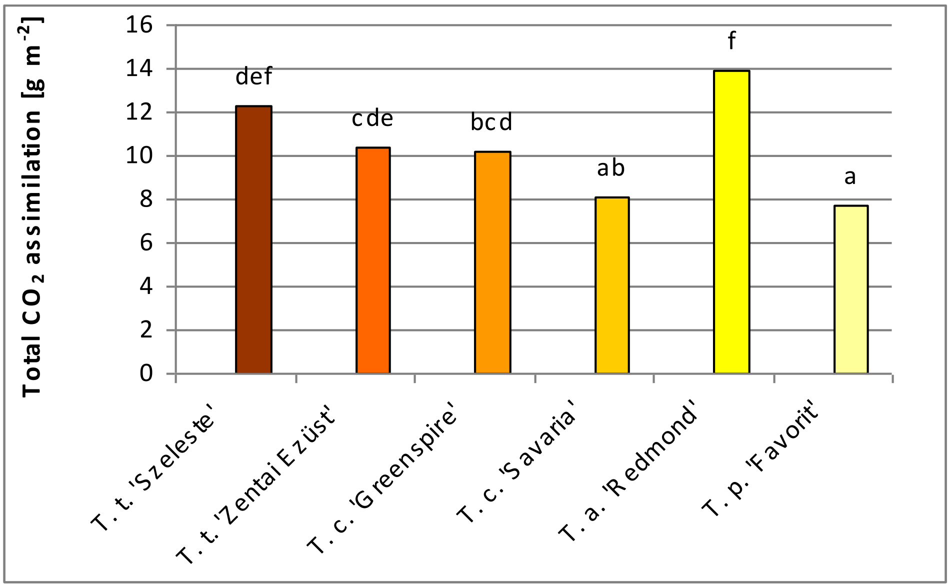

Figure 6. *Daily cumulated CO_2 assimilation of linden leaves (g·m^{-2})*

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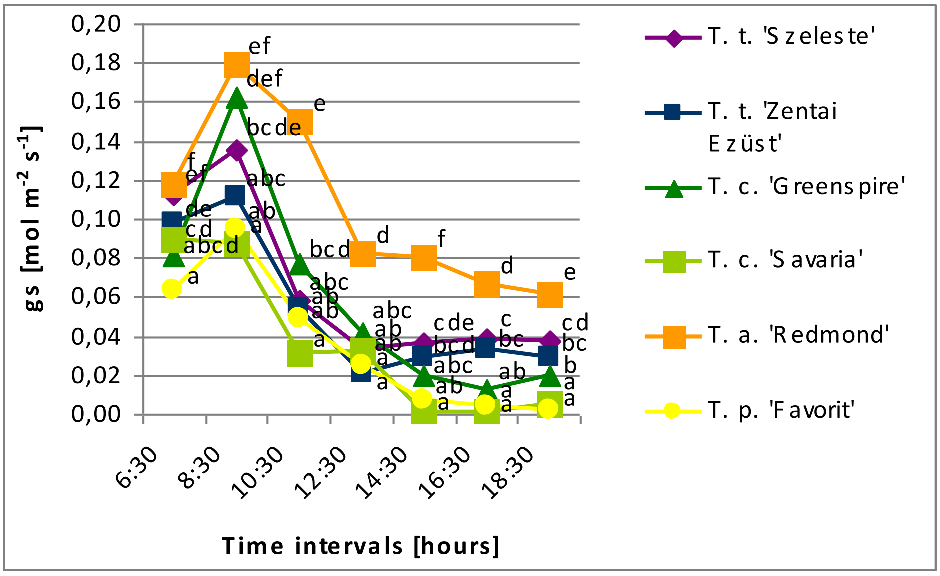

***Figure 7.** Daily course of stomatal conductance (gs) of linden leaves (mol·m^{-2}·s^{-1})*

The daily course of stomatal conductance (*gs*) showed a performance typical to drought stressed plants (*Fig. 7*). The daily maximum was achieved between 8:30 to 9:30, followed by rapid decrease to very low level, but with considerably large cultivar differences. In the early morning (6:30 to 7:30) low stomatal conductance was measured on leaves of *T. platyphyllos* 'Favorit' and on both *T. cordata* cultivars, compared to the control (*T. tomentosa* 'Szeleste'). In the daily maximum period from 8:30 to 9:30 the stomatal conductance of *T. cordata* 'Savaria' was significant lower, while *T. americana* 'Redmond' was higher than that of control. In the following sections of the day until 19:30 the stomatal conductance of *T. americana* 'Redmond' leaves remained higher than that of control during the whole day. On the other hand, *T. cordata* 'Savaria' and *T. platyphyllos* 'Favorit' showed significant lower stomatal conductance in the afternoon period from 14:30 to 19:30 (*Fig. 7*). By the end of the day, as the global radiation reduced to minimum, similar differences remained between cultivars.

The daily course of transpiration (*E*) (*Fig. 8*) on *T. cordata* and *T. platyphyllos* leaves showed the peak in the morning hours, between 8:30 to 9:30, than decreased to low level from 12:30 to 18:30, while the midday drop on *T. tomentosa* and *T. americana* cultivars was followed by a minor peak in the afternoon. Compared to the control *T. americana* 'Redmond' showed higher transpiration between 10:30 to 15:30, and the highest peak between 12:30 to 13:30. Cultivars *T. platyphyllos* 'Favorit', *T. cordata* 'Savaria' and 'Greenspire' showed lower transpiration without any second peak. Between 18:30 to 19:30 cultivars *T. platyphyllos* 'Favorit' and both *T. cordata* cultivars transpired less than the control *T. tomentosa* 'Szeleste'.

The daily course of net CO_2 assimilation rate (*A*) of leaves performed similarly to stomatal conductance: the daily maximum was achieved between 8:30 to 9:30, followed by rapid decrease to low level, with considerably large cultivar differences (*Fig. 9*). In the early morning peak (8:30 to 9:30) highest CO_2 assimilation was measured on *T. cordata* 'Greenspire' and *T. americana* 'Redmond', while *T. cordata* 'Savaria', and *T. tomentosa* 'Zentai Ezüst' showed significant lower values. In the early afternoon hours (14:30) all the cultivars produced less CO_2 fixation compared to *T. americana*

’Redmond’, while at 16:30 both *T. tomentosa* cultivars recovered, showing higher net CO_2 assimilation, than that of *T. cordata* cultivars and *T. platyphyllos* ’Favorit’.

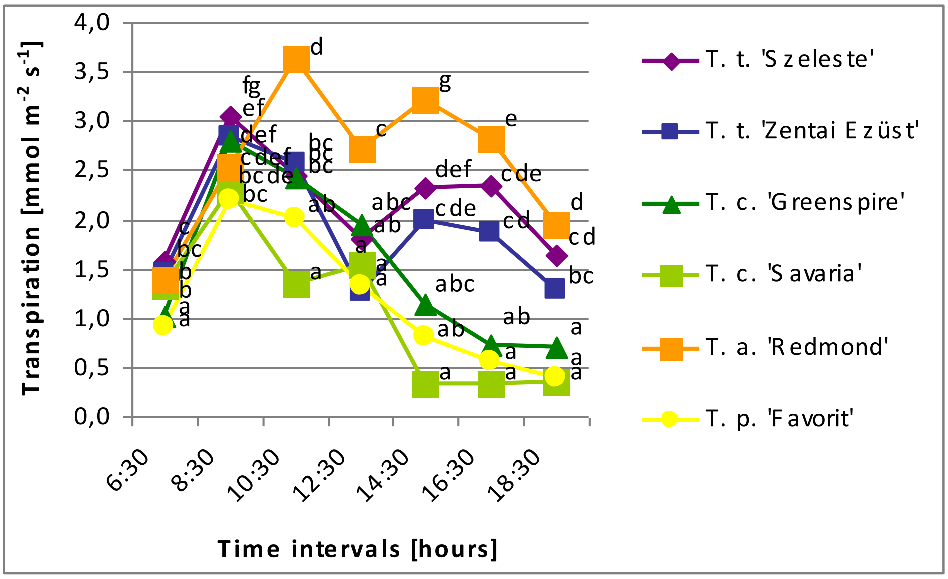

Figure 8. *Daily course of transpiration of linden leaves (mmol·m^{-2}·s^{-1})*

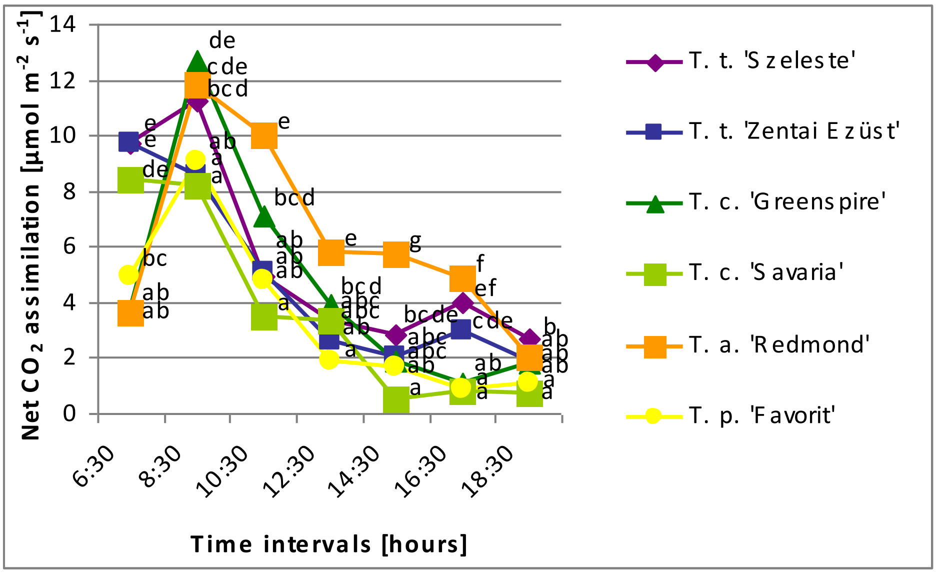

Figure 9. *Daily course of net CO_2 assimilation of linden leaves (µmol·m^{-2}·s^{-1})*

Discussion

Meteorological data measured in August 2011 and on the days of investigations (*Fig. 1, Fig. 2* and *Fig. 3*) indicate that linden trees in establishing stage (planted in 2009) on light sandy soil were exposed to severe drought. Based on the visible symptoms the leaves of *T. cordata* ’Greenspire’ were mostly injured by water stress while *T. tomentosa* ’Szeleste’ could be considered as most tolerant (*Table 1*). This evaluation of

T. tomentosa is in agreement with Schmidt and Tóth (2006) and Radoglou et al. (2008) while the large injuries on both *T. cordata* cultivars do not confirm their adaptability in the investigated establishing stage.

The leaves showed a diurnal course of stomatal conductance typical to water stressed plants: the daily maximum was around 8:30, than decreased to the minimum. The observed performance of stomatal conductance – corresponding to Withlow and Bassuk (1988) – confirms that the stomatal control is a basic element of water balance strategy of linden cultivars but in this trait the linden (*Tilia* sp.) species and cultivars performed differently related to the leaf symptoms (*Fig. 7*). By the stress symptoms most concerned leaves of *T. cordata* 'Greenspire', 'Savaria' and *T. platyphyllos* 'Favorit' showed low level of stomatal conductance during the whole day. This strategy as a short term dynamic adaptation to water stress may efficiently contribute to the water saving (Sperry et al. 2000; Yordanov et al. 2003; Bréda et al., 2006). The largest conductance was measured during the whole day on *T. americana* 'Redmond', which is in correspondence with its daily water use and low leaf temperature (*Fig. 4* and *Fig. 5*). Both *T. tomentosa* cultivars produced a second minor peak in the afternoon, which is in correspondence to their trends of transpiration. The performance of leaf stomatal conductance indicates considerable differences in short term dynamic adaptability of linden cultivars to water stress that would need further investigation.

The cumulated daily water use does not correspond with the severity of visible water stress symptoms. From among the highly stressed cultivars *T. americana* 'Redmond' leaves transpired double amount of water related to the similarly stressed *T. cordata* 'Greenspire' and 'Savaria' (*Fig. 3*). Within *T. cordata* there are significant differences (*Fig. 3*) in favor to 'Savaria'. This performance of daily course of transpiration resulted in more than twofold differences daily vapor release: *T. cordata* 'Savaria' and *T. platyphyllos* 'Favorit' leaves transpired around 1.0 $\mathrm{kg \cdot m^{-2}}$ a day while *T. americana* leaves consumed 2.3 $\mathrm{kg \cdot m^{-2}}$.

The transpiration according to the stress symptoms and performance of stomatal control remained on a low level, 0.5 to 3.5 $\mathrm{mmol \cdot m^{-2} \cdot s^{-1}}$(*Fig. 5*). For leaves of *T. cordata* in forest stands of Southern Moravia Kazda et al. (2000) found at light saturation ($A_{\max}$) as highest mean transpiration rate $E = 3.48\ \mathrm{mmol \cdot m^{-2} \cdot s^{-1}}$; this is similar value to those that we have measured on leaves of *T. americana* 'Redmond'. To the same time *T. cordata* 'Savaria' transpired 60% water amount only, and in the afternoon hours the transpiration decreased to 10%. By the daily course of transpiration of leaves cultivars could classified into two groups: both *T. cordata* and *T. platyphyllos* after the daily maximum in the morning show a rapidly decreasing trend, while *T. americana* and both *T. tomentosa* cultivars showed a second peak in the afternoon. This performance of the last two species suggests a larger water uptake, reserve or supply capacity in the soil-plant-air complex of these cultivars.

When comparing the transpiration of leaves to the leaf temperature (*Fig. 4*), we can state that the significant higher transpiration on *T. americana* leaves is more efficient in cooling the leaves during the daytime. From among the *T. cordata* cultivars we measured highest leaf temperature on 'Savaria' during the daytime, which seems to be in correspondence to its low transpiration capacity, but the low leaf temperature of this cultivar between 16:00 - 20:00 cannot be explained by transpiration activity. It is conspicuous that *T. tomentosa* cultivars with their relatively high transpiration capacity, and late afternoon peak, showed the highest leaf temperature (*Fig. 4*).

The above observation suggests considerable cultivar differences in adaptability to water stress conditions. Cultivars of *T. cordata* and *T. platyphyllos* showed an efficient short term dynamic adaptation to water stress by stomatal control (Sperry 2000; Bréda et al. 2006), while cultivars of *T. tomentosa* and *T. americana* could maintain the transpiration of leaves on a relative higher level and produce a second peak after the midday drop. The low level of visual leaf symptoms by water stress of *T. tomentosa* corresponds to this, but mesic leaf symptoms of *T. americana* are not in line. The high stomatal conductance and transpiration of *T. americana* 'Redmond' leaves support the Gilman and Watson's (2012) statement on drought tolerance of this cultivar but the intermediate level of visual symptoms are in disagreement. Maintaining the steady water status of *T. americana* 'Redmond' leaves requires double amount of water supply.

The daily trend of net CO_2 assimilation (*A*) more or less followed the stomatal conductance; similarly to Forrai et al. (2012) at maximum level we measured 10-14 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which rapidly decreased to 2-6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to the early afternoon hours. Under drought stress the low stomatal conductance strongly controlled the carbon assimilation of *Tilia* leaves, which resulted significant differences in the daily accumulated carbon gain (*Fig 4*.). The leaves of *T. americana* 'Redmond' assimilated maximum quantity CO_2 a day, while *T. cordata* 'Savaria' and *T. platyphyllos* 'Favorit' assimilated lowest. This performance of daily cumulated carbon gain suggest that leaves of *T. americana* 'Redmond' can realize their maximum assimilation at higher transpiration costs. On the other hand, the leaves of *T. cordata* 'Savaria' and *T. platyphyllos* 'Favorit' realized water saving on the cost of strongly reduced carbon gain, which confirms Kazda et al. (2000). Corresponding to their drought and heat adaptability (Schmidt and Tóth 2006), in this concern, the performance of leaves of *T. tomentosa* cultivars showed intermediate level of transpiration and carbon gain. This performance was described by Sperry et al. (2002) and Bréda et al. (2006) as traits of tolerant species or cultivars.

The daily cumulated CO_2 assimilation of leaves was highest on *T. americana* 'Redmond', which is in correspondence with their largest stomatal aperture, transpiration and lower leaf temperature. In contrary, all the *T. tomentosa* cultivars with low stomatal aperture but large transpiration capacity showed the highest leaf temperature. Despite of the highest leaf temperature their leaves produced medium level of cumulated CO_2 assimilation. This suggests a larger heat tolerance of this cultivars. Within the *T. cordata* cultivars the large differences in transpiration and cumulated CO_2 assimilation indicates that the water saving cultivar 'Savaria' realizes this on cost of assimilation capacity.

Conclusion

Although the leaf transpiration alone is not an appropriate indicator of drought tolerance of linden cultivars, the differences between cultivars in leaf gas exchange characteristics suggest need on further investigations. The performance of *T. tomentosa* and *T. americana* indicate that in the soil-plant-air complex of these species there might be a more efficient mechanism in water uptake or larger water reservation and supply capacity, which allows maintaining the higher level of transpiration. However, we are aware of that leaf physiology, alone, is not a good predictor of the whole plant responses to drought (Sperry 2000, Yordanov et al. 2003, Bréda et al., 2006), the above performance of stomatal control and transpiration of leaves on *Tilia* cultivars should be considered in cultivar evaluation for urban forestry. The above leaf gas exchange characteristics strongly influence the drought adaptability, ornamental value and the environmental benefits (CO_2 fixation, O_2 and vapor release) of the investigated *Tilia* cultivars under stress conditions.

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PHYSICO-CHEMICAL PARAMETER VARIABILITY RELATIVE TO SEASONAL DYNAMICS AND COMMUNITY STRUCTURE OF PLANKTON IN THE BOUKOURDANE LAKE DAM (ALGERIA)

BRAHIM ERRAHMANI, M.[1,2*] – HAMAIDI-CHERGUI, F.[2] – HAMAIDI, M. S.[2]

[1]*Department of Chemistry, Faculty of Sciences, Blida 1 University, Algeria.*
(phone/fax: +213-25-433-642)

[2]*Department of Biology, Faculty of Natural and Life Sciences, Blida 1 University, Algeria.*
(phone/fax: +213-25-434-803)

**Corresponding author*
e-mail: m.brahim.errahmani@univ-blida.dz

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Abstract. The impact of seasonal variations in the water physico-chemical parameters on the zooplankton and phytoplankton composition was investigated in the Boukourdane reservoir (Tipaza, Algeria) over two years (April 2005-March 2007). High levels of nitrites were observed during the cold seasons, and the phosphate levels were higher in summer with a very high NO_3^-/PO_4^{3-} ratio (>30) in winter. A significant increase in the level of chlorophyll *a* (Chl *a*) was observed in spring. The relative standard deviations (RSDs) highlighted a large variability during the warm seasons, especially for nitrites, nitrates, phosphates and ammonia. A great species diversity was observed (rotifers: 79, copepods: 13, and phytoplankton: 83). The weighted averages of the species enabled us to determine the optimal values of their physico-chemical parameters, as elevated temperatures for Bacillariophyta and Pyrrhophyta or high concentrations of chlorophyll *a* for Charophyta. The zooplankton was more prevalent during the spring and appeared to be correlated with sulfates and electrical conductivity. A redundancy analysis (RDA) corroborated the previous results and explained 84.1% of the total variance. This analysis highlights the influence of some of the environmental variables on the seasonal distribution of plankton, characterized by a high profusion in spring and scarcity in autumn and winter.

Keywords**:** *environmental parameters, zooplankton, RDA, correlations, weighted average*

Introduction

Dams have been used for thousands of years to regulate river flows and ensure an adequate supply of water during dry periods (McCartney et al., 2001). The steadily increasing demand for water in recent decades poses various problems, both qualitative and quantitative (Ramdani et al., 2012). Changes in land use and management practices can have a considerable impact on water quality parameters (Brainwood et al., 2004). Due to the tremendous development of industry and agriculture, the disposal of untreated public sewage water, and agricultural runoff, the water quality and its biotic resources are in continuous deterioration (Venkatesan, 2007; Elmaci et al., 2008).

Through biomonitoring, the environmental quality of a given site can be judged from its species assemblages, based on knowledge of environment–biota relationships (Pilière et al., 2014).

Knowledge of the characteristics of a habitat, its species composition and the physico-chemical and biological factors that directly or indirectly affect the inhabitants are essential for a proper appraisal of the ecology of the aquatic animal species.

Zooplankton and phytoplankton have recently been used as indicators to observe and understand changes in an ecosystem because they seem to be strongly influenced by climatic

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features (Beaugrand et al., 2000; Al-Ghanim, 2012). Telesh (2004) stated that plankton is an important ecosystem component that responds to ecosystem alterations rather rapidly.

The ecology of aquatic environments has been widely studied elsewhere in the world, but very few limnological studies have been made on the freshwater ecosystem of Algeria (Cherbi et al., 2008; Hamaidi et al., 2008, 2009, 2010; Chaib et al., 2011; Hamaidi-Chergui and Hamaidi, 2013; Hamaidi-Chergui et al., 2013). The aim of this study was to establish relationships between the seasonal variations of environmental and biological variables and to contribute to an understanding of the ecology of the copepod, rotifer and phytoplankton taxonomic composition in the Boukourdane Lake Dam.

Material and methods

Area of study

The Boukourdane rockfill dam (*Fig. 1*) is located in the northwest of Mitidja (Sidi Amar, 36°32’03’’N 02°18’18’’E) and was constructed in 1985. It has a height of 74.4 m and a retained water volume of 97 hm^3. It receives water from the Menaceur and Fedjana rivers and is situated in a sub-humid climate region with 500 mm average pluviometry (Cherbi et al., 2008). It can be considered as a mid-size dam (Foum El Gueiss 0.43 hm^3, Beni Haroun 998 hm^3), and it supplies fresh water to Tipaza, Cherchell and the capital Algiers. Its waters are also used for irrigation of the western Mitidja perimeter.

Physico-chemical analysis

The water samples were collected between 9 and 11 a.m. from three sites of near surface water, three times per month between April 2005 and March 2007, and were then placed in polyethylene containers (2 L).

The pH and the electrical conductivity (EC) were measured in the field using a Hach sensION pH meter and a HD 3406.2 (Delta Ohm) conductivity meter, respectively.

Nitrate, nitrite, chloride and phosphate were analysed according to the standard methods described by APHA (1998) and Rodier (1997).

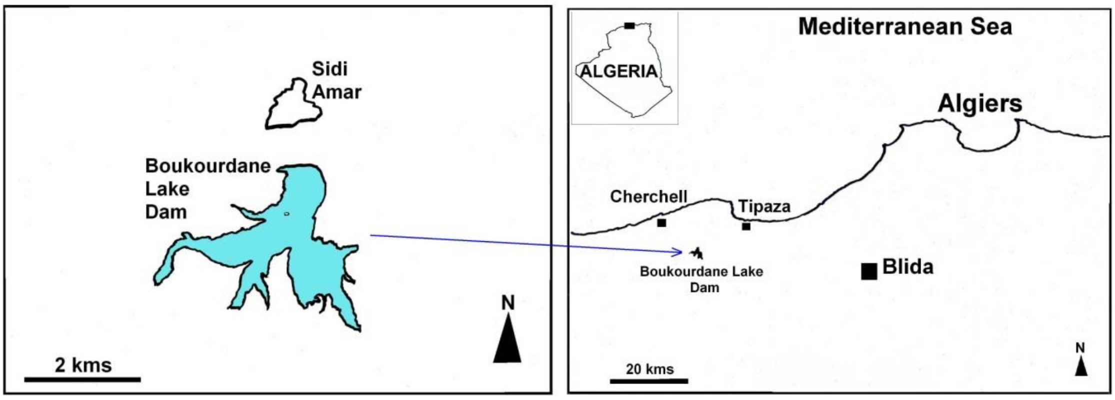

Figure 1. *Location of the Boukourdane Lake Dam (Algeria).*

The calcium and magnesium concentrations were determined using atomic absorbance spectrophotometry (AAS), and the sodium was measured using flame photometry. The permanganate oxidation method was used to determine organic matter.

Sub-samples were filtered (Whatman GF/C membranes) and used to quantify Chl *a* concentrations using spectrofluorimetry.

Plankton identification and enumeration

Sampling was carried out using a plankton net of 105-µm mesh size. One litre of sub-surface collected water was immediately preserved with 5 mL of 4% formalin solution and then kept for 24 h undisturbed to allow the sedimentation of the plankton. The supernatant was pipetted out, and the sample was concentrated to 10 mL.

The drop count method (Trivedy and Goel, 1984) was used for the quantitative estimation of the phytoplankton and zooplankton.

The phytoplankton was identified in accordance with standard methods (Bourelly 1966, 1968, 1970; Sournia, 1968; Compère, 1974, 1975a, 1975b, 1977, 1991, 1992, 2001; Komarek and Anagnostidis, 1986, 1989; Anagnostidis and Komarek 1988, 1990) and more specific literature.

The zooplankton groups were identified according to the literature on copepods (Dussart and Defaye, 1985, 2001; Einsle, 1996; Karaytug, 1999) and rotifers (Pourriot and Francez, 1986; Nogrady et al., 1993).

Data treatment and statistical analysis

The data distributions were first tested for the normality and the homogeneity of variances using the Kolmogorov-Smirnov and Levene tests, respectively. The Pearson's correlation coefficients were determined to identify relationships among the environmental variables and between these variables and species abundances. The sampling variability was estimated by calculating the "relative standard deviation" (RSD), also known as the "coefficient of variation".

The seasonal variations (spring, summer, autumn and winter) were compared using one-way ANOVA, and the significant differences were highlighted using the Newman-Keuls or Tukey post hoc tests. When the assumptions of the normality and homogeneity of variances were violated, the alternative Kruskal-Wallis ANOVA on ranks with multiple comparisons on groups was performed. The analysis was performed using Statistica 10 (Statsoft Inc, Tulsa, OK, USA), and the results are given as the mean±SE (SE: standard error of mean).

Each species has its own optimal environmental conditions for proliferation. An estimate of this optimum value of each parameter for each species was made by calculating the weighted average

$$\mathrm{WA(Sp)} = \sum_i (\mathrm{Env})_i (\mathrm{Abund})_i \Big/ \sum_i (\mathrm{Abund})_i$$

where $(\mathrm{Env})_i$ is the value of the environmental variable in the i^{th} sample and $(\mathrm{Abund})_i$ is the abundance of the species in the same sample (Leps and Smilauer, 2003).

The general relationships between the physico-chemical variables and taxa were studied using the redundancy analysis (RDA) under Canoco 4.5 for Windows (ter Braak and Smilauer, 2002). This technique can summarize the relations between response variables and several predictors, the biological species and environmental variables, respectively (Leps and Smilauer, 2003).

Results

Physico-chemical variables

The seasonal variations of the physico-chemical variables of water over the two years of the experiment are given in *Table 1.*

Table 1. *Seasonal variations of physico-chemical variables in the Boukourdane Lake Dam (EC: Electrical conductivity; DO: Dissolved oxygen; Org. m.: Organic matter; Ch.a: Chlorophyll a, AG: Algerian guidelines). Data are given as the mean±SE*

	T (°C)	**pH**	**EC (µS.cm^{-1})**	**DO (%)**	**Nitrates (mg.L^{-1})**	**Nitrites (mg.L^{-1})**	**Sulfates (mg.L^{-1})**	**Ammonia (mg.L^{-1})**
Spring	21.2±0.6	7.8±0.1	803±31	91.9±2.2	4.5±1.1	0.024±0.011	150.7±7.2	0.034±0.034
Range	18.5-23.0	7.6-8.1	710-900	85-98	2.5-8.0	0-0.068	131-178	0-0.20
Summer	27.2±0.8	7.5±0.3	735±25	95.8±8.8	3.4±1.7	0.023±0.015	146.8±5.7	0.023±0.016
Range	25-30	6.3-8.0	690-840	76-127	0-11	0-0.09	130-164	0-0.10
Autumn	16.8±1.1	7.5±0.1	655±12	75.0±9.3	2.9±1.7	0.038±0.017	127.5±1.8	0.015±0.007
Range	13.7-20.3	7.0-7.8	600-680	44-111	0.30-11.00	0-0.112	122-135	0-0.042
Winter	14.3±0.7	7.7±0.1	600±11	83.6±6.9	4.1±1.2	0.307±0.134	130.2±2.2	0.102±0.045
Range	12.8-16.4	7.5-7.9	580-650	63-104	0-8.00	0-0.817	125-139	0-0.300
p		0.29	0.0002	0.25	0.88	0.019	0.003	0.18
2-years means	19.9±1.1	7.6±0.1	698±18	85.9±3.9	3.7±0.7	0.098±0.041	138.8±3.1	0.043±0.016
AG	30	6.5-8.5	2800		50	0.1		0.5
WHO standards	25-30	6.5-8.5	180-1000		50	≤0.1		≤0.1

	Phosphates (mg.L^{-1})	**Sodium (mg.L^{-1})**	**Chloride (mg.L^{-1})**	**Calcium (mg.L^{-1})**	**Magnesium (mg.L^{-1})**	**Org. m. (mg.L^{-1})**	**Chl*a* (µg.L^{-1})**
Spring	0.24±0.24	47.5±3.0	67.3±6.2	75.7±2.7	31.5±2.0	6.18±1.49	7.72±1.65
Range	0-1.47	40-57	41.84	69-87	24-37	3.2-13.3	4.5-13.3
Summer	0.55±0.34	41.3±2.5	76.8±4.1	67.7±3.3	34.4±2.3	4.18±0.62	5.72±0.96
Range	0-2.14	31-49	58-86	54-76	30-43	2.3-6.1	2.30-8.54
Autumn	0.34±0.17	42.8±3.2	74.7±1.1	67.5±0.7		3.62±0.43	2.40±0.50
Range	0-1.16	35-52	70-78	66-70		2.00-4.60	1.07-4.30
Winter	0.13±0.11	40.8±2.3	69.7±0.6	64.5±2.4		6.32±1.50	3.10±0.98
Range	0-0.67	33-48	68-72	60-75		2.6-11.9	0.12-5.40
p	0.64	0.33	0.11	0.029	0.37	0.24	0.017
2-years means	0.32±0.11	43.2±1.3	72.1±1.9	68.8±1.4	32.8±1.5	5.07±0.58	4.74±0.68
AG	0.5	100	250	75	50		
WHO standards	0.2	150	250	200	50	<5	

The biennial average temperature was 19.9±1.1°C, and the water pH fluctuated slightly between 7.5 and 7.8. The electrical conductivity (mean 698 µS.cm^{-1}) was significantly higher in spring and summer ($p<0.01$).

Despite not significant variations, the nitrate concentration reached high values in spring and winter (2.9 to 4.5 mg.L^{-1}). The nitrite concentration remained low throughout the year but increased sharply in winter to 0.31 mg.L^{-1} (three times the standard value). The phosphate level fluctuated between low levels in winter and high levels in summer, 0.13 and 0.55 mg.L^{-1}, respectively.

The NO_3^-/PO_4^{3-} ratio is an interesting parameter, sometimes used to identify the element that inhibits the development of phytoplankton. It was found to be very high in winter (>30) and low in summer and autumn (<10).

The seasonal average of ammonia remained under standard values, suggesting a low degree of sewage pollution in the lake, with the highest values recorded in winter and the lowest in summer and autumn. The sulfate level was significantly higher in spring and summer (>145 mg.L^{-1}) but decreased in autumn and winter (<128 mg.L^{-1}).

The level of sodium was found to be slightly higher during spring but otherwise moderate. The calcium level was significantly higher in spring (75.7 mg.L^{-1}) and fell during winter to 64.5 mg.L^{-1}, remaining at intermediate values during the rest of the year. The level of magnesium during spring and summer was found to be under standard limits.

Chl *a* was significantly higher in spring (7.72 µg.L^{-1}), remained at intermediate values in summer, and then fell under 3.2 µg.L^{-1} in autumn and winter. The variations in the organic matter content were not significant throughout the period of study (3.62 to 6.32 mg.L^{-1}).

Relative standard deviation (RSD)

Over a low concentration range, the variability is estimated as the average standard deviation of replicates, while over a high concentration range, the variability can be estimated as the average of the factor $RSD=100.(\sigma/\bar{x})$, where σ is the standard deviation and $\bar{x}$ the mean of the variable.

The extreme seasonal fluctuations of the environmental variables were identified through the factor RSD (*Table 2*).

Table 2. *Extreme RSD (relative standard deviation in %) of physico-chemical variables in the Boukourdane Lake Dam for the four seasons.*

	RSD<5%					**RSD>120%**			
	Spring	**Summer**	**Autumn**	**Winter**		**Spring**	**Summer**	**Autumn**	**Winter**
pH	2.1		3.6	1.9	Phosphates	244.9	151.9	126.5	203.2
EC			4.6	4.3	Ammonia	244.9	174.7	120.5	109.1
Sulfates			3.5	4.1	Nitrites	114.9	162.4		106.8
Cl^-			3.6	2.2	Nitrates		121.7	139.2	
Ca^{2+}			2.4						

The variables of pH, EC, sulfates, chloride and calcium had the lowest variabilities, below 5%, during the cold periods (autumn and winter), while the variables of phosphates, ammonia, nitrites and nitrates had the highest variabilities, over 120%, mostly in summer.

Pearson's correlation coefficients

When two variables are correlated, tracking one of them enables measuring the change in the other, reducing the experimental costs of physico-chemical monitoring.

The most significant positive correlations ($p<0.01$, *Table 3*) were observed between T, EC and SO_4^{2-}; EC and Ca^{2+}; pH and Na^+; NO_2^- and NH_4^+; and EC and Chl *a*.

The most significant negative correlations ($p<0.01$) were observed between the pH and PO_4^{3-}, NH_4^+ and Cl^-, and PO_4^{3-} and Cl^-.

Table 3. *Pearson's correlation coefficients among environmental variables ($^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$)*

	T	pH	EC	DO	NO_3^-	NO_2^-	SO_4^{2-}	NH_4^+	PO_4^{3-}	Na^+	Cl^-	Ca^{2+}	Mg^{2+}	Org m	Chl *a*
T	1,00														
pH	-0,06	1,00													
EC	**0,58****	0,24	1,00												
D.O.	0.42	0,21	0,15	1,00											
NO_3^-	0,04	0,36	0,11	0,54**	1,00										
NO_2^-	-0,37	0,13	-0,36	-0,02	0,17	1,00									
SO_4^{2-}	**0,57****	0,36	**0,69*****	0,40	0,22	-0,22	1,00								
NH_4^+	-0,22	0,05	-0,42*	0,18	0,10	**0,53****	-0,30	1,00							
PO_4^{3-}	0.28	**-0,53****	-0,09	-0,24	-0,40	-0,08	-0,22	0,25	1,00						
Na^+	-0,01	**0,63*****	0,42*	0,37	0,42*	-0,06	0,31	-0,04	-0,37	1,00					
Cl^-	0,17	0,04	0,18	0,20	0,17	-0,04	0,39	**-0,52****	**-0,59****	0,06	1,00				
Ca^{2+}	0,26	0,36	**0,64*****	0,29	0,16	-0,22	0,44*	-0,27	-0,05	0,46*	0,03	1,00			
Mg^{2+}	0,28	-0,07	0,20	-0,60	0,37	0,61*	0,64*	-0,21	-0,19	-0,24	0,48	-0,22	1,00		
Org. m.	-0.11	0,34	0,11	0,43*	0,19	0,34	-0,11	0,47*	-0,28	0,43*	-0,12	0,22	-0,67*	1,00	
Chl *a*	0.43*	0,11	**0,61****	0,13	0,10	-0,27	0,31	-0,41*	-0,19	0,16	-0,04	0,44*	-0,20	0,20	1,00

Plankton analysis, seasonal richness of copepods, rotifers and phytoplankton

A total of 92 zooplankton species belonging to the two principal zooplankton groups (13 copepods and 79 rotifers) were found in the Boukourdane Lake Dam.

Some species of rotifers, including *Polyarthra remata, Keratella quadrata quadrata, Lecane monostyla, Lecane luna, Testudinella patina patina, Brachionus quadridentatus quadridentatus* and *Asplanchna priodonta*, occurred in most of the samples (73.3% of the counted total).

The most prevalent species in spring were *Keratella quadrata quadrata, Lecane monostyla, Lecane luna, Brachionus quadridentatus quadridentatus*. Another species present in summer samples was *Hexarthra fennica.*

Polyarthra remata was present, especially in winter (53.0%), together with *Asplanchna priodonta; Testudinella patina patina* and *Filinia longiseta* were found in autumn.

Only three species of copepods, namely *Copidodiaptomus numidicus* (calanoid, 32.2%), *Acanthocyclops trajani* (cyclopoid, 14.8%) and *Eucyclops agiloides* (cyclopoid, 11.6%), were widespread and occurred in most of the samples.

Cyclops strenuus strenuus and *Metacyclops minutus* were also observed, but in smaller quantities.

The average number of copepods (*Fig. 2*) was high in spring (median=251 ind.L^{-1}) and summer (198 ind.L^{-1}) but much lower in autumn (with a large variability) and winter (<100 ind.L^{-1}).

Rotifers (*Fig. 3*) abounded in spring (median=204 ind.L^{-1}), with a large variability during this season (standard error = 83 ind.L^{-1}), but their number sharply dropped in summer (12 ind.L^{-1}).

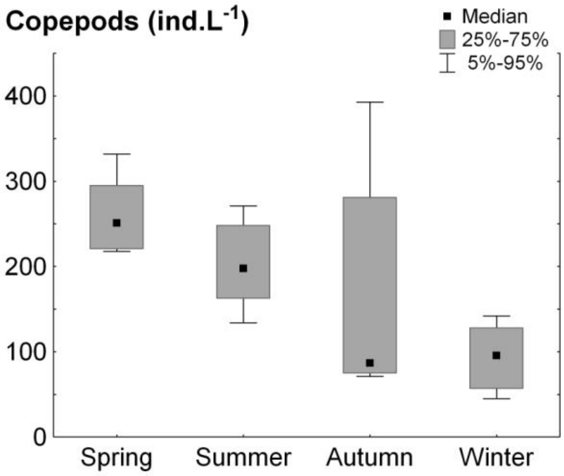

Figure 2. *Seasonal features of copepods*

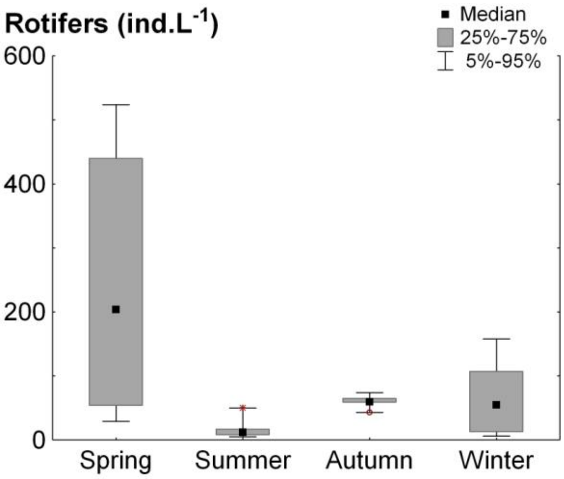

Figure 3. *Seasonal features of rotifers*

A total of 83 algal taxa were identified. The large spring-like richness of the phytoplankton community and its variability are highlighted (*Figs. 4 and 5*) and consisted of Chlorophyceae (38 species), Cyanobacteria (11), Bacillariophyceae (23), Pyrrhophyta (3), Euglenophyceae (5), Rhodophyta (1) and Charophyta (2). They contributed 33.2%, 33.0%, 26.2%, 6.5%, 0.86%, 0.14% and 0.09% of the total phytoplankton abundance, respectively.

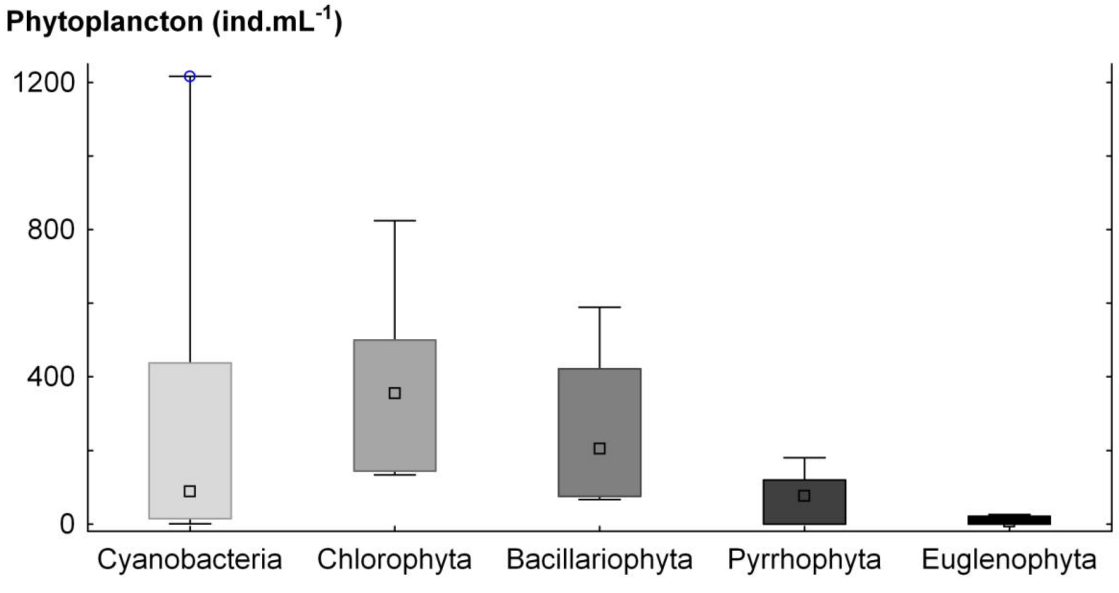

Figure 4. *Distribution of the phytoplankton community (□ median, box 25-75%, I.C. 5-95%).*

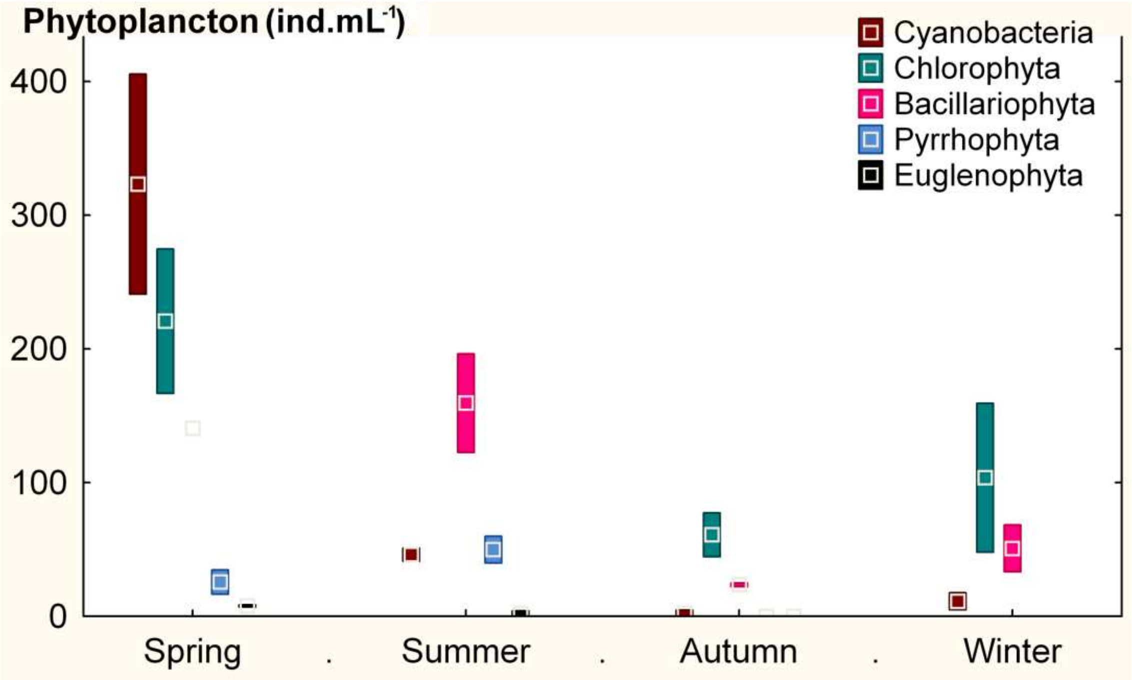

Figure 5. *Seasonal features of phytoplankton (□ median, box 5-95%).*

The density of phytoplankton was distributed from the most common variety, Chlorophyta (median=118 ind.mL^{-1}), to the least common one, Euglenophyta (2.2 ind.mL^{-1}), not including Rhodophyta and Charophyta, which were not considered due to their smaller quantities.

Cyanobacteria had the greatest variability (median=29.5 ind.mL^{-1}, standard error=52.4 ind.mL^{-1}). Among the Chlorophyceae, *Pediastrum simplex, Pediastrum boryanum, Chlorella sp.* and *Crucigeniella rectangularis* were the most abundant. Among the Bacillariophyceae, *Synedra acus* and *S. pulchella* were the most abundant.

Microcystis, Oscillatoria limnetica and *Microcoleus chthonoplastes* were the most prevalent of the Cyanobacteria; *Euglena, Lepocinclis* and *Phacus* were the only observed genera of the Euglenophyceae. Chlorophyta represented a dominant phytoplankton division (33.2% of total), followed by Cyanobacteria (33.0%) and Bacillariophyta (26.2%).

The seasonal variations of the phytoplankton are shown in *Fig. 5*.

Chlorophyta was most abundant in spring (average 221 ind.mL^{-1}). Cyanobacteria were also at maximum density in spring (323 ind.mL^{-1}) but were practically absent in autumn (2 to 3 ind.mL^{-1}). Bacillariophyceae proliferated in July (summer average: 160 ind.mL^{-1}) but fell sharply in autumn (23 to 24 ind.mL^{-1}). Pyrrhophyta and, to a lesser extent, Euglenophyceae were present in spring and summer but were completely absent in autumn and winter.

Weighted average of the species

The weighted average WA(Sp) (*Table 4*) is the simplest way to estimate the species optimum. This method allows determining, for each species, the optimal values of the environmental variables.

Table 4. *Estimation of species optima using the weighted averages of the values of environmental variables (Cop: copepods, Rot: rotifers, Cyan: Cyanobacteria, Baci: Bacillariophyta, Pyrr: Pyrrhophyta, Eugl: Euglenophyta, Chloro: Chlorophyta, Charo: Charophyta, Rhod: Rhodophyta, Alg: algae, Org. m.: organic matter, Chl a: chlorophyll a).*

	Cop	**Rot**	**Cyan**	**Baci**	**Pyrr**	**Eugl**	**Chloro**	**Charo**	**Rhod**	**Alg**
T (°C)	21.0	19.6	21.6	23.0	25.1	22.4	18.8	21.0	16.7	21.3
pH	7.6	7.7	7.8	7.6	7.5	7.8	7.7	7.8	7.5	7.6
E.C. (mS.cm^{-1})	0.72	0.74	0.79	0.73	0.75	0.79	0.73	0.80	0.65	0.73
DO (%)	88.3	89.6	92.6	90.2	93.1	94.2	84.4	93.1	77.7	89.0
NO_3^- (mg.L^{-1})	3.82	4.16	3.93	4.36	3.16	4.62	3.90	3.86	3.41	3.46
NO_2^- (mg.L^{-1})	0.064	0.079	0.029	0.075	0.019	0.027	0.096	0.020	0.038	0.083
SO_4^{2-} (mg.L^{-1})	141.6	142.4	147.2	147.0	145.7	150.5	142.9	147.0	128.1	142.2
NH^+ (mg.L^{-1})	0.035	0.054	0.040	0.023	0.033	0.031	0.033	0.045	0.012	0.043
PO_4^{3-} (mg.L^{-1})	0.33	0.27	0.33	0.28	0.54	0.24	0.22	0.33	0.28	0.38
Cl^- (mg.L^{-1})	71.9	68.7	67.0	75.7	71.9	69.9	70.0	64.8	74.9	70.3
Ca^{2+} (mg.L^{-1})	70.2	72.4	75.0	68.7	70.2	74.9	70.8	76.7	67.5	70.2
Mg^{2+} (mg.L^{-1})	33.0	31.2	31.1	35.9	32.9	32.6	32.5	30.1		32.4
Na^+ (mg.L^{-1})	44.1	45.9	47.1	42.4	43.3	47.1	43.6	48.4	44.3	43.5
Org m (mg.L^{-1})	5.06	6.19	6.24	4.38	5.07	5.88	5.16	6.76	3.51	5.38
Chl *a* (µg.L^{-1})	5.35	5.88	7.30	5.55	6.42	7.22	5.81	7.68	2.46	5.83

The Pyrrhophyta abundance weighted optimum was characterized by elevated temperature, DO and phosphate levels. That of Bacillariophyceae was characterized by elevated temperatures (T>=23.0°C), whereas Chlorophyta was generally more abundant at low temperatures (T<19.0°C). The prevalence of Charophyta was characterized by high optimal levels of Chl *a* and organic matter but low levels of nitrites.

Conversely, Rhodophyta reached its optimal level at low levels of temperature, EC, Chl *a*, organic matter and ammonia. The rotifers showed their abundance-weighted optimum at high concentrations of organic matter, ammonia and nitrites.

Factors regulating the seasonal dynamics of the zooplankton and phytoplankton assemblages

A correlation matrix of environmental-species variables was produced (*Table 5*) to highlight the internal structure that ultimately contributes to the identification of the major sources of growth not visible at first glance.

This table highlights the strong correlations between the copepod population and the variables EC and Chl *a*. The rotifers showed a slightly significant correlation with calcium. Cyanobacteria were highly correlated with calcium, Bacillariophyta with sulfates, and Euglenophyceae with EC, calcium and Chl *a*.

***Table 5.** Correlation matrix of the species-environmental variables of the Boukourdane Lake Dam waters (Cop: copepods, Rot: rotifers, Cyan: Cyanobacteria, Baci: Bacillariophyta, Pyrr: Pyrrhophyta, Eugl: Euglenophyta, Chloro: Chlorophyta, Org. m.: organic matter, Chl a: chlorophyll a). ($^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$)*

	Cop	Rot	Cyan	Baci	Pyrr	Eugl	Chloro
T	0,63	-0,06	0,24	$0,85^{*}$	$0,91^{*}$	0,15	0,72
pH	0,24	0,53	0,48	0,19	-0,25	0,75	0,44
EC	**0,91****	0,54	$0,75^{*}$	$0,81^{*}$	0,36	**0,92****	0,79
DO	0,44	0,27	0,34	0,53	0,55	0,54	0,09
NO_3^-	0,11	0,19	0,06	0,25	-0,20	0,39	0,05
NO_2^-	$-0,80^{*}$	-0,16	-0,38	-0,39	-0,64	-0,06	-0,32
SO_4^{2-}	0,65	0,31	0,47	**0,88****	0,35	0,64	$0,87^{*}$
NH_4^+	-0,42	0,20	-0,04	-0,38	0,42	0,27	-0,42
PO_4^{3-}	0,11	-0,13	0,02	-0,04	0,50	-0,35	-0,09
Na^+	0,53	0,54	0,51	0,07	-0,13	0,54	-0,03
Cl^-	-0,10	-0,59	-0,56	0,58	-0,17	-0,41	-0,16
Ca^{2+}	$0,81^{*}$	$0,79^{*}$	**0,87****	0,52	-0,02	**0,92****	0,49
Mg^{2+}	-0,52	-0,56	-0,63	0,94	-0,23	-0,24	1,00
Org m	-0,02	0,56	0,38	-0,21	0,31	0,77	-0,14
Chl *a*	**0,81***	0,55	$0,80^{*}$	$0,82^{*}$	0,60	**0,84***	$0,90^{*}$

RDA analysis

The redundancy analysis (RDA) was applied to 15 environmental variables and 10 species variables, which were reduced to 11 and 7 variables, respectively, in the final RDA (*Figs. 6A and 6B*). The large number of variables required to efficiently diagnose the water quality is an obstacle to the implementation of a coherent and sustainable monitoring program. Therefore, certain variables were omitted, due to insufficient collected data or errors.

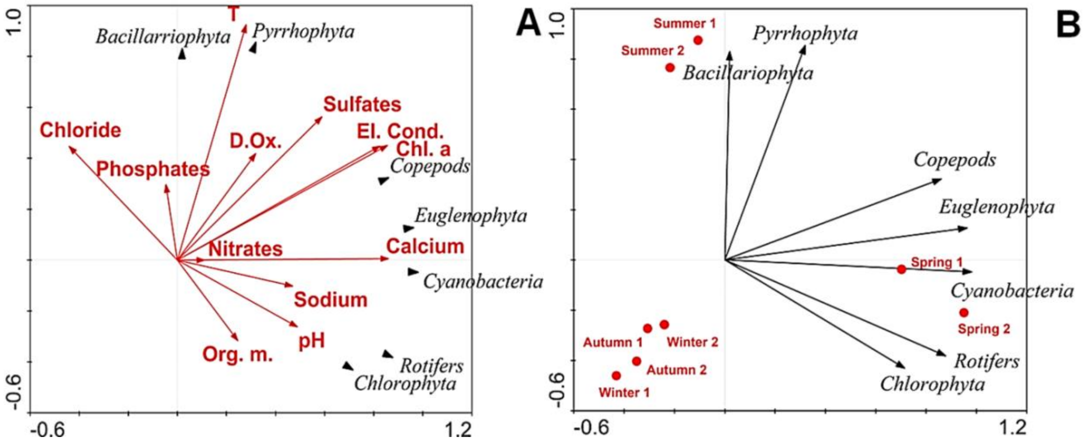

***Figure 6.** Biplot in the redundancy analysis (RDA) of zooplankton and phytoplankton species in relation to environmental scores.*

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The RDA biplot of the plankton species and the environmental scores corroborate our previous observations. The first two axes explain 84.1% of the data variance, which is a satisfactory percentage. Most of the taxa appear on the right side of the factorial graph, corresponding mostly to the spring and related to “intermediate to high” levels of mineral content or trophic state.

The zooplankton and a large part of the phytoplankton abundance were quite sharply influenced by the levels of sulfates, EC, Chl *a* and calcium. This effect was lower for dissolved oxygen, sodium and nitrates. Rotifers and Chlorophyta appeared to be more influenced by pH variability, sodium and organic matter contents. Bacillariophyta and Pyrrhophyta appeared in summer and were influenced by elevated temperatures.

Discussion

Physico-chemical variables and Pearson’s correlations

The pH values classify Boukourdane Lake Dam in the quasi-alkaline range (6.3-8.1), which is indicative of the medium production and alkaliphilous water body category under the criteria of Venkateswarlu (1983). The observed fluctuations in the electrical conductivity could be due to variations in the decomposition rate of organic matter, the low level of water caused by evaporation, or the influx of seepage and nutrients from the drainage basin. This reservoir can be classified as eutrophic according to the Olsen criteria (1950) (conductivity >500 $\mu S.cm^{-1}$).

In general, the solubility of oxygen decreases when the temperature and salinity increase. Throughout the study period, the dissolved oxygen level was generally close to saturation in summer and spring. The relatively low levels in autumn may be due to pollution in the site or to decaying algal cells that used up the available dissolved oxygen at the water surface. These results were identical to those reported by Shah and Pandit (2013).

A high concentration of nitrates, as observed in spring and winter, is useful in irrigation, but their entry into water resources increases the growth of nuisance algae and triggers eutrophication and pollution (Trivedy and Goel, 1984). This could also be due to the high level of dissolved oxygen during these periods. In winter, the high levels of nitrites might be partly due to influx of nutrients from the watershed areas along with the runoff water in this cold period. Using Pearson's correlation, Yu et al. (2014) suggest a strong and positive correlation between toxicity and the total nitrogen in wastewater, including NO_2^- and NH_4^+. The pattern of nitrite-nitrogen concentration was found to be quite similar to that observed by Garg et al. (2010) and Sulthana et al. (2011).

The actual level of phosphates defines the Boukourdane reservoir as a eutrophic water body (Lee et al., 1981). Based on the total phosphorus level, Tammeorg et al. (2014) found a similar evolution, with the highest values during late summer and early autumn and the lowest during the winter and spring periods in different lakes. The very high levels (>30) of the NO_3^-/PO_4^{3-} ratio are related to the poverty of plankton in winter and are in agreement with the results of Herut et al. (2000). A ratio of 20:1 suggests that the algae may be phosphorus-limited, which is typical for most freshwater systems (Sargeant, 1997).

The high concentrations of sodium limit the biological diversity due to osmotic stress; the encountered levels are consistent with the findings of Mathur et al. (2010) and Usharani et al. (2010). The slightly higher levels of chloride in summer and autumn may be attributed to the rise in temperature increasing the rate of evaporation, causing a decline in water cover. These results are in accordance with the reports of Govindasamy et al. (2000) and Moundiotiya et al. (2004). Under the classification of Unni (1983), this reservoir can be categorized as a polluted water body.

The magnesium level, which is essential for chlorophyll growth (Dagaonkar and Saksena, 1992), remained under standard values but could not be measured during autumn and winter.

The chlorophyll in an aquatic body is a direct indication of algal growth. According to the OECD criteria (1982), its concentration was practically in the mesotrophic range (2.5-8.0 $\mu g.L^{-1}$).

A correlations analysis highlighted significant relationships between land use types and water quality during the dry and rainy seasons in a basin (Bu et al., 2014). The correlation analysis in another lake showed that a select number of macroinvertebrate metrics were significantly correlated with environmental variables (Johnson and Ringler, 2014). These relationships have been essentially confirmed in this work for EC correlated to copepod abundance, and for EC, sulfates and calcium correlated with a part of the phytoplankton.

Seasonal richness of copepods, rotifers and phytoplankton

The principal species found in the Boukourdane Lake Dam were consistent with those described by other studies performed on the Algerian aquatic ecosystem (Samraoui et al., 1998; Cherbi et al., 2008; Bouzidi et al., 2010; Hamaidi et al., 2010; Hamaidi-Chergui et al., 2013). The rotifer results are consistent with those of Zanata and Espindola (2002), who found them to be dominant during the dry period. Sartori et al. (2009) and other studies showed that rotifers are always the species with the highest richness.

Because the physico-chemical variables change throughout the year, it is of considerable interest to quantify the seasonal changes in the zooplankton community structure (Enriquez Garcia et al., 2009), and in reservoirs used for water supply, this knowledge can be important for quality management purposes (Garcia et al., 2002).

Polyarthra remata was mostly present in winter, which is in agreement with Cherbi et al. (2008). *Keratella* species are inhabitants of moderately clean (mesotrophic) waters (Saksena, 1986). Generally, *Keratella quadrata* and *Filinia longiseta* are indicators of eutrophic lakes (Geng et al., 2005).

Many rotifer species occurred sporadically, and most of them were found only in the littoral zone. It is known that inorganic nitrogen such as NO_3^- and NO_2^- can help increase the rotifer density, for which an important determinant is the trophic status of lakes (Wang et al., 2010). The oligotrophication in a lake may coincide with decreases in *Polyarthra* or *Keratella* (Barbiero and Warren, 2011). *Hexarthra fennica*, present in summer, is described as euryhaline and seasonal.

The high copepod density in spring was correlated with elevated levels of Chl *a*. The most common species were *Copidodiaptomus numidicus* and *Acanthocyclops trajani*, as found in earlier studies (Cherbi et al., 2008). In general, an adequate food supply in terms of a high Chl *a* concentration favours the copepods, which feed more selectively (Voutilainen et al., 2012). Chl *a* had a greater impact on species common in the open water than on macrophytes (Enriquez Garcia et al., 2009).

In general, the phytoplankton level is a useful tool for assessing long-term changes in rivers, such as those associated with eutrophication and river management (Prygiel et al., 1999). The abundance of the spring species Chlorophyta is consistent with the results reported by Yerli et al. (2012) and Yilmaz and Aykulu (2010). Van den Hoeck et al. (1995) already showed that Chlorophyceae are a large and important group of freshwater algae.

The high rates of productivity encountered during spring can be due to the increase in temperature and the high transparency, speeding up the photosynthetic activity of the phytoplankton (Bhoyar and Tamloorkar, 2012). Moreover, the excess nutrients can cause algal blooms and the possibility of water contamination by toxin producers, if present (Marcé et al., 2010).

The weighted averages of the species were very useful and enabled us to develop an estimated mapping of species for any dataset of environmental variables, which is very helpful for preventive monitoring.

RDA analysis

Multivariate statistical techniques help in the interpretation of complex data matrices to better understand the water quality and ecological status of the systems studied (Shrestha and Kazama, 2007). These techniques have been applied to evaluate the temporal or spatial variations of water quality data and also their usefulness to obtain better information for the effective management of water resources (Singh et al., 2004).

The RDA analysis corroborated a great part of the previous results reported in this work. A low contribution of relationships between some environmental variables and copepod assemblages has been found (Fiasca et al, 2014), corroborating our results for dissolved oxygen, pH and nitrates. The strong presence of Chlorophyta in the spring, which correlated with the pH and the organic matter density, is also in agreement with the results of O'Farell et al. (2002) who noted that the profuse phytoplankton growth led to increases in the pH and suspended matter content.

Conclusions

In conclusion, the Boukourdane Lake Dam can be categorized as eutrophic according to its levels of EC, phosphates and Chl *a* but stands at an intermediate position when compared to other temperate water bodies.

The redundancy analysis revealed that sulfates, EC, Chl *a* and calcium were the most important variables affecting the zooplankton and phytoplankton distribution. Great variability in the levels of nitrites, nitrates, phosphates and ammonia was observed from one season to another.

The values of the environmental parameters for each species optimum were determined through calculation of their weighted averages. This allowed us to fairly accurately determine the distribution of the most prevalent species for every season.

The results confirmed earlier findings stipulating that the Boukourdane reservoir was dominated by rotifer species, but in spite of their lower number and predation from vertebrates, the crustacean zooplankton were more numerically abundant, especially in the spring.

This pilot investigation suggests that more studies are necessary in Algerian lakes to improve our knowledge of zooplankton and phytoplankton distributions, composition and relationships with the water quality, so this report represents the beginning of studies on environmental conditions.

The present study offers a significant base to evaluate the fish production potentialities and to formulate sustainable management policies for aquaculture in the study area.

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ADVENTITIOUS ROOTING OF NATIVE *ERICA MANIPULIFLORA* SALISB., A MEDITERRANEAN ORNAMENTAL SHRUB

ARI, E.[1,2*] – GURBUZ, E.[1,3]

[1]*Bati Akdeniz Agricultural Research Institute, 07100 Antalya, Turkey*

[2]*Permanent Address: Department of Agricultural Biotechnology, Faculty of Agriculture,*
Akdeniz University, 07058, Antalya, Turkey
(phone/fax: +90-242-3102451)

[3]*Permanent Address: Bartın Directorate of Provincial Food Agriculture and Livestock, Bartın, Turkey*

[*]*Corresponding author*
e-mail: esinari@akdeniz.edu.tr

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Abstract. The use of *Erica manipuliflora*, a decorative native ornamental species, is limited in landscaping due to insufficient plantlet production in nurseries. The aim of study was to determine the most effective and practical adventitious rooting (AR) protocol for successful vegetative production of *E. manipuliflora*. Two experiments (Expt.) were established in November and June to reveal the effects of different substances [perlit and perlit:sphagnum peat (1:1 v/v) in Expt.I and peat, peat:sand (1:1), cocopeat:sand (5:1) and perlite in Expt.II] and exogenous auxins including liquid (in Expt.I) and powdered (in Expt.II) formulations of IBA, NAA or combination of both. The results indicated *E. manipuliflora* cuttings taken in June and cultured in cocopeat:sand (5:1) mixture without any auxin treatment presented the highest survival (84%) and rooting (76%) performance. In addition, exogenous auxin usage is not essential for AR of *E. manipuliflora* cuttings. It can be used for the cuttings inserted in cocopeat-free media. But it exhibited reducing effect on rooting in cocopeat-based medium. The use of mycorrhizal inoculation and also minicuttings in eco friendly cocopeat-based media in future studies might increase the efficiency of *E. manipuliflora* AR protocol.

Keywords: *auxin, cocopeat, domestication, heath, ornamental plant*

Abbreviations: AR, adventitious rooting; CPA, commercial powdered auxin; Expt., experiment.

Introduction

High adoption of xeriscape approach laid out the increasing use of native plants. Today, utilization of them has been accepted as an emerging niche market (Hamill, 2005). According to Norcini (2006), the ratio of native plant sales of Florida in 2005 was 11% of all ornamental plant production. The use of native plants is an upward trend in southeastern United States since the 1990s (Waterstrat, 1997; Brzuszek and Harkess, 2009). Thanks to the other factors like growing environment consciousness, changing consumer trends and the global climate changes, this trend has progressed in other parts of the world, too. Also superior functional characteristics like resistance/tolerance to drought, saline and poor soil conditions can be mentioned as main causes in this trend.

In our previous inquiry study (Arı et al., 2010), we determined the perspective and tendency of the representatives of landscape sector for the use of natural plants in Antalya, Turkey. *Erica manipuliflora* Salisb. (Ericaceae), whorled heath, was identified as one of the most desired three native species for large quantity production and sale in the market by both

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landscape architects and nursery managers. Since it has valuable xeriscape plant characters like reducing water use and landscape maintenance cost, it is not only preferred for Antalya but also for other regions under Mediterranean climate.

Native distribution area of *E. manipuliflora* (synonym: *E. verticillata* Forssk.) (UPOV, 2015) covers a region from Croatia to Lebanon, including southeast Italy, Crete and Cyprus (Fagundez and Izco, 2011). *E. manipuliflora* mostly disperses up to 1500 m altitude of the West and South Anatolia of Turkey, particular in open fields, phrygana and macchie groups, *Pinus brutia* forests, limestone or serpentine layered rocks (TPDS, 2015). This evergreen, perennial shrub growing up to 1-2 m inhabits among the widespread woody species of macchie community in Mediterranean territory of Turkey.

E. manipuliflora is a phanerophyt species (TPDS, 2015) that blooms scented (Jones, 1987) and nectar producing flowers with 1–5 in axillary umbels on very short shoots (THS, 2015a) varying from white to dark pink, shades of lilac color between May and November. It has flowers with 8 stamens and 4 corolla lobes and sepals (Stevens, 1978; Güvenç and Kendir, 2012), which are partially fused and bell-shaped, papillate anthers, crystals on the mesophyll and occasionally a sparsely hairy ovary top. Its ellipsoid seeds reach up to 0.8 mm long (Fagundez and Izco, 2011) while needle-like short leaves in whorls of 3 or 4 grow up to 9 mm long (THS, 2015a). Aerials parts of *E. manipuliflora* have been ethnobotanically utilized for preparation of herbal teas as astringent, diuretic and remedy of urinary infections in Turkey (Baytop, 1999; Tuzlaci and Eryasar Aymaz, 2001; Akkol et al., 2008). On the other hand, it can be used also for apiculture making benefit of abundant flowers. However, the main interest for *E. manipuliflora* is for its ornamental value. This attractive species can be used as group or ground-cover plant in landscaping. It has gained more importance for conservation of ecosystems due to its less water and fertilizer need because of the ability to grow in arid and barren lands. The latest demand for *E. manipuliflora* has been improved by the greenkeepers of golf courses in Antalya lately. This formable species is desirable to cover small hills since it is likely to control the plant size and flowering period of *E. manipuliflora* by pruning.

In order to provide this plant species in large quantities, it should be domesticated and domestication of *E. manipuliflora* is also important for the sustainable assessment and exploitation of an underused genetic resource. The first step for domestication is to establish the production systems. It is possible to produce *E. manipuliflora* via generative or vegetative methods. In generative production, germination is not consistent as in other Ericaceous plants species whose germination is often variable and not well defined (Englert et al., 1996). In our previous germination study (Ari et al., 2014), only 8 seedlings could be produced from 0.10 g seeds sown by scattering and without prechilling. Pipinis et al. (2006) also sowed *E. manipuliflora* seeds with scattering and achieved 181 seedlings from 0.15 g seeds treated one month of prechilling. In addition, Piotto et al. (2003) reported the seeds of *E. arborea*, a close relative species of *E. manipuliflora*, could be germinated after being exposed to smoke and high temperatures up to 120°C for 10 minutes. Seemingly, extra treatments might be needed for germination of *E. manipuliflora* seeds. On the other hand, the individual plants which would be derived from native seeds will exhibit segregation in their phenotypes. Moreover, a high morphological variability was reported for *E. manipuliflora* (McClintock, 1989). However, different vegetative production methods have the advantage to supply mass clonal plant production which is important especially for the reproduction of unique plants with distinguished plant characteristics, and also of the mutant genotypes.

The adventitious rooting (AR) of cuttings is the most widely used vegetative production method for woody ornamental plants. There are many factors affecting AR ratio such as plant growth regulator (PGR) type and concentrations, rooting substrates and environmental conditions. Among them, substrate is one of the most important factors. A good substrate should ensure the correct degree of moisture for cutting base while allowing aeration, besides holding the cutting in place (Hartmann et al., 2010; Fornes et al., 2013). Sphagnum peat moss, vermiculite, perlite, sand, clay, coconut peat, and composting of forest waste, and manure, etc, are among the wide variety of inorganic and organic materials for composing different types of growing media used in nursery cultures (Vallejo et al., 2012).

On the other side, exogenous auxins also have a big role in determination of AR capacity (Kotis et al. 2009; Ling et al. 2013). The most common used exogenous auxin is indole-3-butyric acid (IBA) because of its higher root inducing capability. α-naphthalene acetic acid (NAA) is another preferred exogenous auxin. Beside the usage of exogenous auxin alone, combinations of two auxins or auxin and cytokinin have also been used. IBA and NAA are widely preferred for auxin combination owing to their consistency in stimulating AR of cuttings (Hartmann et al., 2010).

Most of the nursery growers use commercial powdered rooting hormones since they are more practical and accessible compared to the experimental pure PGRs used generally in liquid formulations. Thus, it might be more useful to suggest to growers the more accessible production materials.

The main purpose of this study was to establish the most effective and practical AR protocol for successful vegetative production of native *E. manipuliflora*. For this purpose, it was focused on determination of the effects of different rooting substances and exogenous auxins including liquid and powdered formulations on rooting abilities and growth of *E. manipuliflora* cuttings.

Materials and Methods

Plant Material

The study was performed in Bati Akdeniz Agricultural Research Institute (BATEM) in Aksu (lat. 36°56′N, long. 30°53′E), Antalya, Turkey, in 2008-2009. Donor *E. manipuliflora* plants were located in Kumkoy, Aksu, under Mediterranean environment with dry, hot summers and mild, wet winters. The climatic parameters of the months when the cuttings were sampled for two experiments were presented in *Table 1*. The soil type of donor plants was sandy with 32.8% $CaNO_3$ ingredient, 2% organic matter content, electrical conductivity (EC) of 75 µS cm^{-1}, pH 7.7, available phosphorus (P) level of 4 mg kg^{-1}, changeable potassium (K) level of 29 mg kg^{-1}, changeable calcium (Ca) level of 1987 mg kg^{-1}, changeable magnesium (Mg) level of 94 mg kg^{-1}.

Table 1. *The meteorological data* of the months when the cuttings were sampled from donor plants of Erica manipuliflora.*

Parameters	November	June
	2008	**2009**
Mean minimum air temperature (^{0}C)	10.7	18.8
Mean maximum air temperature (^{0}C)	23.2	33.7
Mean air temperature (^{0}C)	16.2	26.5
Mean relative humidity (%)	79.0	63.7
Total precipitation (mm)	29.0	-
Mean maximum precipitation (mm)	14.6	-
Mean wind speed (m.s^{-1})	1.6	2.1

*: The meteorological data were taken from TSMS (2015)

Rooting Experiments

Two experiments (Expt.) were established to determine the effects of different rooting substrates and exogenous auxin types and concentrations on AR of *E. manipuliflora* cuttings. Both experiments were set up in completely randomized design in varying numbers of factorial arrangements. The long semi hardwood shoot cuttings were gathered from a well branched, vigorous *E. manipuliflora* population which consisted from a few uniform shrubs adjacent side by side due to the requirement of high number of cuttings for the experiments. The long cuttings carrying little fresh shoots with light green needle-like leaves were moved to glasshouse within one hour, then immediately shortened to small pieces with sharp pruning shears. The cuttings were 0.5-1 cm in diameter and 10-15 cm length and had at least 5-6 leaf nodes. They were inserted in ≈ 4-5 cm depth into the rooting benches with 20 cm height containing different substrates. The benches were bottom heated at 21°C in Expt. I. The irrigation of cuttings was supplied with an intermittent misting system controlled by a timer. Cycles of mist were arranged based to changing climatic conditions of glasshouse and cuttings' requirement. No fertilizer was treated to cuttings.

Expt. I

Expt. I. was set up on 10 November to determine substantially the effects of different auxin concentrations in liquid formulations in either alone or combinations on rooting of *E.manipuliflora* cuttings in two substrates. The experiment was in a 2 x 10 (substrate x hormone) factorial arrangement with 3 replications, 30 cuttings in each, a total of 1.800 cuttings. The basal ends of the cuttings were quick dipped for 10 s into the different auxin solutions of IBA and NAA (Merck, Darmstadt, Germany) alone and the combinations of IBA and NAA. IBA was dissolved in 96% ethanol (Merck), and then diluted to different concentrations while NAA in 0.1 N NaOH. The tested hormone concentrations were; 1) control, 2) 1000 mg l^{-1} IBA, 3) 2000 mg l^{-1} IBA, 4) 4000 mg l^{-1} IBA, 5) 2000 mg l^{-1} NAA, 6) 4000 mg l^{-1} NAA, 7) 8000 mg l^{-1} NAA, 8) 1000 + 500 mg l^{-1} IBA + NAA, 9) 2000 + 1000 mg l^{-1} IBA + NAA, 10) 4000 + 2000 mg l^{-1} IBA + NAA. The control cuttings were immersed in distilled water. After air dried ≈ 10 min, the treated cuttings were stuck in perlit (Etiper, Izmir, Turkey) or the mixture (1:1 v/v) of perlit and sphagnum peat (Klasmann-Deilmann GmbH, Geeste, Germany), which was denominated as peat hereafter, in the heated rooting benches. Peat was not used alone due to its inefficiency which was experienced in our previous works with different auxin solutions. All the cuttings were pulled out on 26 May to make observation. The survived and rooted cuttings were counted, and then other important parameters representing root and shoot growth of the cuttings were recorded. These parameters included root length (cm), shoot number (n) and the density of shoot and root. "Density" means the degree of shoot and root developments. This parameter was scored based on a 1-5 visual rating scale, where 1 = very poor, 2 = poor, 3 = medium, 4 = good, 5 = very good.

Besides these treatments, a small-scale treatment was set up in the same day in order to see the effect of a commercial powdered rooting hormone (Mass Plant, Adana, Turkey) containing 0.5% IBA + 0.5% NAA. This treatment had 4 replications, in each of which 30 cuttings were placed into perlite media.

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Expt. II

This experiment was set up on 09 June based largely on the results of Expt.I to determine the effects of different substrates and commercial powdered auxins (CPA) on rooting of *E. manipuliflora* cuttings. The experiment was performed in a 4 x 3 (substrate x hormone) factorial design with 5 replications, 10 cuttings in each (a total of 600 cuttings). All of the fresh semi hardwood cuttings were firstly immersed in distilled water. After air dried ≈ 10 min, cuttings excluding the control ones were treated with one of two CPA. The first CPA (I.CPA) contained 0.5% IBA (Rhizopon AA, Hazerswoude-Rijndijk, Netherlands) and the second CPA (II.CPA) had 0.5% IBA + 0.52% NAA content (Mass Plant, mentioned above). Treated cuttings were inserted into 4 substrates; 1) peat, mentioned above, 2) mixture of peat and sand (1-3 mm coarse river sand) (1:1 v/v), 3) mixture of cocopeat (Dutch Plantin, Boekel, Nederland) and sand (5:1 v/v) and 4) perlite media. The cuttings were harvested on 10 August to record the rooting results. Only survival and rooting performances were evaluated in Expt. II.

Statistical analysis

The data of both experiments were first subjected to Levene's test to detect the variance homogeneity. Two-way and also one-way, if required, variance analysis (ANOVA) were implemented for homogeneous variances by General Linear Model procedures to test treatment differences. The means were separated by Tukey's multiple range test using SPSS software (IBM Corp., Armonk, NY, USA) at an alpha 0.05 level. For non-homogeneous survival and rooting percentages of the cuttings, the ratios were transformed using arcsine of the square root to increase homogeneity before analysis. The presented means were based on nontransformed data.

Results

Expt. I

The two-way ANOVA of Expt. I indicated very significant difference ($p < 0.001$) within two substrates (perlite and perlite:peat) for all observation parameters except root length, which was significant at $p < 0.05$ (*Table 2*). The cuttings in perlite media presented superior performance than those of in perlite:peat media (1:1 v/v) for survival (56.44% versus 36.44%) and rooting (56.44% versus 36.11%) ratios, root length (9.87 cm versus 8.90 cm), shoot number (12.25 versus 3.71) and densities for shoot (3.63 versus 2.78) and root (3.66 versus 2.92). Meantime, survival and rooting ratios of the cuttings were determined to be almost the same.

Auxin treatments exhibited very significant difference ($p \leq 0.001$) for only survival and rooting ratios of cuttings, while they showed insignificance for other observation parameters (*Table 2*). Of the auxin treatments, the treatment of 4000 mg l^{-1} IBA significantly generated the highest average survival and rooting ratio (67.8%) regardless of substrate, which followed by the treatment of 2000 + 1000 mg l^{-1} IBA + NAA (60.6%). IBA + NAA treatments displayed higher performance than alone IBA or NAA treatments for the other cutting growth parameters. The highest shoot number (10.4) and both shoot (3.47) and root (3.56) densities occurred in the treatment of 4000 + 2000 mg l^{-1} IBA + NAA, while the highest root length (10.83 cm) in the treatment of 2000 + 1000 mg l^{-1} IBA + NAA. As for interactions between substrate and auxin treatments, there could not be found significant difference for any of observation parameters.

Table 2. *Effects of different substrates and liquid auxin formulations on survival, rooting and other growth parameters of Erica manipuliflora cuttings in Expt.I.*

		Survival (%) M ± SE²	**Rooting (%) M ± SE**	**Root length (cm) M ± SE**	**Shoot number M ± SE**	**Density¹**	
						Shoot M ± SE	**Root M ± SE**
Substrate							
	Perlit	56.44 ± 2.66 a	56.44 ± 2.66 a	9.87 ± 0.27 a	12.25 ± 0.60 a	3.63 ± 0.09 a	3.66 ± 0.07 a
	Perlit:peat (1:1)	36.44 ± 3.64 b	36.11 ± 3.63 b	8.90 ± 0.32 b	3.71 ± 0.30 b	2.78 ± 0.15 b	2.92 ± 0.10 b
Auxin (mg l^{-1})							
Control	0	46.67 ± 7.45 ab	45.00 ± 7.78 ab	9.32 ± 0.42 a	7.91 ± 2.46 a	3.14 ± 0.46 a	3.25 ± 0.39 a
IBA							
	1000	38.33 ± 4.92 b	38.33 ± 4.92 b	8.03 ± 0.68 a	6.38 ± 1.68 a	2.89 ± 0.32 a	3.14 ± 0.17 a
	2000	46.11 ±11.59 ab	46.11 ± 11.59 ab	9.30 ± 0.39 a	7.85 ± 2.31 a	3.27 ± 0.57 a	3.22 ± 0.39 a
	4000	67.78 ± 9.05 a	67.78 ± 9.05 a	8.67 ± 0.80 a	7.96 ± 2.37 a	3.08 ± 0.31 a	3.39 ± 0.21 a
NAA							
	2000	56.67 ± 5.64 ab	56.67 ± 5.64 ab	9.45 ± 0.70 a	8.45 ± 2.64 a	3.13 ± 0.38 a	3.12 ± 0.26 a
	4000	41.67 ± 7.59 ab	41.67 ± 7.59 ab	9.41 ± 0.80 a	6.20 ± 1.84 a	3.08 ± 0.35 a	3.13 ± 0.24 a
	8000	36.11 ± 8.75 b	36.11 ± 8.75 b	9.23 ± 0.17 a	6.97 ± 1.57 a	3.07 ± 0.16 a	3.17 ± 0.10 a
IBA+NAA							
	1000 + 500	34.44 ± 5.14 b	34.44 ± 5.14 b	10.19 ± 0.89 a	7.55 ± 1.37 a	3.46 ± 0.19 a	3.47 ± 0.27 a
	2000 + 1000	60.55 ± 6.58 ab	60.55 ± 6.58 ab	10.83 ± 0.56 a	10.15 ± 2.15 a	3.45 ± 0.19 a	3.45 ± 0.14 a
	4000 + 2000	36.11 ± 4.16 b	36.11 ± 4.16 b	9.40 ± 0.99 a	10.39 ± 2.63 a	3.47 ± 0.25 a	3.56 ± 0.26 a
Significance							
Substrate (S)		***	***	*	***	***	***
Auxin (A)		***	***	NS	NS	NS	NS
S x A		NS	NS	NS	NS	NS	NS

[1] These data were obtained based on a 1-5 visual rating scale (1:very poor, 2:poor, 3:medium, 4:good, 5:very good).

[2] Values are mean (M) and standard error (SE). Two-way ANOVA was used for the comparison of the means and they were separated by using Tukey's multiple range test. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

NS: Nonsignificant; *, ***: Significant at $p \leq 0.05$ and $p \leq 0.001$, respectively.

When IBA and NAA treatments were dealt with separately, IBA alone treatments were found more stimulant than NAA alone treatments to produce higher survival, rooting and other observation parameters except from root length and shoot density. The average values of IBA versus NAA treatments were 50.74% / 44.82% for both survival and rooting ratio, 9 cm / 9.36 cm for root length, 7.40 / 7.21 for shoot number, 3.08 / 3.09 for shoot density and 3.25 / 3.14 for root density, irrespective of the substrate factor. Meanwhile, the average shoot number (7.91) and shoot density (3.14) of the control treatments were superior to those of both IBA and NAA alone treatments. In addition, the rooting ratio (45%) and root density (3.25) of the controls were higher than NAA treatments, while the root length (9.32) was higher than IBA treatments.

The most important observation parameter in this study is AR ratio of the cuttings. Even though the interaction between substrates and auxin concentrations was found nonsignificant for this parameter, the separate one-way variance analysis was performed to show the difference between auxin treatments in each substrate. The analysis results were presented in *Fig. 1*.

The highest rooting ratio (71.11%) was determined in the cuttings treated with 4000 mg l^{-1} IBA in perlite:peat (1:1 v/v) at $p < 0.05$ (*Fig. 1*). The same ratio was also observed in the treatment of 2000 + 1000 mg l^{-1} IBA + NAA in perlite. However, it is not possible to mention statistically about a given favorable influence of exogenous auxin treatments on AR in perlite media ($p = 0.89$) since the control treatment (58.8%) without auxin exhibited higher rooting performance than 4 different auxin treatments in perlite medium. In addition, performance of control treatment (35.6%) in perlite:peat (1:1 v/v) media exceeded those of five auxin treatments in the same substrate.

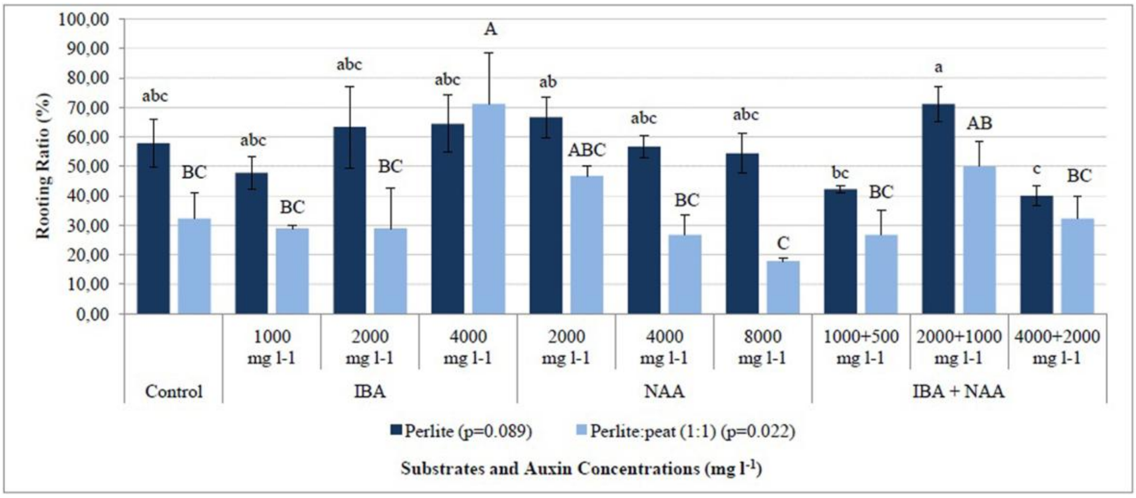

Figure 1. *Effects of different liquid auxin formulations on adventitious rooting ratios (%) of Erica manipuliflora cuttings in the substrates of perlite and perlite:peat (1:1 v/v) in Expt. I (values are mean (M) and ± standard error (SE) bars. Comparison of means in each substrate was determined by separate one-way ANOVA, then TUKEY post hoc test. Means within each substrate followed by the same letter are not significantly different at* $p \leq 0.05$*).*

Meanwhile, rooting ratio of the small-scale rooting treatment in which a commercial powdered auxin (Mass Plant) consisting from 0.5% IBA + 0.5% NAA ingredient was tested in perlite medium was determined as 35.8% . Since the experimental conditions of this rreatment were different, its result was not analyzed statistically together with those of the treatments of Experiment I. Its average rooting ratio (35.8%) was also higher than that of seven liquid auxin treatments in perlite:peat (1:1) medium.

As for the results of other observation parameters in each substrates, they were presented here only in the text, not in a Table or Figure: The longest average root lengths were obtained from the cuttings treated with 2000 + 1000 mg l^{-1} IBA + NAA both in perlite and perlite:peat media (11.41 cm and 10.25 cm, respectively). The same auxin combination in perlite:peat media formed the highest average shoot numbers (5.85). However, the treatment of 4000 + 2000 mg l^{-1} IBA + NAA in perlite media produced superior performance (15.41) for the same parameter. Regarding the densities over 5 according to a 1-5 visual rating scale, the highest shoot and root intensity (3.53 and 3.30, respectively) in perlite:peat media were detected in the treatment of 1000 + 500 mg l^{-1} IBA + NAA combination. Whereas, the most vigorous shoot and root development of the experiment occurred in perlite. The best root density (4.07) was achieved from the treatment of 4000 + 2000 mg l^{-1} IBA + NAA in the same substrate. Interestingly, the most shoot density (4.12) was determined in the control treatment without auxin again in the same medium.

Taken into consideration of all the results of Expt.I, growing substrates appear more effective than liquid auxin treatments on survival and rooting ratios and the other growing parameters of *E. manipuliflora* cuttings, as presented in *Table 2*.

Expt. II

According to two-way ANOVA results of Expt. II, the difference among four substrates was found very significant ($p < 0.001$) for both survival and rooting percentage (*Table 3*).

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Table 3. *Effects of different substrates and commercial powdered auxins (CPA) on survival and rooting ratios (%) of Erica manipuliflora cuttings in Expt.II.*

		Survival (%) **M ± SE [1]**		**Rooting (%)** **M ± SE**	
Substrate					
	Peat	11.33 ± 3.36	c^x	1.33 ± 0.91	c
	Peat:sand (1:1)	22.67 ± 4.62	bc	5.33 ± 2.15	bc
	Cocopeat:sand (5:1)	54.67 ± 7.03	a	43.33 ± 8.01	a
	Perlite	30.00 ± 4.14	b	18.00 ± 4.49	b
CPA					
	Control	36.50 ± 7.26	a	25.50 ± 7.52	a
	I. CPA	32.50 ± 4.03	a	16.00 ± 4.32	ab
	II. CPA	20.00 ± 4.23	b	9.50 ± 3.28	b
Significance					
Substrate (S)		$p = 0.000$ ***		$p = 0.000$ ***	
CPA		$p = 0.003$ **		$p = 0.005$ **	
S x CPA		$p = 0.001$ ***		$p = 0.001$ ***	

[1] Values are mean (M) and standard error (SE). Two-way ANOVA was used for the comparison of the means and they were separated by using Tukey's multiple range test. Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.
, *: Significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

Of the four substrates, cocopeat:sand (5:1 v/v) was the best to exhibit the highest survival (54.7%) and rooting (48.3%) performance, followed by perlite (30% and 18%), irrespective of CPA (*Table 3*). The peat based substrates, peat:sand (1:1 v/v) and peat alone, resulted in the lowest survival (11.3% and 22.7%, respectively) and rooting ratios (1.3% and 5.3%, respectively).

The difference among the CPAs was significant at $p < 0.01$ for survival and rooting percentage (*Table 3*). However, this difference derived from control treatment without auxin. It was superior in terms of survival (36.5%) and rooting (25.5%) percentages compared to both I.CPA with IBA content, and II.CPA containing IBA+NAA combination. Meantime, I.CPA was better than II.CPA for survival (32.5% versus 20%) and rooting (16% versus 9.5%) ratios.

Due to the significant ($p \leq 0.001$) interaction between substrate and CPA, separate one-way variance analysis was performed to show the difference between CPAs in each substrate for AR performance of cuttings, the results of which were presented in *Fig. 2*.

Overall, the control treatment in cocopeat:sand (5:1 v/v) was significantly ($p < 0.01$) the most responsive application for AR formation (76%), while there was no difference between two CPAs in the same substrate (30% and 24%) (*Fig. 2*). Also, no difference could be found between the CPAs including control treatment in other growing substrates. On the other hand, the survival ratio of control cuttings in cocopeat:sand medium was 84%, while 44% and 36%, respectively, in the cuttings treated with I. and II.CPA, in the same substrate. As an additional observation, the root structures generated in cocopeat:sand mixture was much more powerful and intensive than that of other substrates. Therefore, these results revealed the superiority of the mixture of cocopeat:sand (5:1) medium in producing vigorous roots from *E. manipuliflora* cuttings. Furthermore, there is no need to use CPA for successful vegetative production of this native species in cocopeat:sand medium.

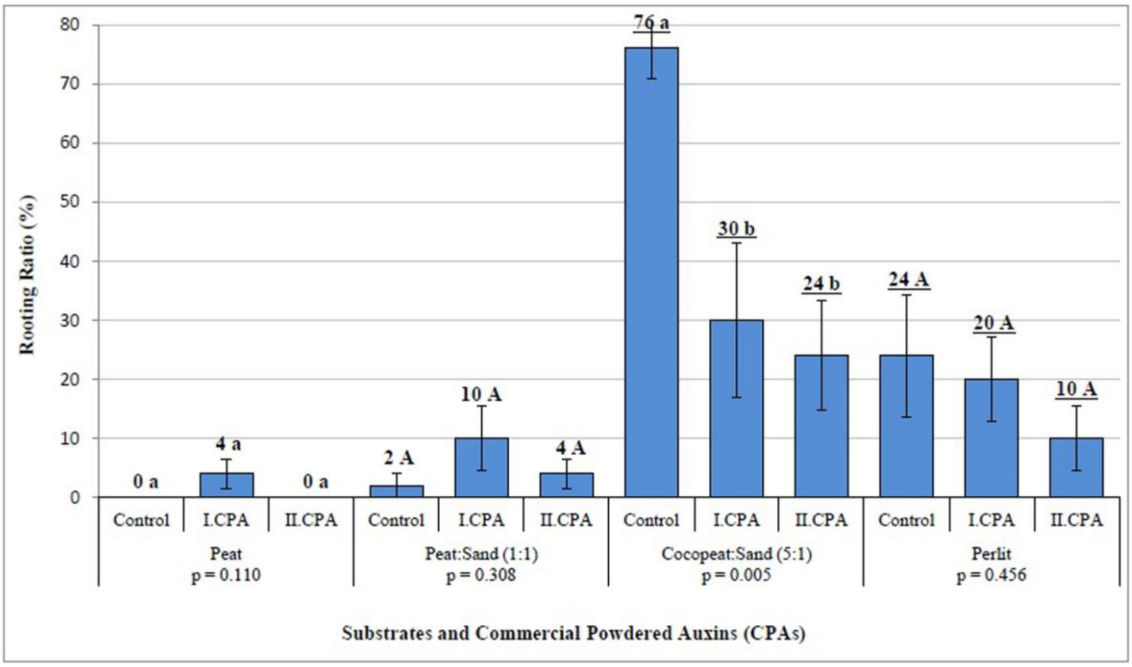

Figure 2. *Effects of different commercial powdered auxins (CPAs) on adventitious rooting of Erica manipuliflora cuttings in different substrates in Expt. II (Values are mean (M) and ± standard error (SE) bars. Comparison of the means in each substrate was estimated by separate one-way ANOVA, then TUKEY post hoc test. Means within each substrate followed by the same letter are not significantly different at $p \leq 0.05$).*

Discussion

Two experiments were performed to reveal the most effective AR protocol for successful vegetative production of native *E. manipuliflora*. Expt. I was set up on 10 November to investigate the effects of ten different liquid auxin formulations on rooting of the cuttings cultured in two rooting substrates, perlite and perlite:peat. Significant difference ($p < 0.001$) was determined among both substrates and the liquid auxin formulations for rooting percentage. However, the interaction between them was nonsignificant. In the substrate base, the highest rooting percentage (71.11%) was identified in the cuttings inserted in perlite:peat medium and treated with 4000 mg l^{-1} IBA ($p < 0.05$). On the other hand, the treatments of IBA + NAA combinations, presented higher performance than alone IBA or NAA treatments for root length, shoot number, shoot and root densities. According to Hartmann et al. (2010), combination of IBA and NAA than either auxin alone produces more rooting and larger root systems in the cuttings of many species, which is compatible with our results.

If it is required to deal with IBA and NAA treatments separately, IBA alone treatments were found more successful than NAA alone treatments to generate higher survival, rooting and other observation parameters except from root length and shoot density. The superiority of IBA compared to NAA for AR of the cuttings has also been revealed in other plant species such as Paeonia ‘Yang Fei Chu Yu’ (Guo et al., 2009) and *Macleania rupestris* (Veloza et al., 2014) because of its several advantages such as greater stability to light than others (Kurepin et al., 2011; Pacurar et al., 2014), localized action, persistence and low toxicity (Fachinello et al., 1995).

Expt. II was designed on 09 June to explore the effects of three CPAs including control and four substrates (perlite, peat, peat: sand, cocopeat:sand) on rooting of *E. manipuliflora* cuttings. The interaction between substrate and CPA was found very significant ($p < 0.001$) for survival and AR formation. Also, very significant differences were detected among the substrates ($p < 0.001$) and CPAs ($p < 0.01$) for both parameters. However, the difference among CPAs derived from the control treatment without auxin. In the separate one-way variance analysis of rooting performance in each substrate, the difference among CPAs was determined significant for only cocopeat-based substrate ($p < 0.01$). The highest survival and also rooting ratio occurred in cocopeat-based medium. Control treatment of cocopeat-based substrate was the superior and showed 2.5 and 3-fold increase in rooting percentage than the I. and II.CPAs, respectively. The survival (84%) and rooting (76%) ratios of the control treatment in cocopeat:sand were the highest values among overall treatments of Expt. I and II.

Cocopeat known also as coconut coir dust is an agricultural by-product (Hume, 1949) obtained after the extraction of fiber from the mesocarp or husk of coconut (*Cocos nucifera* L.) fruit (Abad et al., 2002; Awang et al., 2009). Even though it has been recognized since 1940s (Hume, 1949), it has attracted the attention particularly for the last 20 years (Lowry, 2015). It has been accepted to be a good media component (Awang et al., 2009) and a peat substitute because of its several advantages such as perfect drainage, absence of weeds and pathogens, slower decomposition, acceptable pH, cation exchange capacity, electrical conductivity and easier wettability (Cresswell, 1992; Meerow, 1994). On the other hand, cocopeat is known to have rather high water holding capacity resulting poor air-water relationship and causing to low aeration within the medium (Abad et al., 2002; Awang et al., 2009). However, it is possible to eliminate this disadvantage by incorporation of different coarser materials into cocopeat (Bunt, 1988; Awang et al., 2009). As a suitable substrate, cocopeat has been used in various plant species for different aims such as potting mixture of *Asplenium nidus* (Ainuddin and Najwa, 2009), *Pistacia lentiscus* and *Quercus ilex* (Vallejo et al., 2012), germination of sweet corn seeds (El-Hamed et al., 2011), transplanting of tomato (Arenas et al., 2002) and *Sambucus* seedlings (Cano et al., 2007), acclimatization and hardening of *Mentha arvensis* (Phatak and Heble, 2002), apple (Modgil et al., 2009) and Ericaceous *in vitro* plants from six genera not including Erica (Scagel, 2003), and AR of the cuttings of *Nerium oleander* (Ochoa et al., 2003), *Calluna vulgaris* and *Rhododendron* cultivars (Matysiak and Nowak, 2009). In most of these studies, cocopeat has been used as a media enhancer in low ratios, up to most 50%, in peat, perlite, vermiculite and sand mixtures, not alone or exceeding half of the medium. In current study, we complied the suggestion of seller company and used 5:1 (v/v) cocopeat:sand mixture. This ratio (80%) seems quite higher compared to other combinations in literature. For instance, Matysiak and Nowak (2009) tested six coir substrates having different ratio of coir up to 35%, sphagnum peat moss, perlite and sand for rooting of the cuttings of ornamental ericaceous plants, *Calluna vulgaris* and Rhododendron cultivars. The best rooting mixture for *C. vulgaris* were those containing at least 15% of coir, while 20% for Rhododendron. In addition, increasing amounts of coir above 20% resulted in decreased rooting ratios for ericaceous plants, especially for Rhododendron 'Roseum Elegans'. Arenas et al. (2002) also reported a reduced plant growth for tomato transplants grown in coir amended media exceeding 50%.

Seemingly, the rooting result obtained from the control treatment of cocopeat-based substrate in the present work is not in accordance with them, but revealed the most successful rooting ratios. The reasons for this result might possibly be attributed to the plant type of *E. manipuliflora*, which is an Ericaceous species producing ericoid mycorrhizas, and Scagel (2003)'s statement. According to her, cocopeat contains a great number of free-living phosphate solubilizing, acid phosphatase producing and nitrogen-fixing rhizobacteria, and thus it is a proper media amendment for growing Ericaceous species. In addition, Scagel (1999) found the rooting of Ericaceous plants increased linearly with increasing stem protein content. During the sampling of cuttings, it is not possible to change protein level of cuttings. However, it can be raised with some cultural methods such as special media mixtures, fertilizers or mycorrhizal fungi application. Cocopeat use in media compositions can increase protein levels of stems, leaves and roots of the plants. For instance, Scagel (1999) determined higher protein level in *Kalmia latifolia* plants growing in an amended medium with cocopeat than the plants in the medium without cocopeat. Hence, changing media ingredient or treatment of mycorrhizal fungi may alter tissue protein levels of ericaceous plant cuttings and affect AR of them as suggested by Scagel (1999).

The literature is lacking for isolation and inoculation treatment of ericoid mycorrhizas in *E. manipuliflora*. However, we know how the predominant species of typical sclerophyllous Mediterranean vegetation (Macchia) are rich in mycorrhizal diversity from Maremmani et al. (2003)'s study. In their mycorrhizal screening in 82 species belonging to 46 plant families in two native Mediterranean Macchia sites from Italy and Greece, they determined 83% and 90% of the plant species, respectively, had mycorrhizas. One of the screened species was *E. arborea* L. and ericoid mycorrhizas were found only in this species. *E. arborea* mycorrhizas are not only limited to ericoid ones since plant roots can form multiple mycorrhizal interactions with various fungal partners from different taxa as reported by Bergero et al. (2000). They identified about 150 fungal isolates from *E. arborea* roots. Additionally, Bergero et al. (2003) used *E. arborea* as a bait plant and found ericoid mycorrhizal fungi persist and sustain mycorrhizal ability in habitats lacking ericaceous host as in the case of *Pernettya macrostigma* (Brook, 1952) and *Calluna vulgaris* (Pearson and Read, 1973). Therefore, *E. manipuliflora* as a close relative of *E. arborea* and a predominant sclerophyllous Macchia species is also most likely in mycorrhizal association in nature. Hereby, a mycorrhizal inoculation in culture stage may probably help its successful production as in other Ericaceous plants such as *C. vulgaris* (Gebhardt and Friedrich, 1987), *Vaccinium corymbosum* (Scagel et al., 2005), *V. meridionale* (Ávila Díaz-Granados et al., 2009), *Leucothoe axillaris* and *Pieris japonica* (Zinati et al., 2011). However, as we did not make an inoculation intentionally, the successful survival and rooting results of the control treatment in cocopeat-based media might be explained with again Scagel (2003)'s above mentioned claim. Cocopeat imported from tropical regions and used in our study might presumably have contained some indigenous microbial and mychorrhizal entities even if in low populations, and such quantity is enough to initiate the required mycorrhizal inoculation with an ericaceous species (Ibrahim Ortas, Cukurova University, Adana, Turkey, personal communication). According to Zinati et al. (2011), microbial entities containing natural ericoid roots and soil having a mix of rhizospheric and nonrhizospheric bacteria, mycorrhizal and saprotrophic fungi have potential to improve plant growth by enhancing nutrient uptake. Vosatka et al. (1999) also reported that colonization may reduce transplant mortality and increase crop uniformity and productivity in the soil-less media containing low populations of indigenous mycorrhizal fungi. Indeed, the highest survival (84%) and rooting (76%)

ratio took place in cocopeat-based media in our work. If the information of “more than 90% of terrestrial plants are associated with mycorrhizas installing a permanent and intimate mutualistic symbiosis” (Strack et al., 2003) and “native genotypes are more dependent on mycorrhizas compared to cultivated forms” (Clement and Habte, 1995) is considered accurate, mycorrhizal requirements of native plants should be taken into consideration during their culture. Thus, mycorrhizal inoculation seems required for also *E. manipuliflora* production, and it is recommended for future studies.

In terms of cutting collection time, the cuttings sampled on 09 June (Expt. II) were better than the ones collected on 10 November (Expt. I) for AR formation and growth, which was possibly caused by the cuttings carrying fresher leaf shoots in Expt. II. However, the time span between taking cuttings from donor plant and inserting them to a substrate is another considerable issue for higher AR in *E. manipuliflora*. In our previous small-scale study (Ari et al., 2015) *E. manipuliflora* cuttings were taken from stock plants next to glasshouse on 19 June, inserted in ≈ 20 min in cocopeat:sand (5:1 v/v) mixture and revealed the highest AR (80%). Hence, moisture content of the cuttings should be taken into account and it is recommended to cut off and treat *E. manipuliflora* cuttings in the shortest time for minimum moisture loss especially in summer months.

In regard to useful auxin type, the auxin use in liquid formulations in Expt.I seems more successful than the powdered auxin use in Expt. II to form higher rooting ratio (up to 71% versus 30%). However, it is not possible to talk about an essential requirement for PGR use in either liquid or powdered formulation in present work because of the higher performances of control treatments without auxin. The Heather Society also does not recommend to use rooting hormones for hardy heathers (THS, 2015b) and evaluate *E. manipuliflora* among the hardy Ericas (THS, 2015a).

As a remarkable observation, the resulting AR in the cuttings took place from leaf nodes, not from bottom end of the cuttings in both Expt. I and II. This kind of root formation was also stated by The Heather Society for *E. carnea* initiating to form its roots always from a leaf nodule. However, the same Society stated different rooting style for *E. cinerea*, whose roots appear from the cutting base (THS, 2015b). According to Mahlstede and Watson (1952), AR could be initiated directly from the vascular tissue like cambium and secondary phloem parenchyma in Ericales, as in the case of *Vaccinium corymbosum* (Veloza et al., 2014). In appearance, rooting style is not the same for all Ericaceous species. We do not know the origin of root initials for AR formation in *E. manipuliflora* cuttings since we did not make a histological study. However, Zhang et al. (2009) examined the anatomical origin of *Feijoa sellowiana,* rooted in *E. manipuliflora* style, and reported that there were sequential and cyclical cells in the phloem and no root primordium in the Feijoa cuttings.

We observed another notable genesis that several mini individual detached shootings with a few leaf pieces broken from cuttings and fallen into growing media formed healthy, long adventitious roots. As reported by Ludwig-Muller et al. (2005), adventitious roots are less predictable in their cellular origin than lateral roots. They might occur from the cambium or from calli in the case of detached stem cuttings. According to Hartmann et al. (2010), leaves serve as the resource of endogenous auxins and carbohydrates, which implements a stimulating action on AR initiation in cuttings (Veloza et al., 2014). Ericoid mycorrhizal fungi may also produce hormones or stimulate plant production of hormones inducing AR development and growth (Scagel et al., 2005). On the other hand, using exogenous hormones may diminish the effects of mycorrhizal fungi on AR development as in the cuttings of *Vaccinium meridionale* (Avila Diaz-Granados et al., 2009). All these information might explain how mini individual *E. manipuliflora* shootings brought about AR without hormone requirement and why control cuttings in cocopeat-based medium rooted more successfully than the cuttings treated with auxins. Therefore, the use of 4-5 cm fresh leafy minicuttings consisting of a few leaf nodes with needle leaves is suggested for AR formation and consequently for vegetative production of *E. manipuliflora* for future studies as used in plant production of several ornamental plants lately. Also, it is proposed to culture minicuttings in the viols instead of benches containing cocopeat-based growing media, and may be inoculated with ericoid mycorrhizas, but without any PGR use.

In conclusion, *E. manipuliflora* cuttings taken in June and cultured in cocopeat:sand (5:1) mixture without any auxin usage revealed the highest survival (84%) and rooting (76%) ratios among the cuttings in overall treatments. This result indicated that exogenous auxin use is not essential for adventitious rooting of *E. manipuliflora* cuttings. It might be used as rooting enhancer in the studies including cocopeat-free substrates. But it has a reducing effect in cocopeat-based media. The superiority of cocopeat-based medium to generate the highest adventitious rooting originated most likely from the small amount of indigenous microbial and mychorrhizal entities carried with the cocopeat. Thus, mycorrhizal inoculation seems required for *E. manipuliflora* production. For future studies, using of mycorrhizal inoculation or minicuttings in eco friendly cocopeat-based media has the potential to increase the present AR ratio.

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INFLUENCE OF WILDFIRE AND FIRE SUPPRESSION BY SEAWATER ON SOIL PROPERTIES

BOGUNOVIC, I.[1*] – KISIC, I.[1] – JURISIC, A.[1]

[1]*Department of General Agronomy, Faculty of Agriculture, University of Zagreb*
Svetosimunska 25, 10000 Zagreb, Croatia
(phone: +385-1-239-3815; fax: +385-1-239-3981)

**Corresponding author*
e-mail: ibogunovic@agr.hr; phone: +385-1-239-3815; fax: +385-1-239-3981

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Abstract. During the last decades rapid climate changes have occurred and through frequent fires they have strongly affected the landscape. Wildfires directly influence on soil properties and generally increasing availability of nutrients. Due to the lack of multiple sources of freshwater, most fires in Dalmatia are being extinguished by seawater, which directly affects soil properties. During October 2012 soil samples (0 - 5 cm) were collected at four different locations from a burned and unburned plots in order to observe the impacts of fire on soil properties. Samples were collected 14 and 2 months after the wildfires occurred in 2011 and 2012, depending on the location. This paper analyzes the effects of wildfire and seawater used for fire suppression on soil chemical properties. The results showed significant differences between burned and unburned soil plots for pH, plant available phosphorus (P) and potassium (K), total carbon and total nitrogen. Differences between electrical conductivity (EC), F^-, Cl^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+} in burned and unburned plots also exist. Burned plots contain higher average values of K, P, EC and all water-extractable elements. Soil pH conditions after wildfire favors the solubility of some elements presented in this paper. These investigated parameters are under the direct influence of fire intensity and seawater on the ground. This study is useful for understanding changes in soil chemistry after fire and seawater application.

Keywords: *Dalmatian region, seawater, soil properties, wildfire*

Introduction

Fire is great disturbances in the ecosystems, but it is also an important ecological element (Pereira et al., 2012), fundamental for the landscape sustainability. In forest system wildfire can alter vegetation composition and even promote tree regeneration (Martínez-Sánchez et al., 1999; Cammeraat and Imeson, 1999). Fire was used in landscape management, and there are ecosystems like Mediterranean whose functions cannot be understood without fire (Pereira et al., 2010a; Mataix-Solera and Cerdà 2009). In the period of 1998–2008, out of all the forest fires in Croatia 31.7% were recorded in Dalmatia region (Croatian Mediterranean area that consists of 4 Croatian counties). In burned forest areas of Croatia, Dalmatia participates with as much as 64.3%, and for the most part (50.2%) these areas are overlain by Coppice Forests, shrubs, garrigues and thickets (Mamut, 2011). Wildfires are natural events, but the increased recurrence of wildfires in recent decades has an obvious anthropogenic component (Rovira et al., 2012).

Following wildfire, ash commonly covers the soil surface for some time until it is removed by wind or water erosion. Fires reduce soil organic matter on the surface and leave the soil unprotected from erosion which leads to very fast loss of ash material from the surface (Cerdà and Doerr, 2008; Pereira et al., 2013), especially if the burned areas are at higher inclinations. The erosion moves ash material which affects chemical

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properties of soil. This layer is a key factor for soil chemistry after the fire and can be many centimeters thick and can affect soil erosion (Cerdà, 1998a) and runoff generation processes and rates (Cerdà, 1998b).

Chemical composition of forest fire ash can be very variable and depends on various factors (Dlapa et al., 2013), like burning temperature, type of plant species, part of plant combusted, time of exposure to heat, fuel density and arrangement, and other factors affecting combustion conditions (Liodakis et al., 2005; Pereira et al., 2011). Dominant elements in wood ash are calcium (Ca), potassium (K), magnesium (Mg), silicon (Si), manganese (Mn), aluminium (Al), phosphorus (P), sulphur (S), iron (Fe), sodium (Na) and zinc (Zn) (Etiegni and Campbell, 1991; Liodakis et al., 2007). Many researchers (Andreu et al., 1996; Monleon et al., 1997; Hatten et al., 2005; Certini et al., 2011; Yinghua et al., 2012; Rovira et al., 2012; Bogunovic et al., 2014) have investigated wildfire and their impact on biological and chemical soil characteristics. Some of them have determined the reduction of total carbon (TC) (Certini et al., 2011) and N in soil (Rovira et al., 2012), low K variations and similar pH and P content one year after the fire (Andreu et al., 1996), while others (Yinghua et al., 2012; Bogunovic et al., 2014) observed higher soil pH and P content in burned soils 15 months post fire. Some studies (Hatten et al., 2005) recorded negligible difference in soil properties between burned and unburned areas, while Monleon et al. (1997) noted that 12 years post fire TC and inorganic N content were returned to unburned levels.

A serious problem of fire suppression in Dalmatia is the access to fresh water. Fire brigades use fresh water in wildfire ground suppression, but in many cases due to the inaccessible terrain air forces use seawater for wildfire suppression. According to chemical composition, the seawater is unsaturated homogeneous solution composed of water as a solvent (96.5%), dissolved salts (3.5%), small amounts of particulate matter, dissolved gases and organic ingredients. More than ninety chemical elements are found in seawater, and six major elements dominate: Na^+, Mg^{2+}, Ca^{2+}, K^+, chlorine (Cl^-) and S, while Na^+ and Cl^- account for more than 85% of the total (Martinac, 2010). These elements from seawater and ash material are leached into run-off and into the soil profile and may have impacts on surface soil chemistry.

There are several types of soil salinity and all refer to areas where soils contain high levels of salts (e.g. Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^- and SO_4^{2-}) that can affect plant productivity and soil organisms (Navarro-Pedreño et al., 1997). In this study the saline water application is not a result of arid environment or irrigation, since the source of salts is the seawater used for fire suppression. Seawater affects the natural ecosystem in the form of water and salt stress. Accumulation of dispersive cations, such as Na^+ in soil solution and the exchange phase (K^+, Mg^{2+}, Ca^{2+}) affect the physical properties of soil, such as structural stability, hydraulic conductivity, infiltration rate and erosivity (Juan et al., 2011). A plant in a drying, saline soil is exposed to increased levels of both water stress and osmotic stress, because the matric potential and the osmotic potential decrease simultaneously with decreasing soil moisture (Johnson, 1992). At the same time, the seawater undoubtedly affects burned soil. Researchers have investigated the effects of saline water on crops and soil (Richards, 1992; Sheng et al., 1997; Katerji et al., 2003; Ahmed et al., 2010; Chen et al., 2010; Ben Ahmed et al., 2012) but there is lack of studies of impact of seawater on fire-affected forest soils in the environmental conditions of Dalmatia, a part of Mediterranean.

The aim of this paper is study the effect of seawater on burned soil properties, TC, total nitrogen (TN), K and P, EC and water extractable ions (F^-, Cl^-, SO_4^{2-}, Na^+, Mg^{2+}, Ca^{2+}) after a wildfire in Dalmatia, Croatia.

Material and Methods

Site Characteristics

The study area is located in island Korcula and Peljesac peninsula in southern Dalmatia: Smokvica (42°55' N, 16°54' E, 97 m.a.s.l.), Blato (42°56' N, 16°50' E, 185 m.a.s.l.), Panjika (42°51' N, 17°30' E, 305 m.a.s.l.), Ponikve (42°50' N, 17°37' E, 240 m.a.s.l.). All four areas are in similar ecosystems with a Mediterranean climate characterized by long, dry and hot summers and mild and humid winters. Air temperatures are relatively high throughout the year (mean annual air temperature is 11.2 °C), and only during January and February temperatures are below 10 °C. Average minimum month temperature is 9.8 °C in January and average maximum month temperature is 26.9 °C in July (Meteorological and hydrological institute of Croatia, period 1961 - 1990). Average annual rainfall is 1300 mm and increases from the coastal area to the interior of the island, and from lower to higher altitudes. Average annual number of sunny hours amounts to over 2500 at some locations. The area is covered with Mediterranean flora and sometimes pine trees. All wildfires on investigated locations were supressed from air with seawater, while *Table 1* shows detailed information on the characteristics of individual sampling locations.

Table 1. *Site characteristics for burned and unburned sampling locations on Chromic cambisols. B-burned; C-unburned*

Sample code	Latitude/ longitude	Altitude (meters)	Year of fire	Slope (°)	Aspect	Dominant species
Smokvica B	42°55'25"N 16°54'19"E	82	2011	32	N	Strawberry tree (*Arbutus unedo* L), Holm oak (*Quercus ilex L.*), Heather (*Calluna vulgaris L.*)
Smokvica C	42°55'20"N 16°54'18"E	68		28	S	Black pine (*Pinus nigra* J.F.Arnold), Phillyrea (*Phillyrea latifolia* L)
Blato B	42°56'51"N 16°50'25"E	163	2012	39	N	Different pines, Phillyrea (*Phillyrea latifolia* L)
Blato C	42°56'50"N 16°50'21"E	155		42	N	Aleppo pine (*Pinus halepensis* Miller)
Panjika B	42°51'19"N 17°30'51"E	310	2012	17	W	Strawberry tree (*Arbutus unedo* L), Holm Oak (*Quercus ilex* L), maquis (macchie), Prickly Juniper (*Juniperus oxycedrus* L.)
Panjika C	42°51'24"N 17°30'25"E	336		25	W	Different pines, Strawberry tree (*Arbutus unedo* L.),

Ponikve B	42°50'55"S 17°37'21"E	223	2011	18	NI	Holm Oak (*Quercus ilex* L), Black pine (*Pinus nigra* J.F.Arnold), Prickly Juniper (*Juniperus oxycedrus* L.)
Ponikve C	42°50'57"N 17°37'20"E	236		12	N	Different pines, Prickly Juniper (*Juniperus oxycedrus* L..), Holm oak (*Quercus ilex L.*)

Survey areas consisted mainly of carbonate sedimentary rocks, limestones and dolomites. The karst bedrock in southern Dalmatia is dominantly covered by *terra rossa* - chromic cambisols (FAO, 2006), which cover the largest part of the coastal terrain and most of the islands. Most elevated areas in the highest mountain areas of the Peljesac peninsula are overlain by rendzic and mollic leptosols. Smaller patches on the island of Korcula are overlain by aric anthrosols.

Plot Design and Soil Sampling

At each site we collected 3 soil samples (0 - 5 cm) from burned and 3 from unburned plots (each taken sample consists of 10 individual samples homogenized into one) with similar aspect, slope, elevation and vegetation characteristics and those that have been diversely affected by wildfires in 2011 (areas burned 14 months prior to sampling) and 2012 (areas burned 2 months prior to sampling). The total number of collected samples is 24. Near each burned area unburned sample points were chosen according to their similarity to the burned areas in order to determine the differences between samples. Each composite sample was taken 40 m from another sample and represent an area of approximately 1200 m^2. Samples were taken with spade and stored in plastic bags without air and taken to the laboratory for analysis.

Preparation of Samples and Chemical Analysis

In the laboratory prior to chemical analysis, the samples were air dried, milled and passed through a sieve of 2 mm diameter. The following soil properties were monitored in the <2 mm fraction: soil pH, EC, plant available phosphorus (P) and potassium (K), TC, TN, F^-, Cl^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+}. The soil pH was measured using the electrometric method in 1:2.5 (w/v) ratio with the Beckman pH-meter Φ72, in KCl suspension (according to HRN ISO 11464:2004 norm). TN content was determined by dry combustion method according to HRN ISO 13878:1998. TC content was determined after dry combustion (HRN ISO 10694:1995). Plant available P and K were extracted by ammonium lactate (AL) solution (Egner et al., 1960) and detected by spectrophotometry and flame photometry, respectively. Electrical Conductivity was calculated at 25 ^{o}C on soil water (1:5) extract according to HRN ISO 11265:2004. Water-extractable anions (F^-, Cl^-, SO_4^{2-}) and cations (Na^+, Ca^{2+}, Mg^{2+}) in soil samples were extracted in ultra-pure water in 1:10 (w/v) ratio according to ÖNORM L 1092 norm. After the extraction, samples were centrifuged, filtrated and contents of anions and cations were detected by suppressed conductivity on Doinex ICS-1000 system with an analytical column: for anions - Ion Pac AS 17 (4x250 mm) and for cations - Ion Pac CS 16 (5x250 mm), all in compliance with HRN EN ISO 14911(2001) and HRN EN ISO 10304-1(1998). One-way ANOVA was carried out to test whether the investigated properties varied significantly amongst burned and unburned plots at each location. In case it did, the *post-hoc* Fisher test was applied to separate mean values at $P \leq 0.05$. All statistical analyses were carried out with SAS Institute 9.1.3.

Results

Statistically significant differences were found in all chemical variables measured in soils that have been burned 14 and 2 months prior to sampling in contrast to unburned plots. Detailed results are presented in *Figure 1* and *Table 2*.

TC concentrations were lower in the surface horizon of burned plots on Smokvica and Blato location, while burned plots on Panjika and Ponikve area recorded higher TC results. TC values ranged from 6.04% to 17.10% in burned samples, and from 6.82% to 13.07% in unburned samples. Mean value of TC was 10.19% in burned soils and 9.21% in unburned plots. Burned samples recorded higher value of TN in 3 of the 4 locations. The mean value of TN in burned samples was 0.55% and 0.37% in unburned samples. Burned plots contain higher amounts of TN than unburned and there was no significant difference only at Smokvica location.

The level of plant available P and K was significantly different among all locations. In unburned samples P ranged in very low supply levels (14 to 48 mg kg^{-1} P_2O_5), while the burned samples had moderate to very rich P supply and values from 135 to 424 mg kg^{-1} P_2O_5, except for Blato location where 30 mg kg^{-1} P_2O_5 were found. The average values of soil K supply were 737 mg kg^{-1} K_2O in unburned samples and 425 mg kg^{-1} K_2O in burned samples. These results show that the soil is very well supplied with K, with the exception of unburned samples from Ponikve site.

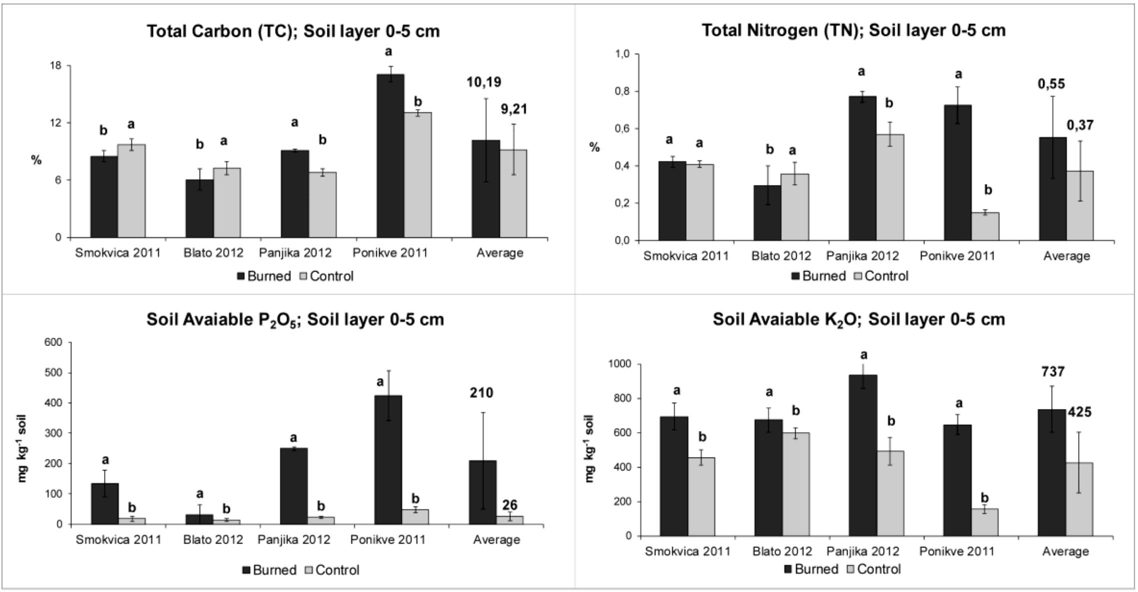

Figure 1. *Mean values of soil chemical properties for burned and control plots. For each chemical property at each location bars with different lowercase letters are statistically different (p<0.05). Hanging bars represent standard deviation.*

Burned plots recorded neutral to alkaline pH which varied from 6.78 to 7.90. The mean soil pH for unburned plots was 6.62, while unburned plots were low acid to neutral and their soil pH varied from 5.97 to 6.67. Statistical differences between unburned and burned plots were observed in the pH in each location. In this study significant differences between burned and unburned samples were identified in F^-, SO_4^{2-}, Na^+, Mg^{2+} and Ca^{2+}, in all investigated locations (*Table 2*). The amounts of Ca^{2+}, Mg^{2+}, Na^+ and SO_4^{2-} were significantly higher in burned plots than in unburned. Relatively high concentrations of SO_4^{2-}, Na^+, Mg^{2+}, F^-, Cl^- and Ca^{2+} were recorded in burned samples from all locations (with the exception of Na^+, F^- in Blato location). EC also showed significantly higher values in all four investigated sites, with almost 87% higher average value (295.88 µS cm^{-1}) in burned than in unburned plots (157.83 µS cm^{-1}).

***Table 2.** Mean concentration of pH, EC and water-extractable elements in the burned (B) and unburned (C) plots.*

Sample code		**pH**	**EC**	**F^-**	**Cl^-**	**SO_4^{2-}**	**Na^+**	**Mg^{2+}**	**Ca^{2+}**
		-log(H+)	**µS cm^{-1}**	**mg kg^{-1}**					
Smokvica 2011	**B**	7.09±0.12a	266.0±9.21a	1.89±0.07a	42.81±1.48a	52.46±1.82a	55.42±1.92a	29.96±1.04a	542.79±18.80a
	C	6.20±0.17b	155.9±5.40b	0.39±0.01b	18.75±0.65b	21.91±0.76b	44.30±1.53b	6.92±0.24b	308.53±10.69b
Blato 2012	**B**	6.78±0.05a	221.0±7.66a	0.26±0.01b	47.88±1.66a	65.97±2.29a	57.19±1.98b	28.61±0.99a	381.44±13.21a
	C	5.97±0.10b	146.5±5.07b	0.33±0.01a	42.19±1.46a	28.89±1.00b	67.91±2.35a	19.45±0.67b	245.64±8.51b
Panjika 2012	**B**	7.31±0.03a	335.5±11.26a	4.29±0.05a	104.33±7.41a	327.66±8.21a	57.17±2.51a	27.30±2.17	607.99±12.88a
	C	6.67±0.11b	173.5±8.26b	0.10±0.01b	22.12±1.22b	39.08±2.36b	42.13±1.32b	<0.01	374.96±17.52b
Ponikve 2011	**B**	7.90±0.04a	361.0±12.51a	5.84±0.20a	43.39±1.50a	219.81±7.61a	23.96±0.83a	175.56±6.08a	447.59±15.51a
	C	6.62±0.02b	155.4±4.16b	0.33±0.08b	31.83±2.66b	34.55±0.66b	12.95±0.91b	46.79±2.55b	215.90±10.85b
Average	**B**	7.27±0.13	295.91±17.29	3.07±0.65	59.60±7.98	166.48±34.44	48.43±4.34	65.36±19.23	494.95±26.99
	C	6.62±0.20	157.8±3.89	0.29±0.04	28.72±2.85	31.10±2.02	41.82±5.92	18.29±5.42	286.26±19.18

* Values of standard error of mean are given after ±. Different letters within a row for each location indicate significant differences ($p<0.05$).

Discusion

Carbon movement in the soil depends on fire severity (Pereira et al., 2010b), fire intensity, fire type (canopy or aboveground, underground fires), and even slope (González-Pérez et al., 2004). Low-intensity fires have little effect on the soil carbon while wildfires drastically reduce the amount of carbon in the soil (Johnson, 1992). Lower levels of carbon in burned samples one year after the fire in pine forests was recorded (Johnson, 1992), while other research (Binkley et al., 1992) observed lower amount of TC in burned soil than in unburned samples one and three years after the fire. Other studies recorded a nearly 50% increase of soil carbon content in burned area than in unburned area (Rovira et al., 2012 ; Choromanska and DeLuca, 2002). Increases in soil carbon are also reported due to an increased deposition of dry leaves and charred plant materials in fires that affect the tree canopy (González-Pérez et al., 2004). Our findings also show contradictory results of TC on investigated locations.These differences could be explained with differences in vegetation characteristics between locations. Control plots on each location have different dominant species and therefore we can presume that recorded wildifers had an uneven amount of fuel for burning, different combustion process, such as air temperature and humidity, wind speed, and topography of the site. All these factors had an influence on fire severity and therefore on TC. Average values (all four locations) of TC between burned and unburned plots were small. These trends may be due to the lower temperatures and incomplete combustion of soil organic matter at Smokvica and Blato location. It is already noted that severity of wildfires strongly influence on soil organic matter. Fire does not necessarily reduce topsoil organic matter content in a significant manner and low intensity fires have even been reported to increase organic matter content (Varela et al., 2010). Furthermore, in time of sampling, 14 and 2 months after fire it is visible generally fast recovery on vegetation (*Figure 2*) in all locations, but especially at Blato and Smokvica location. We can presume that small differences in average values of TC between burned and unburned plots are consequence of fast organic matter recovery in burned plots which affects on C sequestration. It is also noted in others studies (Johnson and Curtis, 2001; Wang et al., 2012) that fire had little effect on soil carbon. Even though it is evident that burned areas record generally fast vegetation recovery, on Blato location burned plot recorded the lowest TC concentration compared to all other locations, likely due to erosion removing material from the soil surface. Furthermore, these different observations between locations may be attributed to differences in sampling time after fire, soil properties, vegetation differences between investigated locations and fire severity.

A) *B)* *C)*

Figure 2. *From left to the right. Vegetation recovery on Blato (A, B), and Smokvica (C) location*

Total nitrogen follow similar pattern on investigated locations like TC. Our results agree with previous studies carried out by Weston and Attiwill (1990), Rovira et al. (2012) and Choromanska and DeLuca (2002) who also observed differences in TN depending on fire severity and the time that has passed since the fire. Permanent study shows that fire can increase and decrease the amount of N in soil. Different study results are also reported in studies of Schoch and Binkley (1986) and Yinghua et al. (2012) who observed minimal N loss from a fire, but decomposition of the forest floor was stimulated by fire, releasing additional N during the following growing season, after the regeneration of vegetation. Results of TN in this study are different because N is soil after fire depends on many factors. Immediate after fire soil organic N is losing through volatilization (Binkley and Fisher, 2012), but some substantial portion of soil organic N can survive low intensity fires. Moderate to high intensity fires convert most soil organic N to inorganic forms. Ammonium is a consequence of the combustion, while nitrate forms are created from ammonium some weeks or months after fire as a result of nitrification (Covington and Sackett, 1992). These N-forms if are not taken by plants can leach (nitrate) or held by negatively charged minerals in soil (ammonium), thus affecting the overall result of TN in the soil.

It is generally expected that the pH increases in forests after the fire and decreases over time at the rate which it does so being dependent on precipitation (Woodmansee and Wallach, 1981). During wildfires the combustion of wood causes the mineralization of organic matter which increases the amount of basic elements, carbonates, oxides, hydroxides and base cations compounds that are rich in alkaline metals (Etiegni and Campbell, 1991; Ulery et al., 1993) that raise and explains the increase of soil pH and EC in our study. Furthermore, pH values increase as a result of oxidation of soil organic matter by combustion of some organic acids (Certini, 2005), and EC increase as result of release of inorganic ions from combusted organic matter (Hernandez et al., 1997). Also, topsoil pH could increase as much as three units immediately after burning (Ulery et al., 1993). This rise was essentially due to the production of K and Na oxides, hydroxides, and carbonates, which did not persist through the rainy season in contrast to neo-formed calcite that was still present 3 years after burning, and maintained moderately alkaline soil pH. It is estimated that capacity of ash to neutralize soil acidity is well correlated with the sum of the concentrations of K^+, Ca^{2+}, and Mg^{2+} in the ash itself (Khanna et al., 1994). Except ash extracts that are rich in K (Badía and Martí, 2003; Ùbeda et al., 2009) higher noted levels of soil K supply were also result of natural content for *terra rosa*. Ca^{2+} also comes from limestone which is parent material on investigated areas. After fire, precipitation trough leaching and erosion move nutrient rich materials, washes away basics elements which finally leads to a decrease in pH during time. Even though the research areas were in the zones of mixed vegetation, forests, and low vegetation, the obtained results confirmed the above assertion. Investigated areas have an uneven precipitation distribution which is reflected in the long spring-summer dry season and rainy winters, and further research should provide insight into the time needed for the soil pH decreasing to pre-fire levels.

Average P supply value was eight times higher in burned samples than in unburned samples, as shown in other study (Yinghua et al., 2012). Some studies found no differences between burned and unburned samples (Binkley et al., 1992) and others (McKee, 1982) recorded significantly higher amounts of P in burned soil. These differences in burned and unburned soil are result of soil pH and fire. After fire, P in ash can leach into run-off and into the soil profile and may have impacts on surface chemistry. Burning converts the organic pool of soil phosphorus to orthophosphate (Cade-Menun et al., 2000), the sole form of P available to biota. The maximum P bioavailability values were recorded at pH of about 6.5 (Sharpely, 2000). But generally, fire-induced change in soil pH toward neutrality has a positive effect on P bioavailability, as shown in this study. Our results recorded higher pH values in burned plot than in unburned which directly affects on correlation with measured higher P values in burned plots in contrast to the unburned.In our work, we observed that all water-extractable base cations were significantly higher in burned soil compared with unburned soil solutions. Water extractable elements concentrations in burned plots are result of higher soil EC as a consequence of nutrient rich ash material on surface. Furtermore, fire suppression by seawater are reason for aditional source of Na^+, Mg^{2+}, Ca^{2+}, K^+, Cl^- which explains the higher values of these ions in burned soil compared to unburned soil solution. Increased concentrations of soluble elements helps increased alkalinity of burned soils. There are a number of factors that influence the vulnerability of sites to total salt accumulation (Oster et al., 1996). These factors include the position of a site within a landscape (Manning et al., 2001), soil type and rainfall. Soil type was the same - *terra rossa*, in all four investigated locations. Average annual precipitation rate was relatively high with 1300 mm (Meteorological and hydrological institute of Croatia), but with extremes in distribution, because almost 80% of rain falls in a period from September through May. The rain did not reduce the amount of salt in the soil, not even in locations that burned two years ago. Although, this depends of several factors as rainfall intensity, topography, vegetation cover etc. Our results show that time that has passed after the fire didn't have effects on salt accumulation in investigated locations. At all sites burned plots recorded higher total salts (EC) values compared to control although the sites burned in 2011 had one more rainy season. Also, the magnitude of salt accumulation in a single growth season is often small and may not be readily detectable by the routine soil sampling in the field (Chen et al., 2010). However, it should be noted that data of the size and intensity of rainfall at each investigated location were not available, so it is based on assumption. Thus, further research should clarify this situation. It remains to been seen how many years it will take for the soil that was treated with seawater to return to pre-burned conditions. In future research will be interesting to observe separated influence of seawater on burned soil properties compared to burn soil suppressed by freshwater on soil.

Conclusions

This study was conducted in southern Dalmatia fire-sensitive coniferous forests of pine trees with maquis and it shows that there is a difference in terms of soil properties between areas burned by wildfires suppressed by seawater and similar areas that remained unburned. The results show a difference in almost all investigated soil properties. Higher values of P, K, TN and TC have been recorded in burned plots. After the fire soil pH, EC and extractable cations and anions increase and this is attributed to the effect of ash and salt water used for suppression. Soil pH conditions after wildfire favors the solubility of some elements presented in this paper. This study cannot separate effect of seawater and fire influence on soil chemistry. The fact that the fire is extinguished with seawater may have implications on soil chemistry in the post fire period, but this can be clearer in a long-term study that compares burned soils extinguished with seawater and fresh water. This study is useful for understanding changes in soil chemistry after fire and seawater application. However, further studies with better establish experiment that includes divided causes on soil, fire and salt water, and continuous monitoring of soil changes is needed.

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THE EFFECT OF BIODIESEL BY-PRODUCTS ON GERMINATION AND PLANT GROWTH

KOVÁCS, A.[1]* – TÓTH, M.[1] – SOMOGYI, F.[2] – TOLNER, L.[1] – CZINKOTA, I.[1] – BÉRES, A.[1] – WILK, T.[1] – ALEKSZA, L.[1]

[1]*Institute of Environmental Science, Szent István University,*
H-2100 Gödöllő, Páter K. u. 1., Hungary
(phone: +36-28-522-000, fax: +36-28-410-804)

[2]*Institute of Mathematics and Informatics, Szent István University,*
H-2100 Gödöllő, Páter K. u. 1., Hungary
(phone: +36-28-522-000, fax: +36-28-410-804)

**Corresponding author*
e-mail: attila.balazs.kovacs@gmail.com

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Abstract. The aim of this paper is to investigate the effects of agricultural utilization of biodiesel by-products. The hydrophilous by-products of transesterification, such as proteins, carbohydrates, minerals, vitamins, potassium hydroxide used as catalyser and methanol are concentrated in the phase of glycerol during biodiesel production. Agriculture can utilize these components but some effects of glycerol in soil do not serve the needs of plants. Several studies have investigated the different effects of glycerol in soil. The specific focus of our research is to analyse the relationship between seed germination and glycerol content of soil. During this research the inhibitor effect of glycerol, biodiesel by-product, and methanol on seed germination of ryegrass (*Lolium perenne* L.) and oilseed rape (*Brassica napus*) were investigated. In the case of some treatments, a different percentage of glycerol and methanol was applied, in order to establish the methanol content of soil that can balance the inhibitor effect of glycerol. Based on the obtained information, pot experiment was established with ryegrass to examine the effects on plant growth. Our research has not only studied the impact of biomass production, but variations in the rate of plant growth as an effect of different treatments were observed as well.

Keywords: *biodiesel by-product, glycerol, germination, microbe*

Introduction

The industry of biofuels is one part of reneweable energy sources which is gaining ground in leaps and bounds. Between 2000 and 2007, global biofuel production tripled from 4.8 billion gallons to 16 billion gallons. Biodiesel production was about 3 billion gallons in 2007 and more than 50% of this quantity was produced by EU (Coyle, 2007). By prognosis, this process will increase, which raises the issue of by-product utilization. The newest, third generation possibility is to produce biofuels from algae. Algae were cultivated as biofuel feedstock mainly due to their high productivity of oil and less land requirement. Microalgae do not compete for land and can grow anywhere, even in brackish saline water (Sanjay et al., 2013; Piyushi et al., 2014*)*.

During biodiesel production a huge amount of useable by-products are produced with high glycerol content. Outputs of biodiesel production depend largely on oil type and quality. Approximately 100 litres of vegetable oil, 25 litres of methanol, and 0.8 kg KOH catalyser are used to produce 75 litres of biodiesel and 25 litres of crude glycerol (Wilkie, 2008). Generally the glycerol is used by the cosmetic and chemical industry but biodiesel by-product is contaminated by vegetal parts. But this „vegetal

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contamination" makes it perfect for utilizing this by-product as a fertilizer on agricultural fields.

The glycerol is an easily available and adequate carbon source for microorganisms (Lee et al., 2001; Tickell, 2003). It can intensify microbial activity which can help to increase the availability of vegetal nutrient. The large quantities of microbes increase organic nutrient content, the organic part of soil, which can increase humus content and results good conditions in soil. One part of nutrients can concentrate in the phase of glycerol, so it can balance the vegetal absorption. To sum up, glycerol has direct and indirect effects on productivity and nutrient content of soil.

Review of literature

During biodiesel production vegetable oils and animal fats are transesterificated by methanol or ethanol in the presence of a KOH or NaOH catalyser. At the end of this process, the results are alkyl ester and glycerol (Furfari, 2008). Solubility behaviour of the two residues can help to separate the hydrophilous glycerol from hydrophobic ester (Holser, 2008). If methanol is used during transesterification, the result is fatty acid methyl ester (FAME), whereas if ethanol is used, the result is fatty acid ethyl ester (FAEE). In the European Union biodiesel is rapeseed-oil methyl ester (Kovács, 2000).

Glycerol of biodiesel production is well utilized by microorganisms (Papanikolaou et al., 2008; Temudo et al., 2008). Studies have investigated whether the utilization of glycerol by microorganisms is a great possibility to convert that into value-added products (Barbirato et al., 1998; Johnson and Taconi, 2007; Yazdani and Gonzalez, 2007). The measurment of biologically active functions of organic matter, such as microbial biomass C, N, P and potential C, N and P mineralization were better able to reflect changes in soil quality and productivity (Patel et al., 2010). Biebl et al. (1998) have analysed fermentation of glycerol by *Klebsiella pneumoniae* (Biebl et al., 1998; Zeng et al., 1993). Biebl also investigated fermentation of glycerol in the case of *Clostridium pasteurianum* batch (Biebl, 2001).

Soil microorganisms adsorb nitrogen instead of plants and retard their development. Provisionally, microbes immobilize nitrogen of chemical fertilizers and the mineral nitrogen part of soil, but later mobilize them (Tolner et al., 2010b). This process prevents the wash-out of nitrogen and subsequently the destruction of microbes nitrogen will be available for plants again. The glycerol increases the storage capacity of soil and helps adsorption of nutrients by solvent action. Dispersive capacity and coagulability of glycerol can cause friable texture of soil (Czinkota, 2007).

The nutrition providing ability of the soil can be tested with soil tests and plant experiments. The growth rate of plants reacts sensitively to the current nutrition supply. The development of plants, thus the dry matter accumulation, is not linear in time, in the vegetation period it is connected to certain stages of development (Lásztity et al., 1984; Waldren and Flowerday, 1979; Prew et al., 1985), showing changes which are genetically determined yet influenced by external ecological factors, it is the result of the interaction of all these (Lásztity, 2006). The growth rate depends significantly on the available nutrient (Jocic 1981; Lásztity and Kádár, 1978). The development of the plants can be followed by visual bonitation and computer processing. Narumalani et al. (2009) and Auda et al. (2008) tried to gather information on the spread of the invasive plant species. Sanyal and Patel (2008) estimated the health and nutrition ability of rice plant by the shape and size of the plant. Timmermnas and Hulzebosch (1996) used image analysis for isolating the growth stages and plant parts. Behrens and Diepenbrock (2006) scanned the development of the swede rape with the help of image analysis. It was established in a former experiment that linear relationship stands between the number of green pixels and plant green mass.

Materials and Methods

Two plant species were investigated in complex experimental series to gain information about the reaction of plants for the glycerol content of soil which is the most determinant component of biodiesel by-product.

In our paper, on the one hand **germination tests** of ryegrass (*Lolium perenne* L.) and oilseed rape (*Brassica napus*) will be presented, which were carried out in order to study the germination retarding effect of glycerol. After the results, on the other hand, **plant growth test** was carried out which was performed only in the case of ryegrass (*Lolium perenne* L.) in order to investigate the growth inhibitor effect of glycerol in the later stage of plant life.

Arenosol from Fót was applied for the treatments. The main parameters of this soil were: plasticity index of soil according to Arany $K_A = 28.33$, lime content ($CaCO_3\%$) = 8%, $pH(H_2O) = 8.2$, humus content (H%) = 1.4%., AL-P_2O_5 = 95 ppm, AL-K_2O = 120 ppm. The water content of the soil was 60% of field capacity. We used analytical quality materials for treatments in both experiments (*Table 1*).

Table 1. *Different treatments applied for experiments*

Treatments in germination experiments	Treatments in growth experiments
PK as control	
NPK as control	Control
0.5% C glycerol	0.5% C glycerol
0.5% C methanol	0.5% C methanol
0.5% C 50% glycerol + 50% methanol	0.5% C 50% glycerol + 50% methanol
0.5% C 90% glycerol + 10% methanol	0.5% C 90% glycerol + 10% methanol
0.5% C by-product	0.5% C by-product
1% C glycerol	
1% C methanol	
1% C 50% glycerol + 50% methanol	
1% C 90% glycerol + 10% methanol	
1% C by-product	
0.25% C by-product	

Germination experiment

90 grams of soil and 100 pieces of seed were placed in every single Petri dish. We did not use pretreatment to break the resting period of plants. After this Petri dishes were set into a refrigerator to reach the required moisture content for seed germination.

In the first stage, the effects of glycerol, glycerol-methanol combination, methanol and biodiesel by-product were investigated. In the second stage, the modification effect of a two-week incubation period was analysed. After the preparation of treatments, seeds of ryegrass and oilseed rape were not placed into soil, only after the two-week incubation.

We wanted to ascertain that the two-week incubation is enough for microorganisms to metabolize glycerol content of soil.

The first experiment was accomplished in four replications. The second experiment, in the case of the two-week incubation, was carried out in two replications, and the immediate seeding was also carried out in two replications. Plantlets were counted in Petri dishes on the fifth day. We tookthist as a basis of absolute germination.

Plant growth experiment

The second column of *Table 1* shows the treatments that we started to apply in pot experiments on 2nd of September 2010. Every pot contained 900g soil which were treated before the sowing of a 1 g seed of *Lolium perenne* L. (ryegrass). The water content of soil was 60% of field capacity which was kept continuously at this value by sprinkling. The culture pots were set in a closed place and were kept under artificial daylight. Treatments were carried out in four replications.

The images were taken at certain times depending on the plant growing status. There were 24 pots and 6 treatments. All experiments were accomplished in four replications. Eight pictures were taken of each pot from different angles (at 45° degrees). On the plant images the image processing program counts the green pixels representing the colour of leaves and thus, we could convert the plant growing status to numerical data. After the pixel numbers were gained, a calibration was made which was able to transform the numbers to plant leaf mass measured in grams. In all treatments the soil samples were mixed with solutions. The calculated amount of materials for the treatments were put in the soil, dissolved in this amount of water. The observation started on the 5th day and ended depending on the plant growing status (Tolner et al., 2010a).

Evaluations

The outlier mean square deviations were examined by Cochran test (Sváb, 1981). The effect of treatments was evaluated by analysis of variance. We used the ANOVA program on Visual Basic algorithm in Microsoft Office Excel which was based on Sváb (1981). This algorythm has been successfully applied in several studies (Kátai et al., 2013; Kovács et al., 2013; Szabó et al., 2013).

Results

Results of germination experiment

The effect of treatment combinations were evaluated by ANOVA with 0.1% probability of error. Reduction in the number of germs were significant in almost every treatment in the case of both species, where glycerol or biodiesel by-product were incorporated into the soil as compared to NPK control.

The inhibitor effect of glycerol was total in 1% C glycerol and the number of germs was zero. There was no significant difference between the results of 0.5% C glycerol and 0.5% C by-product, both of them had inhibitor effect. Moreover there was no significant difference between the results of 1% C glycerol and 1% C by-product

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(*Figure 1*). Thus, these results verified that the inhibitor effect on germination was caused by the glycerol content of by-product.

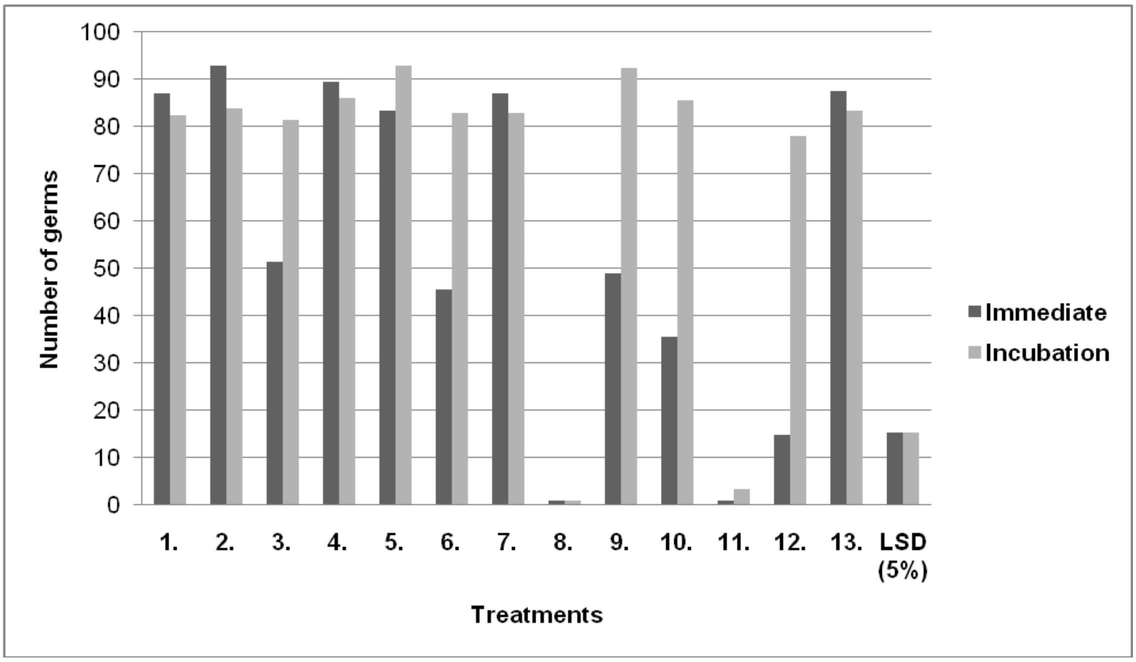

Figure 1. *Number of germs in case of ryegrass*

Blight was experienced in treatments where by-product or glycerol were appiled. Generally Petri dishes of the by-product included more colonies than Petri dishes of glycerol and glycerol-methanol combinations. The reason for this phenomenon was the nutrient content of by-product. During biodiesel production, by-products of transesterification (proteins, carbohydrates, minerals, vitamins, potassium hydroxide used as catalyser, in the case of transesterification used methanol) were concentrated in the phase of glycerol which could increase the effect of glycerol as a carbon source. The smell of blight was typical which was also sign of microbial intensification.

The treatments having methanol content did not inhibit the germination (*Table 2*). Since the methanol does not have a significant inhibition effect on germination, glycerol treatments became comparable to each other.

Table 2. *Inhibitor effect of the pure methanol treatmennts compared to control*

Treatments	Immediate	Incubation	**Mean**	LSD(5%)
2. Nitrogen (NPK)	77	88	**82**	11
4. 0,5% C Methanol	74	87	**81**	
9. 1% C Methanol	64	80	**72**	
Mean	**72**	**85**		
LSD(5%)	9			

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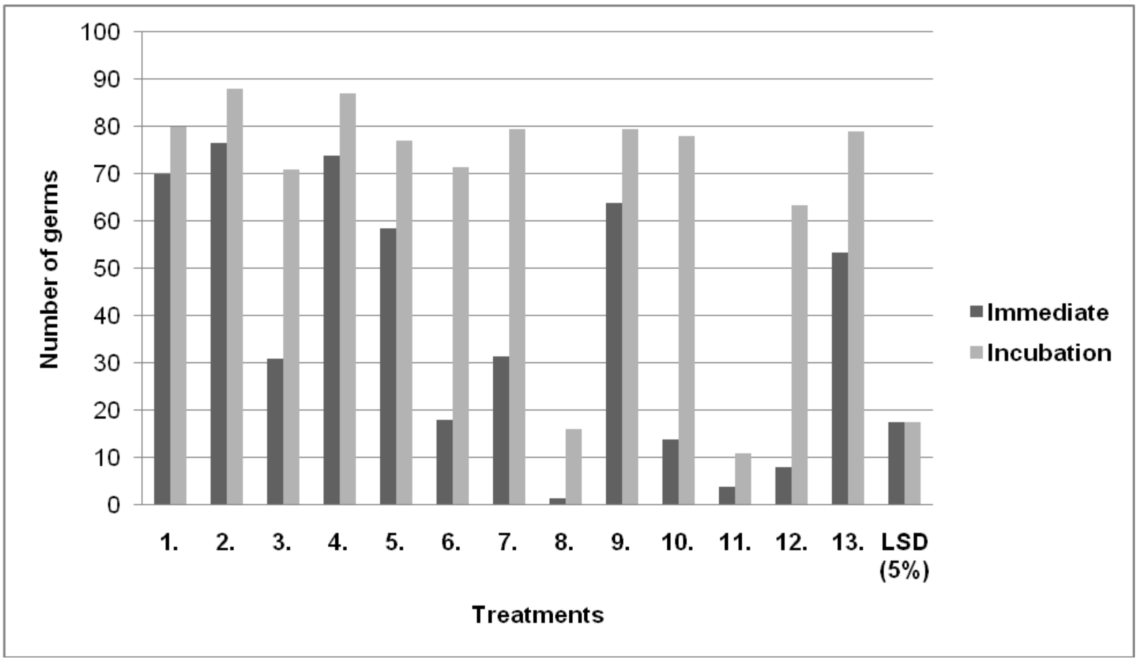

***Figure 2.** Number of germs in case* of oilseed rape

The experimental dates of 13 levels of treatments in two incubation periods were evaluated using two-way ANOVA. There were significant differences between the germination of seeds in the functions of treatments and incubation periods as well (p<0,1%).

The two factors had a strong interaction (F-rate=5,69 p<0,1%). The case of immediate sowing treatments resulted in significant differences, but after the incubation period the dependency by treatments became less harmful.

There was a strong interaction between the number of germinating seeds and the C% ratio in the glycerol phase. It can be seen in *Figure 1* and *2* that without incubation the germination was more sensitive to C% amount in glycerol, than after the two-week incubation.

However, this negative effect of glycerol could be moderated by two-week incubation. Comparing the reactions of biodiesel by-product of the two plants, it was obvious that *Lolium perenne* L. (ryegrass) has a higher tolerance for negative conditions than *Brassica napus* (oilseed rape).

Results of plant growth experiment

After analysiing the pictures a lot of information was gained about plant growth. The complex analysis of the huge database gave appraisable information. During the experiments, the reactions of plants were intensive to different stresses. That was experienced in differences between length of germination times and velocity of growth. Also there were differences between plant individuals within the same culture plates. For quantity determination of differences, photos were taken from eight different angles.

Table 3. shows thousandths of average number of pixels of eight photos in four replications, with different treatments, and at different times of the experiment. Variations of gray backgrounds demonstrate the coefficient of variation (CV) of data

originated from the eight different angles. White shows CV lower than 10%, light gray shows CV between 10% and 20%, dark gray shows CV greater than 20%.

Table 3. *Development of plants between 11th September to 3rd October (number of pixels/1000)*

Sowing 2.09	**11.09**	**12.09**	**15.09**	**18.09**	**21.09**	**24.09**	**27.09**	**30.09**	**03.10**
	768	956	988	1048	1017	1088	1146	1210	898
Control	733	880	911	968	828	857	**881**	1098	973
	721	850	817	905	891	958	1141	1279	1092
	815	981	1117	1241	960	1292	1384	1361	1408
	22	36	**108**	261	339	469	539	705	815
0,5% C	**26**	**64**	**230**	428	507	626	763	869	929
100% glycerol	**22**	**25**	**44**	**142**	215	336	465	539	647
	22	**31**	**40**	129	205	320	471	514	614
	254	374	533	662	726	913	1025	985	1185
0,5% C	**146**	**263**	457	593	645	823	1113	1094	1107
100% methanol	200	320	486	619	694	891	1117	1124	967
	233	354	524	649	743	933	1162	1049	1147
	284	424	624	776	815	937	1208	1291	1043
0,5% C	227	353	542	685	694	786	963	1200	1032
50% methanol	243	376	597	745	795	938	1104	1178	834
50% glycerol	267	409	639	794	810	1018	1275	1132	1160
	24	**62**	**216**	388	498	596	757	840	949
0,5% C	**47**	**111**	**258**	413	495	564	700	751	862
10% methanol	34	**69**	**208**	342	404	469	608	636	718
90% glycerol	**32**	**77**	**257**	**401**	470	567	736	821	826
	417	530	678	788	833	775	1037	1053	1045
0,5% C	406	503	605	725	744	764	973	879	**773**
by-product	385	500	606	688	710	730	925	955	883
	467	595	778	908	876	1035	992	940	801

At every measuring moment, the values of mean-square deviation of standardized data of photos from 6 treatments, from 4 replications were studied using the Cochran test at value P = 5%. In calculation of standardized data, the number of pixels of every single photo was divided by the average number of pixels of eight photos. So the standard deviation of different development stages of plants could be comparable. After that the first 24 mean-square values were investigated to determine outlier data. That process was continued until outlier data was found.

The heterogeneity of plants stand of culture plates were characterized by relative deviation. So the number of pixels were normalized by the mean of pixels of eight photos. The standard deviation and mean-square deviation of normalized values of pixels also were determined.

Data shows that an increase in stress caused not only retardation of growth of germinated plants but also augmentation of irregularity in shooting and in growth. That effect is mainly perceptible in the first section of the growing phase. Between elder plants differences would be equalized progressively. In the case of elder plants the different treatments (Control, By-product) resulted outlier deviation values sporadically which was caused by technical measuring problems. The longer spears were bent over so the optical observing based investigation would become improper.

In the following analysis the standard deviation of pixels of 8 photos was disregarded, the different growth status of plants were characterized only by means of thousandth pixel number of 8 photos.

The homogeneity of standard deviations of different treatments at different times was investigated using the Cochran test but outlier values were not received. After that the whole experiment was evaluated by two-way analysis of variance.

The effect of treatments and changes in time were strongly significant. In accordance with control every treatment caused depression. This was lower in the case of 100% methanol, 50% methanol-50% glycerol and 100% by-product. Significant difference was not experienced between the harmful effects of 100% methanol and by-product. The most retarding effect was observed in the case of treatment 100% glycerol. The changes in time are demonstrated in *Figure 3*.

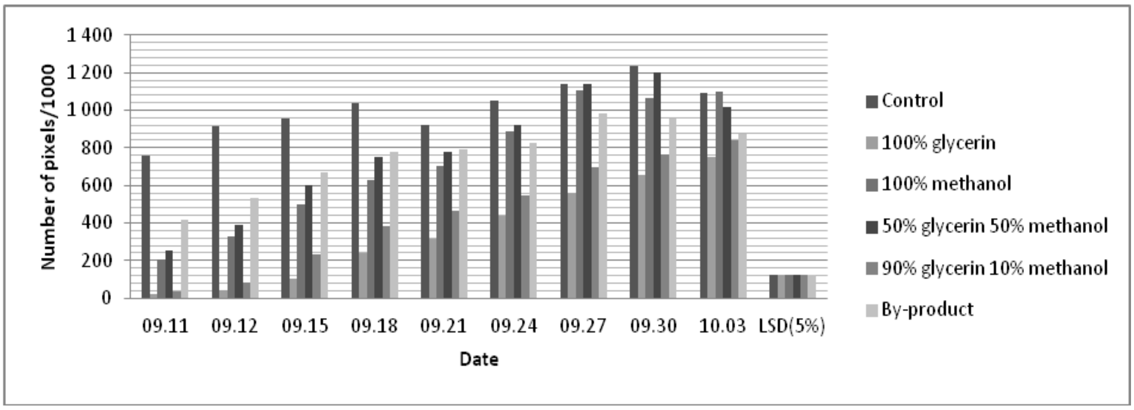

Figure 3. Result of the plant growth experiment with ryegrass

Conclusions

Theresults of our research are important to define the best time for fertilization of by-product.

Glycerol and biodiesel by-product had a significant negative effect on the germination of ryegrass in an arenosol, 1% concentration of glycerol inhibited the germination.

The same inhibitor effect on germination of glycerol and by-product verified the glycerol content of by-product.

The microbial stimulant effect of glycerol bred blight (*Aspergillus* spp.) colonising Petri dishes where glycerol and by-product were applied.

The inhibitor effect of glycerol was decreased in treatments of 50% glycerol - 50% methanol combination. This combination resulted good conditions for plants. In treatments of 1% C 50% glycerol - 50% methanol the improving effect of two-week incubation was significant.

During the research we could not verify clearly the inhibitor effect of methanol in treatments.

Generally we can state that biodiesel by-product was more useful for microorganisms of soil than for plants.

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OCCURRENCE PATTERN ANALYSIS OF DRAGONFLIES (ODONATA) ON THE RIVER TISZA BETWEEN VILOK AND HUSZT BASED ON EXUVIAE

KOLOZSVÁRI, I.[1,2*] – SZABÓ, L.J.[1] – DÉVAI, GY.[1]

[1] *Department of Hydrobiology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*

[2] *Ferenc Rákóczi II. Transcarpathian Hungarian Institute, István Fodor Research Institute, Kossuth Square 6, UA-90202 Beregove, Ukraine*
(phone: +36 (52) 512-900/22621; fax: +36 (52) 431-148)

**Corresponding author*
e-mail: kolozsvaros@gmail.com

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Abstract. Dragonfly exuviae were collected between 2010 and 2013 in the following sections of River Tisza: Vilok (Вилок, Tiszaújlak), Nove Szelo (Нове Село, Tiszaújhely), Tiszobikeny (Тисабікень, Tiszabökény), Vinohragyiv (Виноградів, Nagyszőlős) and Huszt (Хуст, Huszt). Based on the examination of the 1965 exuviae, collected from 13 main channel sections, 6 dragonfly species were identified [*Gomphus vulgatissimus* (Linnaeus, 1758), *Gomphus flavipes* (Charpentier, 1825), *Onychogomphus forcipatus* (Linnaeus, 1758), *Ophiogomphus cecilia* (Fourcroy, 1785), *Calopteryx splendens* (Harris, 1782), *Platycnemis pennipes* (Pallas, 1771)]. Summing up the collected exuviae, the two most frequent species were *G. vulgatissimus* (57.3%) and *O. forcipatus* (39.08%), however significant assemblages of *O. cecilia* (1.93%), *G. flavipes* (1.17%), *C. splendens* (0.36%) and *P. pennipes* (0.15%) could be found as well. In compliance with the Mantel test the channel and riverbank characteristics show a significant correlation with the composition of dragonfly assemblages (R=0.309, p=0 024). According to the canonical correspondence analysis (15 habitat characteristics) the exuviae data of *G. vulgatissimus* are connected with the extent of plant coverage, the closure of foliage and the characteristics of riverbanks; the exuviae data of *O. forcipatus* are associated with water depth, channel deepening tendency, water temperature and type of plant coverage on the river bank.

Keywords: *Upper-Tisza, Ukraine, Odonata, Gomphidae, exuviae*

Introduction

Little is known about the structure of dragonfly assemblages in the Ukrainian section of River Tisza. The earliest published data concerning dragonflies in Transcarpathia (Zakarpatska region) are from the second half of the 19th century by Kohaut (1896). At the beginning of the 20th century their work was continued by Hrabár (1905). Besides, interesting observations were carried out also by Fudakowski (1935) in the area of Chornohora massif, as well as the results of the Transcarpathian data publishing by Павлюк (1990), Mauersberger (1994), Добей and Бондарчук (1998), Vizslán and Huber (2001), Рязанова (2007), Holuša (2009), Martynov and Martynov (2010). Unfortunately, the published sampling sites concerning River Tisza are quite insufficient and they are often difficult to find. Further problematic issue is that a significant portion of the published data is about the results of imago observations. Imago data is not appropriate for a reliable description of an aquatic habitat as dragonfly imagos usually fly kilometres away from their place of emergence.

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Partial information can be obtained about the dragonfly species of the Ukrainian Tisza section from Павлюк'S (1990) imago observations on the dead channel of Tisza at Huszt; from the results of the occasional sampling of macroinvertebrate monitoring by Kovács et al. (Kovács and Godunko, 2008; Kovács et al., 2008), and from Kolozsvári and Illár (2009), as well as from Kolozsvári and Dévai (2013) from the earlier investigations on River Tisza between Vilok and Huszt.

Dragonflies are excellent indicator organisms (Schindler et al., 2003). The River Tisza section that we examined, has still preserved its natural anastomosing characteristics in many ways. From the point of biodiversity research the significance of the so naturally preserved habitats is increasing (Noss, 1990; Ward et al., 1999; Amoros, 2001). The particular ecological relationships are threatened by the strengthening civilizational interventions, so exploring the natural value of the upper parts of the River Tisza needs priority. Earlier investigations have proved that dragonflies are quite sensitive to those changes that affect their habitats. The composition of riverine dragonfly assemblages may be influenced by the riverbed conditions, the stream characteristics, the plant coverage and the river bank transformation (Chovanec and Waringer, 2001; Schindler et al., 2003). In our work we set the aim to make a survey on the composition of dragonfly species on the main channel of River Tisza between Vilok and Huszt. While collecting exuviae it was often experienced that the place of emergence was preferred to a different degree in the river sections and on the different parts of the river bank by the dragonflies (Brookes, 1994; Beisel, et al., 1998; Brunke et. al., 2001; D'Amico et al., 2004; Hardersen, 2008; Raebel et al., 2010; Bereczki et. al., 2012 Giugliano et al., 2012). The terrain objects in the riverbed and on the river banks, the driftwood, the plants, etc., all affect the stream, the alluvium transportation, the erosion, and riverbed depth (Montgomery and Buffington, 1997; Robert 2011; Ward et al., 2002). These previously mentioned factors influence the abundance and density of dragonflies (Petts, 1984 cit. Brookes, 1994). Unfortunately, the habitat factors and their connections in the background of the phenomenon are not exactly known therefore while collecting exuviae, relations between dragonfly assemblages, river banks, and channel characteristics were examined. Water quality degradation and immoderate fluctuation of water regime throughout Europe are serious dangers to the survival of riverine dragonflies, therefore the exploration and protection of their natural habitat is of great importance (Jakab and Dévai, 2008).

Materials and methods

Between 2011 and 2013 exuviae were collected in the section between Vilok (Вилок, Tiszaújlak) and Huszt (Хуст, Huszt) from the River Tisza. Collections were carried out along 30 m long stretches that were marked out at Vilok (Вилок, Tiszaújlak), Nove Szelo (Нове Село, Tiszaújhely), Tiszobikeny (Тисабікень, Tiszabökény), Vinohragyiv (Виноградів, Nagyszőlős) and Huszt (Хуст, Huszt) on the main channel of River Tisza (*Fig. 1*).

Typical river banks in this section were point bars [F1TL (Vilok), F2TL (Vilok), F4TL (Vilok), F1TB (Tiszobikeny), F1NSZ (Vinohragyiv), F1H (Huszt)]; cut banks [F1TH (Nove Szelo), F2TH (Nove Szelo), F3H (Huszt)] and artificially reconstructed protected banks [F3TL (Vilok), F2TB (Tiszobikeny), F2NSZ (Vinohragyiv), F2H (Huszt)]. Our sampling sites were divided into two main groups based on the features of the terrain. The sites at Vilok, Nove Szelo and Tiszobikeny were mainly flat while at Vinohragyiv and Huszt they were at the foot of the mountain (*Fig. 1*). The transliteration of settlement names follows the form suggested by Molnár and Molnár (2005).

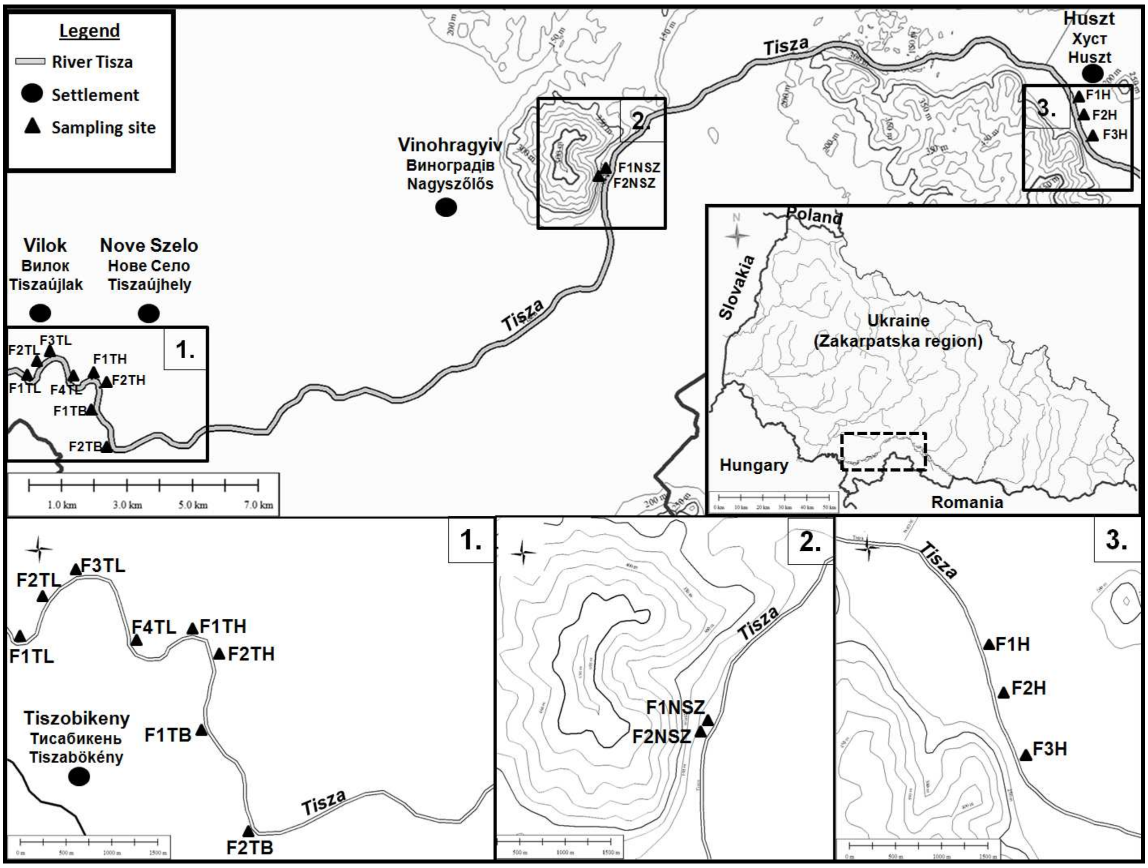

***Figure 1.** The examined section of River Tisza between Vilok and Huszt*

In the examined section of River Tisza heavy rains are followed by quick water level fluctuations (Gönczy and Molnár, 2004). Regularly occurring spates contribute to alluvium movement, bow deepening, alluvial filling of other channel parts, and river bank change (Németh, 1954; Brookes, 1994; Church, 1994; Robert, 2003; Richard et al., 2005; Hoffmann and Gabet, 2007).

When the sampling sites were marked out the main aim was to most completely cover and examine the typical characteristics of the variegated bank and channel structures of the dragonfly habitats. The collection of exuviae can be hindered by the quick rise of water level causing their removal from the river bank (Farkas et. al., 2011, 2012). Exuviae were collected with hand-picking method from riverbanks and plants on the channel and banks. Our collections in the years of investigation were carried out from May to July. Because of the great distances between the sampling sites, samplings were done on every fourth day on the same sampling site.

Some exuviae were presumably washed away on days when water level rose significantly. The identification of the cast off cases (exoskeleton) was based on the works of Askew (2004), Bellmann (2007), Gerken and Sternberg (1999).

It was confirmed earlier that the composition of aquatic macroinvertebrate assemblages is influenced by biotic and abiotic factors of the channel, thus water flow speed, composition of the channel bottom and river bank, plant coverage rate and its characteristics (Corbet, 1983; Gibbons and Pain, 1992; Brookes, 1994; Beisel et al., 1998; Brunke et al., 2001; Müller, 2002; Angelier, 2003; Ward and Mill, 2005; Bereczki et al., 2012). Examining the river bank and channel characteristics of the sampling sites the following factors were taken into consideration: channel width (1), water depth in the stream-channel (2), deepening tendency of the channel (3), mosaic-like nature of the channel (4), condition of the channel near the river banks (5), type of plant coverage on the river bank (6), rate of plant coverage of the riverbank (7), rate of foliage closure (8), intensity of water flow near the river banks (9), water colour (10), water temperature (11), type of the river bank (12), in case of river bank protection, characteristics of the work (13), characteristics of the river bank (14), and forms of the river banks (15).

Before data processing Mardia’s test was used to test multiple normality of the abundance data. The composition of dragonfly assemblages was compared with cluster analysis (Ward’s method), principal component analysis (Bray-Curtis similarity) and canonical variate analysis (CVA). The SIMPER method was applied to demonstrate which species resulted the differences among groups of assemblages. The relationship between 15 river characteristics and the examined species was analysed with Mantel test and canonical correspondence analysis (CCA). For statistical analyses PAST program package was used (Hammer, Harper and Ryan, 2001).

The cartographic representation of the sampling sites was carried out using the Environmental Systems Research Institute (*ESRI*) *ArcGIS* 10.0 – ArcMap geoinformatics software.

Results and Evaluation

During the study a total of 1965 exuviae were collected from 13 sampling sites on the main channel of River Tisza between Vilok and Huszt. Based on the examination of the main channel 6 dragonfly species were identified [*Gomphus vulgatissimus* (Linnaeus, 1758), *Gomphus flavipes* (Charpentier, 1825), *Onychogomphus forcipatus* (Linnaeus, 1758), *Ophiogomphus cecilia* (Fourcroy, 1785), *Calopteryx splendens* (Harris, 1782) and *Platycnemis pennipes* (Pallas, 1771)]. There were great differences in the species composition of the habitats and in the qualitative abundance of the species.

In the area of Vilok 681 exuviae were collected from four main channel sampling sites. The given section of the river shows great variety and dynamically changing bank structure and channel material. From ecological viewpoint, river banks play an important role in habitat characterization, because they mean transition between aquatic and terrestrial habitats (Bravard et al., 1986; Large and Petts, 1994) that is of vital concernment for amphibian insects. The affect of the water stream on the river bank alteration, among natural circumstances, firstly depends on the water speed, the direction and degree of the turbulent water streams appearing on the given river section, as well as on the river bed and river bank material resistance characteristics (Lászlóffy, 1949; Brookes, 1994; Carling, 1994; Robert, 2003). These conditions can be deeply affected by river bank protection and regulation works. These interventions influence the life opportunities of macroinvertebrates in a still partially unknown way. The linkage of dragonfly larvae to the certain types and compositions of riverbed material has not been completely revealed yet. In many cases river bed material preference differences can be observed among the larvae of the same species (Suhling, 1996). On the F1TL, F2TL and F4TL stretches the channel is gradually deepening with point bars. Differences could be found in the bank and channel material. The F2TL stretch is uniformly pebbled, the F4TL stretch is pebbled and sandy-soiled, while the F1TL site bears a completely sandy and muddy character. The bank of the F3TL stretch is steep; the bottom is abruptly deepening with earlier signs of river bank protection. The shoreline was strengthened by huge built-in stone blocks and steel net that is thickly covered with plants. The findings showed the presence of *G. vulgatissimus* (N: 440), *O. forcipatus* (N: 223), *O. cecilia* (N: 10), *G. flavipes* (N: 3), *P. pennipes* (N: 3) and *C. splendens* (N: 2). The species of *O. cecilia* is on the Red List of Threatened Species in Ukraine and it is listed in the Bern Convention (Титар, 2009).The F3TL stretch was the most popular place of emergence of the dragonfly larvae which was surprising considering its disturbed, artificially reconstructed character (*Table 1, Table 2*).

Table 1. *The composition of dragonfly assemblages based on the exuvial samples on the examined habitats (N: number of exuviae)*

Sampling site	**Coordinates (UTM)**	***G. vulgatissimus***		***G. flavipes***		***O. forcipatus***		***O. cecilia***		***C. splendens***		***P. pennipes***	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
F1TL	34U 0637707 5328548	33	50.00	0	0	30	45.45	3	4.55	0	0	0	0
F2TL	34U 0638005 5328971	18	81.82	0	0	3	13.64	1	4.55	0	0	0	0
F3TL	34U 0638333 5329270	224	68.92	3	0.92	89	27.38	5	1.54	1	0.31	3	0.92
F4TL	34U 0638966 5328503	165	61.57	0	0	101	37.69	1	0.37	1	0.37	0	0
F1TB	34U 0639748 5327518	48	48.48	14	14.14	25	25.25	12	12.12	0	0	0	0
F2TB	34U 0640224 5326415	97	80.83	6	5.00	15	12.50	1	0.83	1	0.83	0	0
F1TH	34U 0639600 5328618	1	1.32	0	0	72	94.74	2	2.63	1	1.32	0	0
F2TH	34U 0639861 5328351	143	47.67	0	0	142	47.33	13	4.33	2	0.67	0	0
F1NSZ	34U 0655369 5334794	66	73.33	0	0	24	26.67	0	0	0	0	0	0
F2NSZ	34U 0655288 5334678	210	65.42	0	0	110	34.27	0	0	1	0.31	0	0
F1H	34U 0669587 5337028	31	88.57	0	0	4	11.43	0	0	0	0	0	0
F2H	34U 0669743 5336511	17	22.67	0	0	58	77.33	0	0	0	0	0	0
F3H	34U 0669993 5335854	73	43.45	0	0	95	56.55	0	0	0	0	0	0
Total		**1126**	**57.30**	**23**	**1.17**	**768**	**39.08**	**38**	**1.93**	**7**	**0.36**	**3**	**0.15**

Table 2. *The composition of dragonfly assemblages based on the exuvial samples examined in River Tisza sections belonging to settlements (N: number of exuviae)*

Settlement	***G. vulgatissimus***		***G. flavipes***		***O. forcipatus***		***O. cecilia***		***C. splendens***		***P. pennipes***	
	N	%	N	%	N	%	N	%		%	N	%
Vilok **(Вилок, Tiszaújlak)**	440	71.54	3	0.49	223	36.26	10	1.63	2	0.33	3	0.49
Tiszobikeny **(Тисабикень, Tiszabökény)**	145	66.21	20	9.13	40	18.26	13	5.94	1	0.46	0	0
Nove Szelo **(Нове Село, Tiszaújhely)**	144	38.30	0	0	214	56.91	15	3.99	3	0.80	0	0
Vinohragyiv **(Виноградів, Nagyszőlős)**	276	67.15	0	0	134	32.60	0	0	1	0.24	0	0
Huszt **(Хуст, Huszt)**	121	43.53	0	0	157	56.47	0	0	0	0	0	0

On the main channel of River Tisza at Tiszobikeny collections were carried out on two stretches F1TB and F2TB. The F1TB stretch had point bars and accompanied by the channel they were covered with sandy soil while the F2TB sampling site can be found on the side of a coastal defence dam, made up of stone blocks. The plant cover was sparse on both of the sampling sites. At the Tiszobikeny stretch the species of *G. vulgatissimus* (N: 145) appeared most frequently. *O. forcipatus* (N: 40), *G. flavipes* (N: 20), *O. cecilia* (N: 13) and *C. splendens* (N: 1) also could be found in the area. During the collections this was the place where G. flavipes appeared most often and in the greatest number (*Table 1, Table 2*).

At Nove Szelo on River Tisza a total number of 376 exuviae could be collected. *O. forcipatus* (N: 214) was the most frequent species on the two sampling sites. Significant assemblages could be found of *G. vulgatissimus* (N: 144), *O. cecilia* (N: 15) and *C. splendens* (N: 3). Exuviae of *G. flavipes* could not be found at this sampling site; however their presence was confirmed in the earlier works of Kolozsvári and Dévai (2013) on the side channel of River Tisza at Nove Szelo. Cut banks were dominating on the F1TH and F2TH stretches. The bank material of the F1TH stretch was rough (coarse-grained) pebbled with stone blocks while the F2TH stretch was sandy and pebbly. At the foot of the cut banks the plant cover is sparse and mosaic-like.

Differences could be found in the number of exuviae on the apparently similar sampling sites. On the F1TH stretch the species of *O. forcipatus* (N: 72) were dominant, while on the F2TH stretch the species of *G. vulgatissimus* (N: 143) and *O. forcipatus* (N: 142) could be found almost equally in number (*Table 1, Table 2*).

In the Vinohragyiv section of River Tisza altogether 411 exuviae were collected from two sampling sites. The F1NSZ stretch has point bar, gradually deepening with pebbly bottom while on the F2NSZ stretch coastal defence was carried out earlier. At both sampling sites the river banks were covered with ligneous and bushy vegetation. Exuviae of *G. vulgatissimus* (N: 276), and *O. forcipatus* (N: 134) could be found on the main channel of River Tisza at Vinohragyiv (*Table 1, Table 2*). The presence of *O. cecilia* was revealed by the earlier collections of Kolozsvári and Dévai (2013).

Exuviae collection was carried out on three stretches in the area of Huszt. At the F1H stretch point bars were observed, at the F2H stretch protected banks and the F3H stretch cut banks were found. In the Tisza section of Huszt, *O. forcipatus* (N: 157) and *G. vulgatissimus* (N: 121) riverine dragonflies could be found, similarly to the results of Vinohragyiv (*Table 1, Table 2*).

Differences could be found in the occurrence of the species of the Gomphidae family. While in the section of River Tisza between Vilok and Tiszobikeny all the four identified species of riverine dragonflies were present, in the area of Vinohragyiv and Huszt, only *G. vulgatissimus and O. forcipatus* species could be detected. Our investigation showed, that in the river sections of Vilok, Tiszobikeny and Vinohragyiv the species of *G. vulgatissimus* were the most frequent while in the sections of Nove Szelo and Huszt the species of *O. forcipatus* were the most recurrent.

When choosing the place of emergence the types of river banks were preferred differently by the different species. Point bars [F1TL, F2TL, F4TL, F1TB, F1NSZ, F1H] and artificially reconstructed protected banks [F3TL, F2TB, F2NSZ, F2H] were chosen mostly by the species of *G. vulgatissimus*. In the case of cut banks [F1TH, F2TH, F3H] the species of *O. forcipatus* (56.80%) was the most frequent (*Table 3*).

Table 3. *The composition of dragonfly assemblages based on the exuvial samples on the examined types of river banks (N: number of exuviae)*

Types of river banks	***G. vulgatissimus***		***G. flavipes***		***O. forcipatus***		***O. cecilia***		***C. splendens***		***P. pennipes***	
	N	*%*	**N**	*%*	**N**	*%*	**N**	*%*	**N**	*%*	**N**	*%*
Point bars	361	62.24	14	2.41	187	32.24	17	2.93	1	0.17	0	0
Cutbanks	217	39.89	0	0	309	56.80	15	2.76	3	0.55	0	0
Reconstructed banks	548	65.16	9	1.07	272	32.34	6	0.71	3	0.36	3	0.36

By cluster analysis (Ward's method) three clusters are separated that is confirmed by principal coordinate analysis based on Bray&Curtis similarity (the two axes explain 76.84% of the variation). Examinations carried out by multivariate normality test (Mardia's test) show normal distribution of exuviae number. According to the canonical variate analysis the division of the 3 groups (*Fig. 2*) is rather strong (Wilks' lambda=0.002, F=19.96, $p<0.001$)**.** Based on the result of SIMPER the exuviae of *G. vulgatissimus* (72.67%) are responsible for the division of the 3 groups to a large extent while the exuviae of *O. forcipatus* (27.00%) are responsible to a smaller degree.

In compliance with the Mantel test the channel and riverbank characteristics show a significant correlation with the composition of dragonfly assemblages (R=0.309, p=0.024). According to the canonical correspondence analysis (15 habitat characteristics) the exuviae data of *G. vulgatissimus* are connected with the extent of plant coverage (7), the rate of foliage closure (8) and the characteristics of riverbanks (14); the exuviae data of *O. forcipatus* are associated with water depth (2), channel deepening tendency (3), type of plant coverage on the river bank (6) and water temperature (11); the exuviae data of *G. flavipes* and *O. cecilia* are related to the mosaic-like bottom of the channel (4), the intensity of water flow near the river banks

(9), the rate of foliage closure (8), the rate of plant coverage of the riverbank (7) and type of the river bank (12) (*Fig. 3*).

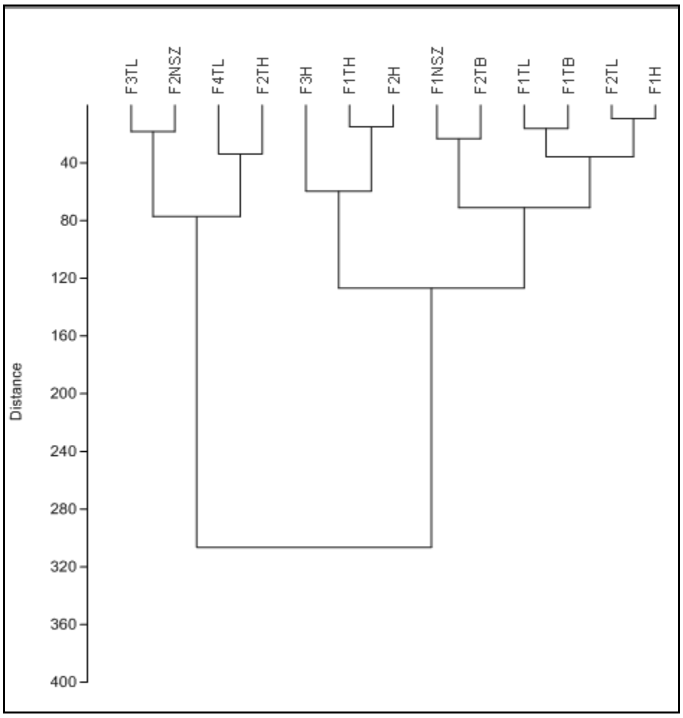

Figure 2. *By cluster analysis (Ward's method) three clusters are separated based on dragonfly assemblages and sampling sites*

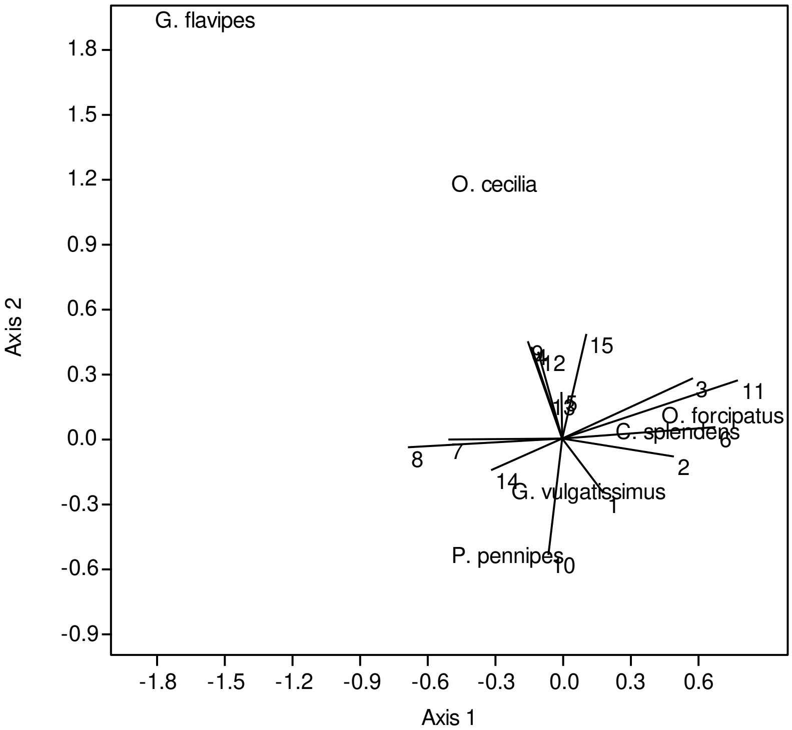

Figure 3. *Result of canonical correspondance analysis based on species and 15 habitat characteristics*

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Discussion

The aim of our work was to get an overview of the dragonfly fauna of River Tisza in the section between Vilok and Huszt and to gather information about the characteristics of their occurrence and quantity based on the data of exuviae collections (*Fig. 1*). If river regulation extends to the given section of the river, there will not be any opportunity for studying these natural habitats along the River Tisza. Our samplings were carried out on 13 main channel sites on 30 metres long stretches in the area of Vilok (Вилок, Tiszaújlak), Nove Szelo (Нове Село, Tiszaújhely), Tiszobikeny (Тисабікень, Tiszabökény), Vinohragyiv (Виноградів, Nagyszőlős) and Huszt (Хуст, Huszt). Based on our exuvial data altogether 6 dragonfly species could be identified [*Gomphus vulgatissimus*, *Gomphus flavipes*, *Onychogomphus forcipatus*, *Ophiogomphus cecilia, Calopteryx splendens*, *Platycnemis pennipes*].

The species of *G. vulgatissimus* and *O. forcipatus* could be found at all of the 13 sampling sites. More than half of our exuviae samplings belonged to the species of *G. vulgatissimus* (57.3%), but significant assemblages of *O. forcipatus* (39.08%) could be found as well. In case of *G. vulgatissimus* most of the exuviae were collected on the stretches of Vilok F3TL (N: 224) and Vinohragyiv F2NSZ (N: 210), the main channel of River Tisza. River bank protection was carried out in both cases of the sampling sites. The exuviae of *O. cecilia* (1.93%) and *G. flavipes* (1.17%) were scarcely found, but seemed to be present in the examined section of the river. *O. cecilia* could be found on the stretches of Vilok, Tiszobikeny and Nove Szelo and exuviae of *G. flavipes* could be gathered at the sampling sites of Vilok and Tiszobikeny. Two specimens of the Zygoptera suborder were isolated: *C. splendens* (0.36%) and *P. pennipes* (0.15%). The mosaic-like composition and occurrence of the dragonfly fauna was general (*Table 1, Table 2*). The background of this phenomenon is not exactly known, but presumably there are connections between the dynamically changing channel, the river bank structures and the abundance of the habitats in the area.

According to our investigations significant correlation can be found between the occurrence of the dragonfly assemblages and the characteristics of natural habitats in the examined sections. The occurrence of *G. vulgatissimus* exuviae is connected with the rate of plant coverage (7), the rate of foliage closure (8) and the characteristics of the river bank (14); the exuviae of *O. forcipatus* are associated with the water depth (2), the deepening tendency of the channel (3), the type of plant coverage on the river bank (6) and water temperature (11). The assemblages of *G. flavipes* and *O. cecilia* exuviae are related to the mosaic-like nature of the channel (4), the intensity of water flow near the river banks (9), the rate of foliage closure (8) and the rate of plant coverage (7) (*Fig. 3*). The collection of exuviae is hindered by their considerable fragility (Bennett and Mill, 1993; Corbet and Hoess, 1998; Corbet, 1999).

As similar investigations were not conducted on our collecting area, the results of our research cannot be compared to previous data of the Upper-Tisza area. It has already been proved that the composition of aquatic macroinvertebrate communities is largely influenced by the abiotic and biotic factors of the habitat. (Corbet, 1983; Gibbons and Pain, 1992; Brookes, 1994; Beisel et al., 1998; Brunke et al., 2001; Angelier, 2003; Ward and Mill, 2005). This has been confirmed by several detailed odonatological surveys in respect of dragonflies as well (Müller, 1995; Lohr, 2010), however the variegation of the habitat factors and the adaptability of dragonflies, make it more difficult to learn the habitat factors on the level of species. It is often observable that some specimens belonging to the same dragonfly species show different sediment and emergence substrate preference (Suhling, 1996; Farkas et al., 2011). The characteristics of the river bank and channel structure, as well as the river current factors greatly influence the ecological peculiarities of a given habitat (sediment composition, oxygen level, temperature, etc.), and thus the composition of the dragonfly fauna (Lutz, 1974; Baker, 1980; Spence et al., 1980; Baker and Feltmate, 1989; Corkum and Hanes, 1992; Wood, 1998; Müller, 2002; Corbet, 2003). On the fast-moving river sections the deepest section is in the streamline, there the habitat conditions are the least favorable for the dragonfly larvae. Despite of the fact, that dragonfly larvae usually prefer the slower watercourses and more protected habitats, it is also observable in case of the riverine dargonflies (Gomphidae) that the species of *O. forcipatus* and *O. cecili*a tolerate the fast-moving river sections better than the species of *G. vulgatissimus* and *G. flavipes* (Corbet, 1983; Suhling, 1996; Suhling and Müller, 1996; Gerken and Sternberg, 1999; Burcher and Smock, 2002; Müller, 2002). Müller's (2002, 2004) samplings carried out on the Odera also showed much larger larval presence on the river bank area than in the streamline in case of the Gomphidae species. Vegetation is also a key component in the stabilization of the river banks (Hickin, 1984; Davis and Gregory, 1994), furthermore it influences the emergence strategy of the riverine dragonflies, the selection of the place of emergence and the extent of larvae departure from water (Corbet, 1983, 1999; Farkas et al., 2011). According to our experience, scientific literature is not always unified in the peculiarities of dragonfly larvae habitat selection as well as in the selection of their place of emergence (Trottier, 1973; Mathavan and Pandian, 1977; Geissen, 2000; Jakab, 2006; Hardersen, 2008).

The typically changing channel and river bank structure of the upper Tisza section hinders the tracing of dragonfly assemblages and the recognition of quantitative changes. In case of species of the Gomphidae family there is a great diversity because of the continuously changing river channel. In the area of Vilok and Tiszobikeny all the four species of riverine dragonflies were found but in the area of Huszt, only the species of *G. vulgatissimus* and *O. forcipatus* were present. The section of the River Tisza between Vilok and Huszt is mostly untouched and an anastomosing riverine main-side-dead channels system is preserved. The given section is unique along the whole channel of the River Tisza. From the point of biodiversity research the significance of the so naturally preserved habitats is increasing.

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SYNTAXONOMY OF THE ROCKY GRASSLANDS ON CARBONATE BEDROCKS IN THE WEST AND SOUTHWEST OF THE REPUBLIC OF MACEDONIA

MATEVSKI, V.[1,2]* – ČARNI, A.[3,4] – ĆUŠTEREVSKA, R.[1] – KOSTADINOVSKI, M.[1] – MUCINA, L.[5,6,7]

[1]*Institute of Biology, Faculty of Natural Sciences and Mathematics, University of Ss. Cyril and Methodius, Arhimedova 3, 1000 Skopje, Republic of Macedonia*

[2]*Macedonian Academy of Sciences and Arts, Bul. Krste Misirkov 2, 1000 Skopje, Republic of Macedonia*

[3]*Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Novi trg 2,1000 Ljubljana, Slovenia;* [4]*University of Nova Gorica, Vipavska 13, 5000 Nova Gorica, Slovenia*

[5]*Iluka Chair, School of Plant Biology, The University of Western Australia, 35 Stirling Hwy, Crawley 6009, Perth, Australia;* [6]*Department of Geography & Environmental Studies, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa;* [7]*Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*

**Corresponding author*
e-mail: vladom@pmf.ukim.mk

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Abstract. We classify and describe plant communities of rocky dry grasslands on carbonate bedrock in the southwestern regions of the Republic of Macedonia. We used our own field data and literature sources to compile a set of vegetation plots that have been stored in a vegetation database, data-mined and analyzed using numerical-analytical tools such as cluster analysis and ordination. Five distinct vegetation types, here interpreted as associations, were revealed and characterized using floristic composition, ecological conditions, life forms, chorological spectrum, and topological attributes. Four associations, namely the *Petrorhagio haynaldianae-Chrysopogonetum grylli*, the *Scorzonero-Stipetum endotrichae*, the *Globulario-Centaureetum grbavacensis* and the *Astragalo-Helianthemetum marmorei*, were recognized as new. All these associations belong to the *Saturejo-Thymion* (*Astragalo-Potentilletalia*, *Festuco-Brometea*).

Keywords: *Balkan Peninsula, dry grasslands, Festuco-Brometea, syntaxonomy, vegetation classification*

Introduction

Dry grasslands are one of the most endangered habitats in Europe (Janišová et al., 2011; Vassilev et al., 2011) due to changes of traditional grassland management and a number of other threats, such as urban sprawl, afforestation and the like. This vegetation is widespread in the central and southern regions of the Balkan Peninsula, since this region experiences relatively dry and warm climate; grazing has long been in these grasslands an important agricultural activity securing the existence of these grasslands for centuries. Although there have been some older (see below) and more recent (Bergmeier et al., 2009; Aćić et al., 2013; Pedashenko et al., 2013) vegetation-classification studies of the dry grasslands in the central and southern Balkans, our knowledge of their floristic and geographic variability is far from sufficient.

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Micevski (1970, 1971a) initiated the research on the vegetation of dry grasslands in the region. This author classified the dry grasslands on carbonate bedrock into then a new alliance – the *Saturejo-Thymion,* and a new order – the *Astragalo-Potentilletalia*. Micevski later described several associations, mainly on silicate bedrocks in Macedonia (Micevski, 1972, 1977, 1978; Micevski and Matevski, 1984). V. Matevski and his collaborators (Matevski and Kostadinovski, 1998; Matevski et al., 2007) published studies from western Macedonia where the dry grasslands are fairly fragmented. More recently, research was carried out in dry grasslands of northern Macedonia on non-carbonate bedrocks (Ćušterevska et al., 2012).

In this study, we aimed to collect all high-quality vegetation-plot data on dry grasslands on calcareous bedrock in southwestern Macedonia and to investigate whether an analysis of this larger data set would recover known vegetation-classification patterns or reveal new syntaxonomic entities.

Materials and methods

Study area

We focused on the collection of vegetation plot data in grasslands on carbonate bedrocks the western and southwestern regions of the Republic of Macedonia (*Fig. 1*); the eastern part of the country is mainly composed of non-carbonate bedrock.

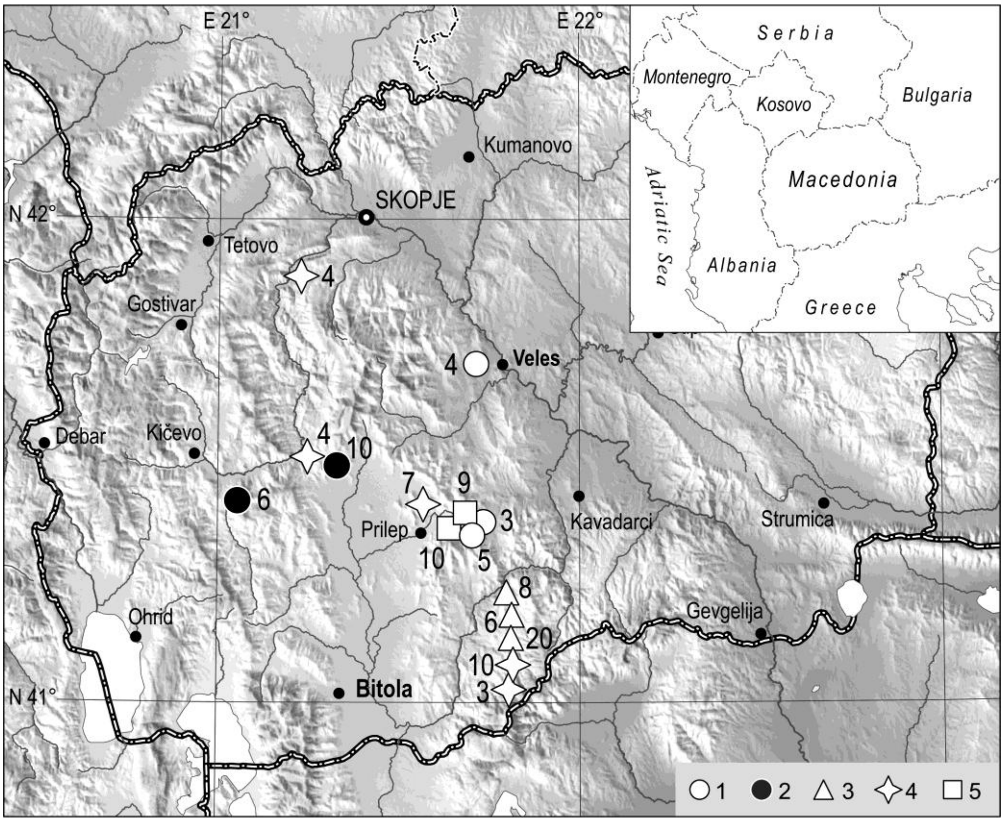

Figure 1*. Location of the studied area. 1 – Petrorhagio-Chrysopogonetum grylli, 2 – Sileno-Thymetum ciliatopubescentis, 3 – Scorzonero-Stipetum endotrichae, 4 – Globulario-Centaureetum grbavacensis, 5 – Astragalo-Helianthemetum marmorei. Numbers at signs indicate number of relevés made at each locality.*

The dry grasslands here are secondary as they were formed by long-lasting degradation of the original forest vegetation, including oak forests dominated by *Quercus pubescens, Q. cerris, Q. frainetto*, *Q. trojana*, and, in places, also by *Carpinus orientalis* (Rizovski, 1974, 1978; Matevski et al., 2008). The potential natural vegetation of the region could be classified as the *Quercetea pubescentis* forests (Horvat et al., 1974; Filipovski et al., 1996; Bohn et al 2004, Čarni et al., 2009).

The climate of the region has submediterranean character, with a pronounced dry summer season. Prilep, situated in the center of the study area, has a mean annual precipitation of 557 mm (most of the rainfall is concentrated to May and November) and mean annual temperature of 11.2° C. The sampled grasslands occurred at altitudes ranging from 540 m to 1300 m.

Vegetation sampling

Original vegetation plot data were collected according to the standard Braun-Blanquet method of field-plot sampling (Braun-Blanquet, 1964). The sampling was done during the optimal phenological period of the year – from the second half of May to mid-June. The size of the sampled plots was 100 m^2. The choice of this sample size was motivated by the relatively homogenous vegetation at this scale and the preponderance of vegetation plot data collected at that scale in the past. The plots were located within targeted remnant patches of dry grasslands in such a way that edge effects (close forest edge, roads, and settlements) were excluded.

Data analysis

The sampled 109 relevés were entered into TURBOVEG (Hennekens and Schaminée, 2001). Classification was made using the PC-ORD 4 package (McCune and Mefford, 1999) integrated in JUICE software, using Ward's method (clustering) and Relative Euclidean Distance (known also as Chord Distance) as the measure of resemblance among relevés. The table sorting based on this clustering was then performed in JUICE 7.0 (Tichý and Holt, 2006). Diagnostic species of each cluster (interpreted as associations) were defined in JUICE by calculating the fidelity of each species to each group using the *phi* coefficient as the fidelity measure (Chytrý et al., 2002). The threshold of the *phi* value was selected at level 0.55. Diagnostic, constant, and dominant species were identified by JUICE (see the descriptions of the plant communities); the *diagnostic species* were recognised using the the *phi* coefficient greater than 0.55 (those more than 0.90 as highly diagnostic in the list of the diagnostic species in the description of the identified associations are given in bold); the *constant species* occur in 90% of relevés (those occurring in 100% of the relevés are also given in bold) while the *dominant species* are those having cover of the category 4 and 5 of the original Braun-Blanquet scale.

We have ordinated the species X relevé matrix using non-metric multidimensional scaling (NMDS), using Mass package (Venables and Ripley, 2002) in R version 2.13 software (http://www.r-project.org/), based on Relative Euclidean Distance. Cover values were transformed to mean cover percentage and square rooted. In order to assist ecological interpretation of the ordinated patterns, average bioindicator values (Pignatti, 2005) for the relevés were calculated based on presence-absence data and overlaid as supplementary environmental data.

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We used Raunkiaer's (1934) system of life forms and we determined the chorological spectra using data of Gajić (1980) and Pignatti (2005). The chorological spectrum is presented as percentages of each group of species within the entire species composition. Box-whiskers diagrams of altitude and slope are also presented in order to document differences between clusters (associations); these were calculated in Statistica (STATSOFT, 2007).

The analytic table presents the results of the cluster analysis and subsequent JUICE tabular sorting. The syntaxonomic affiliation of taxa other than diagnostic ones was decided upon using expert knowledge and the unpublished database of the European Vegetation Checklist (L. Mucina et al., in prep.). Species with low frequency in the analytical table, as well as relevant locality and sampling metadata on each relevé, are listed in the Appendix.

The nomenclature follows in principle the Flora Europaea (Tutin et al., 1964-1993) however it was adjusted using the Med-Check List (Greuter et al., 1984-1989; Greuter et al., 2008) and new taxonomic and nomenclatural findings as featured in the Euro+Med Database (www.emplantbase.org). Some taxonomic concepts follow regional floras (Hayek, 1927, 1931, 1933; Micevski, 1985-2005; Matevski, 2010; Josifović, 1970-1986; Jordanov, 1963-1979; Velchev, 1982-1989; Kozuharov, 1995). Formation of the names of the new syntaxa follows the International Code of Phytosociological Nomenclature (Weber et al., 2000).

Results and discussion

Major vegetation patterns

The cluster analysis suggested five well separated clusters (*Fig. 2*). Since they show a high degree of floristic (and geographic) integrity, they are interpreted as associations (*Elect. App.*), such as the *Petrorhagio-Chrysopogonetum grylli* (Cluster 1), the *Sileno-Thymetum ciliatopubescentis* (Cluster 2), the *Scorzonero-Stipetum endotrichae* (Cluster 3), the *Globulario-Centaureetum grbavacensis* (Cluster 4), and the *Astragalo-Helianthemetum marmorei* (Cluster 5).

NMDS with passively projected bioindicator values (*Fig. 3*) illustrates the ecological differences between the associations. The *Sileno-Thymetum ciliatopubescens* is typically found on the wet extreme of the moisture gradient revealed along the axis 1 of NMDS. This community is found in the westernmost, precipitation-richest region of the studied area. The *Scorzonero-Stipetum endotrichae* is found in habitats in the southern part of the area. The stands of the *Globulario-Centaureetum grbavacensis* are typically found in disturbed habitats where the bedrock shows possibly the greatest influence on the floristic composition. The *Petrorhagio-Chrysopogonetum grylli* and *Sileno-Thymetum ciliatopubescentis* can be found at winter-mild low altitudes, whereas the *Scorzonero-Stipetum endotrichae,* the *Globulario-Centaureetum grbavacensis* and the *Astragalo-Helianthemetum marmorei* are confined to higher altitudes characterized by climatic extremes such as pronounced continentality.

The life-form analysis (*Fig. 4*) revealed that the *Petrorhagio-Chrysopogonetum grylli* and the *Sileno-Thymetum ciliatopubescentis* are richer in therophytes and are found in lowlands as is the *Scorzonero-Stipetum endotrichae* found in the southern part of the studied area. Hemicryptophytes prevail in the dry grassland communities confined to the high altitudes in the central part of the study area (*Globulario-Centaureetum grbavacensis* and *Astragalo-Helianthemetum marmorei*).

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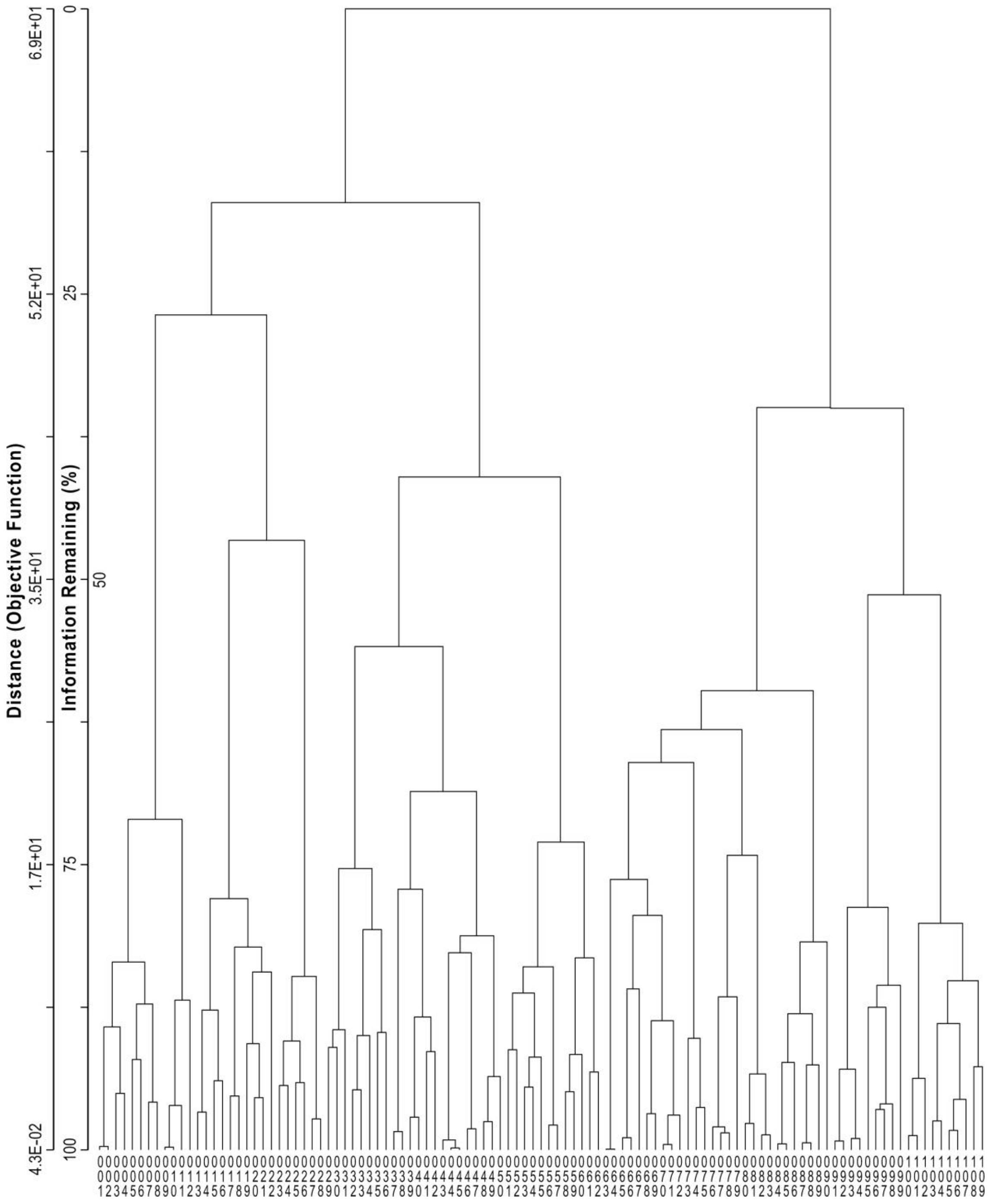

Figure 2. *Classification of the studied dry grasslands (Ward's clustering method, relative Euclidean distance). Legende: Cluster 1 (rels. 1-12): Petrorhagio - Chrysopogonetum grylli, Cluster 2 (rels. 13-28): Sileno-Thymetum ciliatopubescentis, Cluster 3 (rel. 29-62): Scorzonero-Stipetum endotrichae, Cluster 4 (rels. 63-90): Globulario-Centaureetum grbavacensis, Cluster 5 (rels. 91-109): Astragalo-Helianthemetum marmorei.*

The results of chorological analysis are shown in *Fig. 5*. Balkan and Sub-Balkan geo-elements are dominant in all associations. Most Balkan and South-European orophytes can be found at high altitudes in the central part of the studied area (*Globulario-Centaureetum grbavacensis* and *Astragalo-Helianthemetum marmorei*) whereas Euri-Mediterranean and Eurasian species are more common at lower altitudes and in the southern regions (*Petrorhagio-Chrysopogonetum grylli, Sileno-Thymetum ciliatopubescentis* and *Scorzonero-Stipetum endotrichae).* Steno-Mediterranean species are most common in the *Petrorhagio-Chrysopogonetum grylli* occurring at the lowest altitudes of all dry grasslands studied.

Fig. 6. focuses on the Balkan and Sub-Balkan geo-elements. Species of the former two geoelements are most common at higher altitudes in the central regions of the studied area (*Globulario-Centaureetum grbavacensis, Astragalo-Helianthemetum marmorei)*, while many South-Balkan species can also be found here in degraded habitats − in the *Globulario-Centaureetum grbavacensis*. A high proportion of Scardo-Pindian elements can be found in the *Scorzonero-Stipetum endotrichae* at higher altitudes in the south of the region.

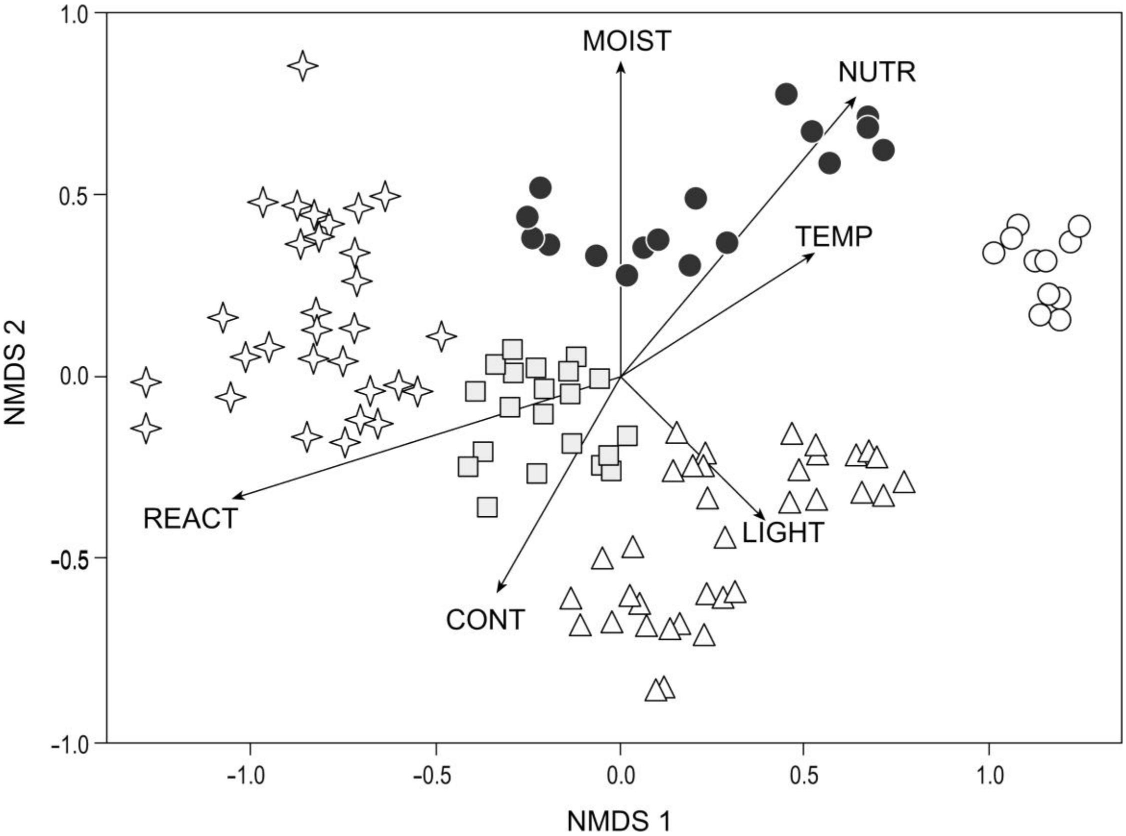

Figure 3. *NMDS ordination (axes 1 and 2) based on relative Euclidean distance, with passive projection of bioindicator values for 5 communities. ○ Petrorhagio-Chrysopogonetum grylli; ● Sileno-Thymetum ciliatopubescentis; Δ Scorzonero-Stipetum endotrichae; ✧ Globulario-Centaureetum grbavacensis; □ Astragalo-Helianthemetum marmorei.*

The *Petrorhagio-Chrysopogonetum grylli* and the *Sileno-Thymetum ciliatopubescentis* are found on moderate slopes (*Fig. 7*), while the *Scorzonero-Stipetum endotrichae,* the *Globulario-Centaureetum grbavacensis* and the *Astragalo-Helianthemetum marmorei* are confined to steeper slopes. No significant differences between communities were found in relation to aspect of the slope (data not shown).

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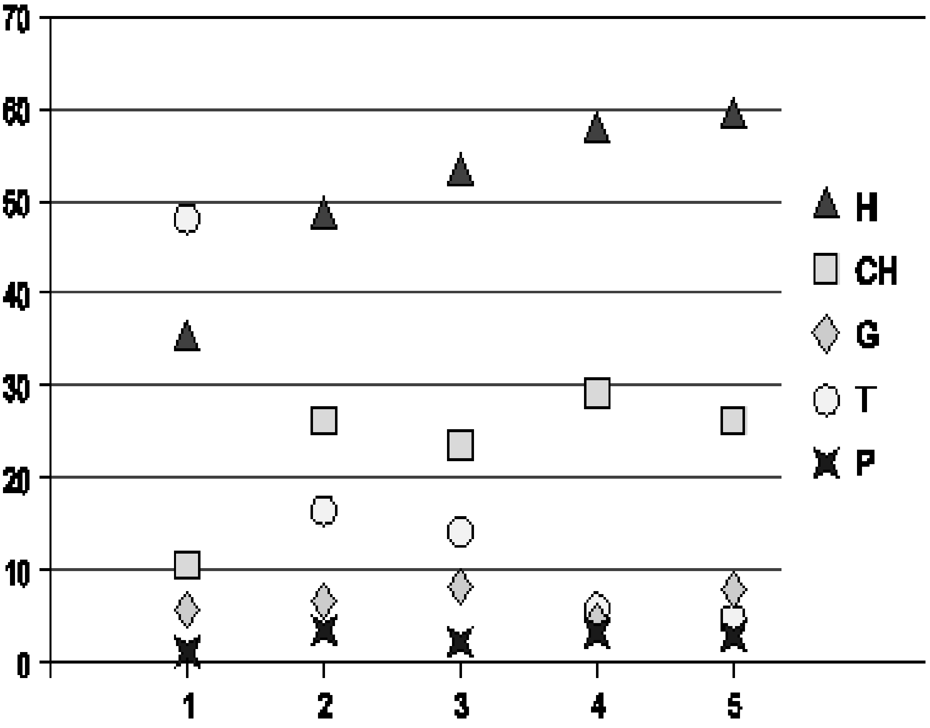

Figure 4*. Life-form spectra. The numbering of clusters corresponds to that in Fig. 2. G: geophytes, Ch: chamaephytes, H: hemicryptophytes, P: phanerophytes, T: therophytes.*

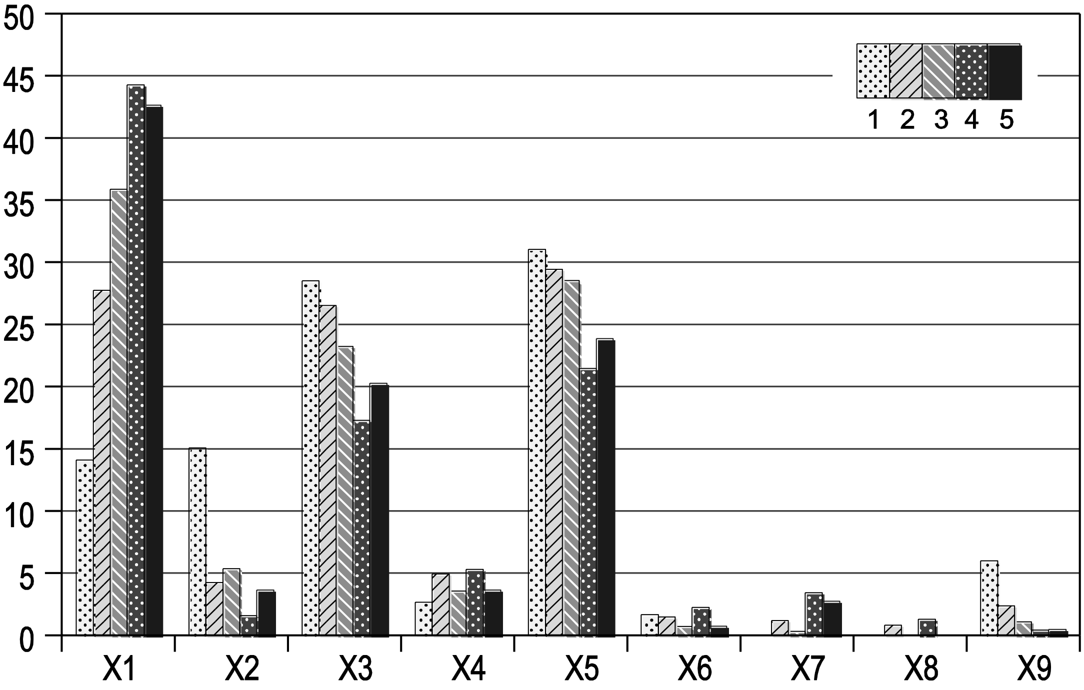

Figure 5. *General chorological spectra. The numbering of clusters corresponds to that in Fig. 2. Geoelements: X1: Balkan, X2: Steno-Mediterranean, X3: Eury-Mediterranean, X4: Mediterranean-montane, X5: Eurasian, X6: Atlantic, X7: Orophilous South-European, X8: Boreal, X9: Cosmopolitan.*

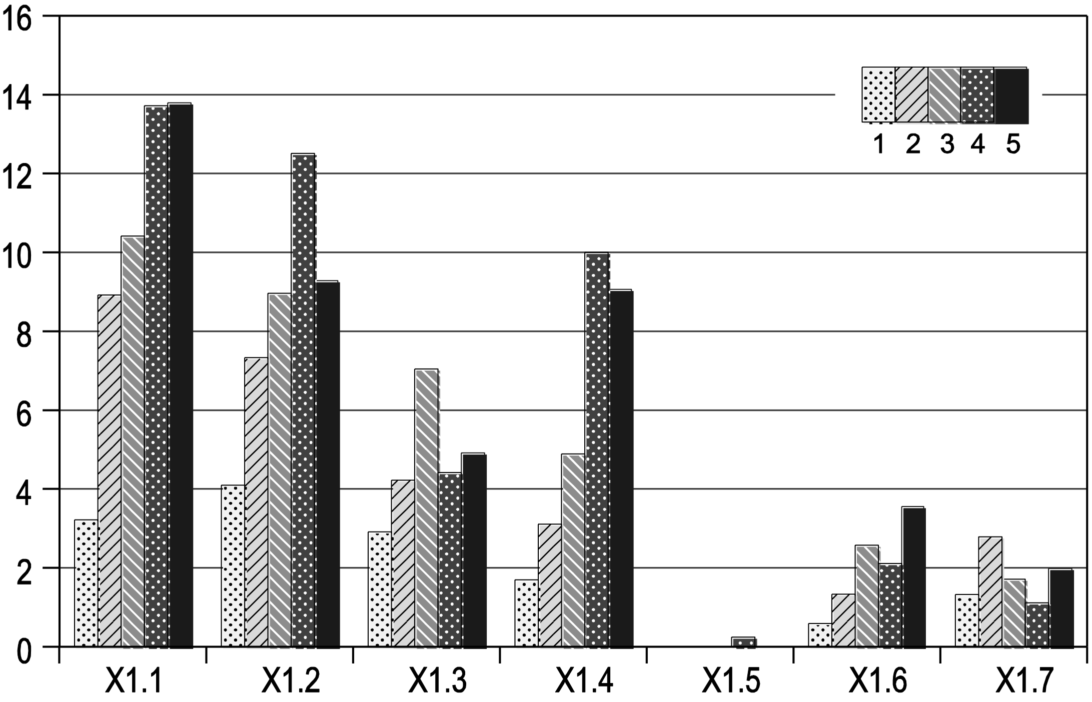

Figure 6. *Chorological spectra the Balkan and Sub-Balkan elements. The numbering of clusters corresponds to that in Fig. 2. X1.1: Balkan (sensu lato), X1.2: South Balkan, X1.3: Scardo-Pindian, X1.4: Macedonian (sub) endemic, X1.5: Balkan-Apennine, X1.6: South Balkan-Asia Minor, X1.7: South Balkan-Caucasian.*

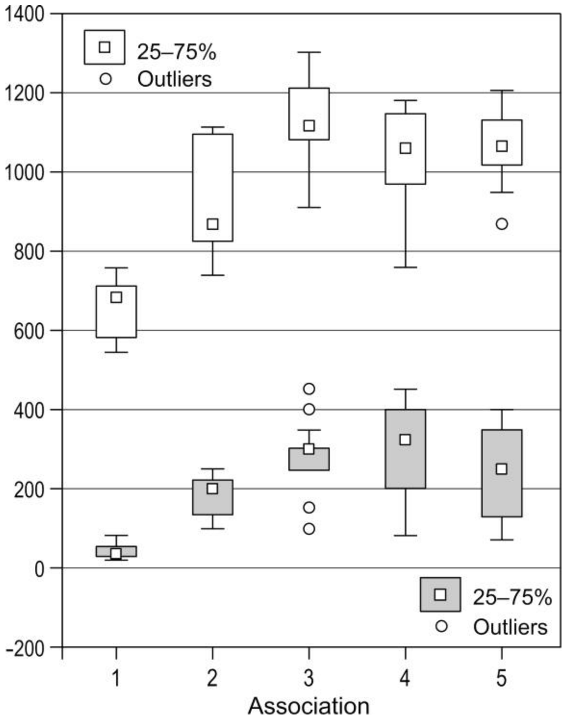

Figure 7*. Altitudinal (empty boxes) and slope (shaded boxes) profiles for the studied communities. The numbering of clusters corresponds to that in Fig. 2. The upper case present altitude and lower slope.*

The comparison of these syntaxonomic units with the existing relevant literature revealed that all associations, except for the *Sileno-Thymetum ciliatopubescentis* Matevski et al. 2007, should be described as new.

Petrorhagio haynaldianae-Chrysopogonetum grylli Matevski, Čarni, Ćušterevska, Kostadinovski et Mucina ass. nova hoc loco

Holotypus *hoc loco*: Elect. App., relevé 1; the relevé is presented in the printed Appendix at the end of this paper.

Diagnostic species: *Achillea coarctata, Aegilops neglecta, Ajuga chamaepitys* subsp. *chia, Alyssum simplex, Arenaria serpyllifolia,* ***Astragalus onobrychis,*** *Bombycilaena erecta, Bothriochloa ischaemum, Bromus squarrosus, Bupleurum apiculatum, Carduus nutans, Centaurea stoebe* subsp. *australis,* ***Cerastium glomeratum,*** *Chrysopogon gryllus, Clinopodium suaveolens, Clypeola jonthlaspi, Convolvulus canthabrica, Crepis sancta, Crucianella graeca, Crupina vulgaris, Dasypyrum villosum,* ***Echinops sphaerocephalus,*** *Euphorbia taurinensis, Festuca callieri, Galium verticillatum, Helianthemum salicifolium,* ***Hippocrepis ciliata,*** *Linaria simplex, Linum corymbulosum,* ***Medicago coronata,*** *Medicago glomerata, Medicago minima, Medicago rigidula, Micromeria juliana, Minuartia glomerata* subsp. *glomerata, Minuartia hamata, Minuartia hybrida,* ***Nigella arvensis,*** *Onobrychis aequidentata, Ononis reclinata,* ***Orlaya daucorlaya,*** *Parentucellia latifolia,* ***Petrorhagia illyrica* subsp. *haynaldiana,*** *Petrorhagia prolifera, Picris pauciflora, Potentilla pedata, Psilurus incurvus, Sanguisorba minor, Satureja montana* subsp. *pisidica,* ***Scabiosa divaricata,*** *Sherardia arvensis, Sideritis montana, Silene radicosa, Stipa pennata, Thymus striatus, Trachynia distachya, Tragopogon dubius,* ***Trifolium campestre, Trifolium scabrum,*** *Trigonella gladiata, Trigonella monspeliaca, Valerianella dentata, Velezia rigida, Veronica arvensis, Xeranthemum annuum, Ziziphora capitata*

Constant species: ***Eryngium campestre, Euphorbia myrsinites, Hypericum rumeliacum, Koeleria splendens, Leontodon crispus, Melica ciliata, Ornithogalum comosum, Poa bulbosa, Potentilla astracanica, Sedum urvillei, Teucrium capitatum***

Dominant species: *Chrysopogon gryllus*

Distribution: central part of the Republic of Macedonia (surroundings of Veles, Negotino and Prilep)

This community develops on deeper soils in sunny, slightly inclined habitats, at altitudes of 120–550 (750) m. There are various opinions about the floristic diversity and syntaxonomic affiliation of the *Chrysopogon gryllus*-dominated communities in the Balkans (Kojić 1955, 1957, 1959; Ilijanić and Topić, 1989; Bergmeier et al., 2009). In the continental parts of the Balkan Peninsula, situated more to the north and east of our study area (in Serbia and Bulgaria), the communities in which *Chrysopogon gryllus* appears as dominant are classified within the *Chrysopogono-Danthonion* Kojić 1957, the *Festucetalia valesiacae* (Horvat et. al., 1974; Kojić et al., 1998; Jovanović et al., 1986; Redžić, 1999; Meshinev et al., 2005). Bergmeier et al. (2009) suggested that the syntaxonomic position of the *Chrysopogono-Danthonion* within the *Astragalo-Potentilletalia* is more acceptable than within the *Festucetalia valesiacae (Festuco-Brometea).* It may also well be that the *Chrysopogono-Danthonion* does not belong to the *Festuco*-Brometea, but should rather be classified within the *Koelerio-Corynephoretea.*

We suggest that the *Petrorhagio-Chrysopogonetum grylli* belongs to the *Saturejo-Thymion* (*Astragalo-Potentilletalia, Festuco-Brometea).* It cannot be classified within the *Chrysopogono-Danthonion* since most of the taxa typical of the *Chrysopogono-Danthonion* (*Danthonia alpina, Achillea chrysocoma, Silene bupleuroides* subsp. *staticifolia, Trifolium montanum, Trifolium ochroleucon, Luzula campestris, Leucanthemum vulgare* etc.) are found on nutrient-poor (non-carbonate) soils and in Macedonia they rather prefer higher altitudes.

The *Petrorhagio-Chrysopogonetum grylli* represents a therophytic-hemicryptophytic community, whereas other communities of dry grasslands from the southwestern Macedonia on carbonate bedrocks are of chamaephytic-hemicryptophytic character. A larger proportion of Steno-Mediterranean and Eury-Mediterranean species can be found in this community (*Fig. 5*), and the number of Balkan, South-Balkan, Scardo-Pindian and endemic Macedonian species (*Fig. 6*) is lower than in the other studied dry grassland communities. The *Petrorhagio-Chrysopogonetum grylli*, limited to region of pronounced sub-mediterranean climate which expands to the Central Balkans along the valley of the Vardar River.

Scorzonero-Stipetum endotrichae Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina ass. nova hoc loco

Holotypus *hoc loco*: Elect. App., relevé 54; the relevé is presented in the printed Appendix at the end of this paper.

Distribution: Republic of Macedonia (Mariovo: Crna Reka Gorge, Vitolishte, Zivovo, Beshishte)

Diagnostic species: *Astragalus mariovoense, Helianthemum canum, Helianthemum nummularium* subsp. *numummularium, Helictochloa aetolica, Hellenocarum strictum, Leontodon biscutellifolius, Minuartia verna* subsp. *collina, Scorzonera mariovoensis, Sedum acre, Sideritis raeseri, Stipa endotricha, Thymus parnassicus,* ***Trinia daleschampii,*** *Veronica praecox*

Constant species: *Agropyron cristatum, Eryngium campestre,* ***Festuca hirtovaginata, Hypericum rumeliacum,*** *Koeleria splendens,* ***Potentilla astracanica,*** *Scabiosa triniifolia,* ***Stachys iva, Teucrium capitatum***

Dominant species: *Inula aschersoniana*

This community is found in rocky (marble) steep slopes, with very shallow layer of soil at altitudes spanning 900−1280 m. The region of the occurrence of this dry grassland type is a sub-mediterranean oasis located along the course of the river Crna Reka. This community is home of several Macedonian endemic species such as *Astragalus mariovoense, Stachys iva* and *Scorzonera mariovoensis*. The surrounding forest vegetation is dominated by Macedonian oak forests (*Quercetum trojanae*) and hornbeam forests of the *Phyllireo-Carpinetum orientalis* (*Fraxino Ostryion, Quercetalia pubescentis*).

Globulario-Centaureetum grbavacensis Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina ass. nova hoc loco

Holotypus *hoc loco*: Elect. App., relevé 83; the relevé is presented in the printed Appendix at the end of this paper.

Diagnostic species: *Anthericum ramosum, Centaurea grbavacensis, Globularia cordifolia, Matthiola fruticulosa* subsp. *vallesiaca, Paronychia chionea, Saxifraga federici-augusti* subsp. *grisebachii, Scorzonera austriaca, Viola herzogii*

Constant species: ***Fumana procumbens,*** *Juniperus oxycedrus*

Dominant species: *Anthyllis aurea, Centaurea grbavacensis*

Distribution: Republic of Macedonia (surroundings of Prilep-Kozjak, Sivec, Debreshte, M. Brod-Barbaras, Mariovo-Sekulova Tumba, Labinica, Toplik, Skopje-Nova Breznica)

This community is found typically on dolomitised limestone at altitudes between 800 and 1180 m, on relatively steep slopes spanning 15° and 45°. The best example of this vegetation can be found in habitats formerly occupied by the Macedonian oak forests (*Quercetum trojanae*) or those previously dominated by *Pinus nigra* (*Fago-Pinetum nigrae).* The Macedonian (sub) endemic taxa, *Centaurea grbavacensis* and *Viola herzogi,* are of particular importance in this community.

Astragalo-Helianthemetum marmorei Matevski, Čarni, Ćušterevska, Kostadinovski et Mucina ass. nova hoc loco

Holotypus *hoc loco*: Elect. App., relevé 103; the relevé is presented in the printed Appendix at the end of this paper.

Diagnostic species: *Achillea agaretifolia* subsp. *aizoon, Allium bornmulleri, Asperula purpurea, Astragalus sericophyllus,* ***Bromopsis riparia,*** *Helianthemum marmoreum, Helianthemum nummularium* subsp. *tomentosum, Hyssopus officinalis* subsp. *aristatus, Linum austriacum, Medicago prostrata, Micromeria cristata* subsp. *kosaninii, Minuartia setacea, Odontites glutinosa, Phelypaea boissieri, Polygala vulgaris, Trinia glauca*

Constant species: ***Agropyron cristatum,*** *Alyssum corymbosoides,* ***Anthyllis aurea, Asperula aristata* subsp. *scabra, Asyneuma limonifolium, Carex liparocarpos,*** *Centaurea grbavacensis,* ***Clinopodium alpinum* subsp. *hungaricum,*** *Dianthus haematocalyx,* ***Festuca hirtovaginata, Fumana procumbens, Galium oreophilum, Hypericum rumeliacum, Iris pumila, Jurinea polycephala, Koeleria splendens, Leontodon crispus, Melica ciliata, Ornithogalum comosum, Potentilla astracanica, Scabiosa triniifolia, Sedum ochroleucum, Sedum urvillei, Stachys iva, Stipa pulcherrima,*** *Teucrium capitatum, Teucrium montanum,* ***Thymus parnassicus, Vincetoxicum hirundinaria* subsp. *nivale,*** *Viola herzogii*

Dominant species: *Centaurea grbavacensis*

Distribution: Republic of Macedonia (surroundings of Prilep-Kozjak, Pletvar, Raec Reka)

This community develops on dolomitised limestone at altitudes spanning 950–1200 m, on slopes 7°–25° (sometimes up to 40°) steep. It is a very thermophilous grassland supporting several (sub)endemics including *Astragalus sericophyllus, Helianthemum marmoreum, Seseli vandasii* and *Stachys iva.*

Sileno-Thymetum ciliatopubescentis Matevski et al. 2007

Distribution: Republic of Macedonia (around Prilep: Debreška Krasta; below mountain Baba Sač: Cer; Kičevsko).

The *Sileno-Thymetum ciliatopubescentis* has already been formally described by Matevski et al. (2007). This community develops on carbonate bedrocks at altitudes of 700–1100 m. The stands of this association are found in the nutrient-richest and moisture-supplied habitats of all the communities concerned. This vegetation is found at relatively low altitudes and therefore is considerably thermophilous.

Syntaxonomic conclusions

The dry grasslands on carbonate bedrocks in the Republic of Macedonia belong to the *Saturejo-Thymion* (Micevski, 1970, 1971a, 1971b; Matevski el al., 2007). The syntaxonomically regionally important taxa of the *Saturejo-Thymion* include *Agropyron cristatum, Anthyllis vulneraria* subsp. *rubriflora, Carex liparocarpos, Dianthus haematocalyx* subsp. *haematocalyx, Dianthus kapinaensis, Genista sessilifolia, Potentilla astracanica, Silene radicosa, Stachys iva, Thymus parnassicus, T.. skopjensis, Viola herzogii,* and others. They are calcicolous elements, and many are endemic to Macedonia or to the Central Balkans.

The grasslands of the *Saturejo-Thymion* cover extensive areas throughout the Republic of Macedonia, especially in northern, western, southwestern and central regions of the country as well as in northern Greece (Bergmeier et al., 2009). They are of secondary origin and are a result of the destruction and degradation of various zonal forest communities, such as the *Phillireo-Carpinetum orientalis*, the *Quercetum trojanae*, the *Quercetum frainetto-cerris* and the like (Micevski, 1971; Matevski et al., 2007).

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APPENDIX

Typification of new associations presented in the publication:

Holotypus relevé of *Petrorhagio haynaldianae-Chrysopogonetum grylli* Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina *ass. nova hoc loco*, rel. 1 in Elect. App. Site: Prilep-Kozjak, date: 15. 06. 2004, Altitude: 704 m, Aspect: SE, Slope 3°, Cover: 97 %, Area: 100 m^2, Coordinates: 41°22'7.07"N, 21°43'2.93"E.

Chrysopogon gryllus 4, *Anthyllis vulneraria* subsp. *rubriflora* 2, *Convolvulus cantabrica* 2, *Koeleria splendens* 2, *Potentilla astracanica* 2, *Teucrium capitatum* 2, *Asphodeline lutea* 1, *Crupina vulgaris* 1, *Helianthemum nummularium* subsp. *tomentosum* 1, *Helianthemum salicifolium* 1, *Hippocrepis ciliata* 1, *Medicago coronata* 1, *Onobrychis aequidentata* 1, *Ononis reclinata* 1, *Poa bulbosa* 1, *Satureja montana* subsp. *pisidica* 1, *Sedum urvillei* 1, *Stachys iva* 1, *Stipa pennata* 1, *Achillea coarctata* +, *Aegilops neglecta* +, *Alyssum simplex* +, *Arabis auriculata* +, *Arabis sagittata* +, *Arenaria serpyllifolia* +, *Asperula aristata* subsp. *scabra* +, *Astragalus onobrychis* +, *Asyneuma limonifolium* +, *Bombycilaena erecta* +, *Bothriochloa ischaemum* +, *Bromus squarrosus* +, *Bupleurum apiculatum* +, *Centaurea stoebe* subsp. *australis* +, *Cerastium glomeratum* +, *Clinopodium suaveolens* +, *Clypeola jonthlaspi* subsp. *jonthlaspi* +, *Coronilla scorpioides* +, *Crepis sancta* +, *Echinops sphaerocephalus* +, *Eryngium campestre* +, *Erysimum diffusum* +, *Euphorbia myrsinites* +, *Euphorbia taurinensis* +, *Fumana procumbens* +, *Galium oreophilum* +, *Galium verticillatum* +, *Hypericum rumeliacum* +, *Inula oculus-christi* +, *Leontodon crispus* +, *Linaria simplex* +, *Linum corymbulosum* +, *Medicago minima* +, *Medicago rigidula* +, *Melica ciliata* subsp. *ciliata* +, *Minuartia glomerata* subsp. *glomerata* +, *Minuartia hamata* +, *Minuartia hybrida* subsp. *hybrida* +, *Muscari racemosum* +, *Myosotis incrassata* +, *Nigella arvensis* +, *Onosma visianii* +, *Orlaya daucorlaya* +, *Ornithogalum comosum* +, *Parentucellia latifolia* +, *Petrorhagia illyrica* subsp. *haynaldiana* +, *Petrorhagia prolifera* +, *Petrorhagia thessala* +, *Picris pauciflora* +, *Pilosella piloselloides* +, *Potentilla pedata* +, *Psilurus incurvus* +, *Ranunculus sprunerianus* +, *Rhamnus saxatilis* subsp. *tinctorius* +, *Sanguisorba minor* subsp. *muricata* +, *Scabiosa divaricata* +, *Scorzonera hispanica* subsp. *asphodeloides* +, *Sherardia arvensis* +, *Sideritis montana* +, *Silene radicosa* +, *Thesium macedonicum* +, *Thymelaea passerina* +, *Thymus striatus* +, *Trachynia distachya* +, *Tragopogon dubius* subsp. *dubius* +, *Trifolium campestre* +, *Trifolium scabrum* +, *Trigonella gladiata* +, *Valerianella dentata* +, *Verbascum herzogii* +, *Veronica arvensis* +, *Ziziphora capitata* +.

Holotypus relevé of the *Scorzonero-Stipetum endotrichae* Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina *ass. nova hoc loco*, rel. 54 in Elect. App. Site: Pantelejmon, above quarry, date: 01. 06. 1993, Altitude: 1130 m, Aspect: S, Slope 25°, Cover: 65 %, Area: 100 m^2, Coordinates: 41° 7'56.64"N, 21°48'1.57"E.

Chrysopogon gryllus 2, Festuca hirtovaginata 2, Scorzonera mariovoensis 2, Stachys iva 2, Stipa endotricha 2, Agropyron cristatum 1, Eryngium campestre 1, Euphorbia taurinensis 1, Helianthemum canum subsp. canum 1, Iris pumila 1, Koeleria splendens 1, Potentilla *astracanica 1, Thymus parnassicus 1, Trinia daleschampii 1, Achillea fraasii +, Aethionema saxatile +, Alyssum doerfleri +, Anthericum liliago +, Anthyllis vulneraria subsp. rubriflora +, Arabis auriculata +, Arenaria serpyllifolia +, Asperula aristata subsp. scabra +, Astragalus mariovoense +, Asyneuma limonifolium +, Bombycilaena erecta +, Bromopsis cappadocica +, Carex liparocarpos +, Caucalis platycarpos +, Centaurea grisebachii +, Clinopodium alpinum subsp. hungaricum +, Clypeola jonthlaspi subsp. jonthlaspi +, Crupina vulgaris +, Dianthus haematocalyx subsp. haematocalyx +, Euphorbia myrsinites +, Fumana procumbens +, Galium verticillatum +, Helianthemum ummularium subsp. numummularium +, Helictochloa aetolica +, Hellenocarum strictum +, Hornungia petraea +, Hypericum rumeliacum +, Juniperus oxycedrus +, Jurinea polycephala +, Lactuca perennis +, Leontodon biscutellifolius +, Linaria simplex +, Medicago medicaginoides +, Minuartia verna subsp. collina +, Myosotis incrassata +, Neatostema apulum +, Onobrychis alba subsp. alba +, Petrorhagia thessala +, Ranunculus sprunerianus +, Rhamnus saxatilis subsp. tinctorius +, Scabiosa triniifolia +, Sedum ochroleucum +, Sideritis montana +, Silene radicosa +, Teucrium capitatum +, Thymelaea passerina +, Tragopogon pterodes +, Valerianella coronata +, Valerianella pumila +, Veronica praecox +.*

Holotypus relevé of *Globulario-Centaureetum grbavacensis* Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina *ass. nova hoc loco*, rel. 83 in Elect. App. Site: M. Brod-Barbaras, date: 10. 06. 2005, Altitude: 780 m, Aspect: S, Slope 25°, Cover: 85 %, Area: 100 m^2, Coordinates: 41°30'31.93"N, 21°15'45.47"E.

Anthyllis aurea 3, Centaurea grbavacensis 3, Fumana procumbens 2, Globularia cordifolia 2, Thymus skopjensis 2, Achillea ageratifolia subsp. aizoon 1, Dianthus kapinaensis 1, Eryngium wiegandii 1, Festuca hirtovaginata 1, Helianthemum canum subsp. canum 1, Linum tenuifolium 1, Matthiola fruticulosa subsp. valesiaca 1, Scorzonera austriaca 1, Stipa pulcherrima 1, Teucrium montanum 1, Alyssum stribrnyi +, Anthericum ramosum +, Anthyllis vulneraria subsp. bulgarica +, Asperula aristata subsp. scabra +, Asplenium ruta-muraria subsp. ruta-muraria +, Asyneuma limonifolium +, Cerastium decalvans subsp. dollineri +, Comandra umbellata subsp. elegans +, Cytisus procumbens +, Erysimum diffusum +, Euphorbia barrelieri subsp. hercegovina +, Fraxinus ornus +, Galium oreophilum +, Haplophyllum albanicum +, Hieracium pannosum +, Hippocrepis glauca +, Inula ensifolia +, Juniperus oxycedrus +, Jurinea polycephala +, Koeleria splendens +, Leontodon crispus +, Linum austriacum +, Onosma heterophylla +, Paronychia chionaea +, Polygala vulgaris +, Quercus trojana subsp. trojana +, Salvia ringens +, Saponaria bellidifolia +, Saxifraga federici-augusti subsp. grisebachii +, Seseli rigidum +, Thalictrum minus subsp. minus +, Thymelaea passerina +, Vincetoxicum hirundinaria subsp. nivale +, Viola herzogii +.

Holotypus relevé of *Astragalo-Helianthemetum marmorei* Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina *ass. nova hoc loco*, rel. 103 in Elect. App. Site: Prilep-Pletvar, date: 16. 06. 2004, Altitude: 1083 m, Aspect: S, Slope 30°, Cover: 65 %, Area: 100 m^2, Coordinates: 41°22'21.81"N, 21°39'21.14"E.

Anthyllis aurea 3, *Astragalus sericophyllus* 3, *Fumana procumbens* 3, *Helianthemum marmoreum* 3, *Thymus parnassicus* 3, *Achillea ageratifolia* subsp. *aizoon* 2, *Agropyron cristatum* 2, *Stipa pulcherrima* 2, *Anthyllis vulneraria* subsp. *rubriflora* 1, *Bromopsis riparia* 1, *Festuca hirtovaginata* 1, *Linum hirsutum* subsp. *hirsutum* 1, *Micromeria cristata* subsp. *kosaninii* 1, *Potentilla astracanica* 1, *Alkanna pulmonaria* +, *Allium bornmulleri* +, *Alyssum corymbosoides* +, *Asperula aristata* subsp. *scabra* +, *Asperula purpurea* +, *Asyneuma limonifolium* +, *Carex liparocarpos* +, *Centaurea marmorea* +, *Chrysopogon gryllus* +, *Clinopodium alpinum* subsp. *hungaricum* +, *Cruciata laevipes* +, *Dianthus haematocalyx* subsp.

haematocalyx +, *Echinops ritro* +, *Erodium absinthoides* +, *Euphorbia barrelieri* subsp. *thessala* +, *Euphorbia myrsinites* +, *Galium oreophilum* +, *Helianthemum nummularium* subsp. *tomentosum* +, *Hieracium pannosum* +, *Hippocrepis glauca* +, *Hypericum rumeliacum* +, *Iris pumila* +, *Juniperus oxycedrus* +, *Jurinea polycephala* +, *Koeleria splendens* +, *Leontodon crispus* +, *Linaria simplex* +, *Matthiola fruticulosa* subsp. *valesiaca* +, *Melica ciliata* subsp. *ciliata* +,*Minuartia setacea* +, *Muscari racemosum* +, *Odontites glutinosa* +, *Onosma heterophylla* +, *Ornithogalum comosum* +, *Paronychia macedonica* subsp. *macedonica* +, *Pilosella piloselloides* +, *Polygala vulgaris* +, *Scorzonera austriaca* +, *Seseli vandasii* +, *Stachys iva* +, *Teucrium montanum* +, *Thesium macedonicum* +, *Thymelaea passerina* +, *Thymus striatus* +, *Vincetoxicum hirundinaria* subsp. *nivale* +, *Viola herzogii* +.

Electronic Appendix: The analytical phytosociological table of the four new associations of the Macedonian dry grasslands. * Life forms: P – phanerophyte, Ch – chamaephyte, H – hemicriptophyte, G – geophyte, T – therophyte. ** Geoelements: 1.1 – Balkan (s. lat.), 1.2 - South Balkan, 1.3 – Scardo-Pindian, 1.4 – Macedonian (sub) endemic, 1.5 – Balkan-Apennine, 1.6 – South Balkan-Asia Minor, 1.7 – South Balkan-Caucasian 2 - Steno-Mediterranean, 3 – Eury-Mediterranean, 4 – Mediterranean-montane, 5 – Eurasian, 6 – Atlantic, 7 - Orophilous-South European, 8 - Boreal, 9 – Cosmopolitan; H – holotype; Associations: P – *Petrorhagio-Chrysopogonetum grylli*, S – *Scorzonero-Stipetum endotrichae,* G – *Globulario-Centaureetum grbavacensis*, A – *Astragalo-Helianthemetum marmorei.*

Geographic and sampling-relevant data in the analytical Electronic Appendix: *Each entry consists of the following:* ***Relevé number****, locality, area of relevés (m²), cover %, slope in degree, aspect, altitude in m, coordinates: latitude and longitude, and date:*

1: Prilep-Kozjak, 100, 97, 3, SE, 704, 41°22'7.07"N, 21°43'2.93"E, 15.06.2004; **2**: Prilep-Kozjak, 100, 98, 3, SE, 715, 41°22'13.73"N, 21°43'4.13"E, 15.06.2004; **3**: Prilep-Kozjak, 100, 93, 3, SE, 693, 41°22'6.26"N, 21°43'7.18"E, 15.06.2004; **4**: Prilep-Kozjak, 100, 98, 4, SE, 745, 41°22'16.80"N, 21°42'57.88"E, 15.06.2004; **5**: Prilep-Kozjak, 100, 98, 5, SE, 756, 41°22'16.25"N, 21°42'51.55"E, 15.06.2004; **6**: Prilep-Kozjak, 100, 97, 2, S, 666, 41°22'49.97"N, 21°43'52.20"E, 22.06.2004; **7:**Prilep-Kozjak, 100, 97, 3, SE, 680, 41°22'49.85"N, 21°43'46.04"E, 22.06.2004; **8**: Prilep-Kozjak, 100, 99, 3, S, 682, 41°22'52.35"N, 21°43'46.37"E, 22.06.2004; **9**: Veles-Groot, 100, 90, 8, W, 543, 41°42'57.04"N, 21°44'10.18"E, 17.06.2005; **10**: Veles-Groot, 100, 95, 5, S, 563, 41°42'59.21"N, 21°44'08.05"E, 17.06.2005; **11**: Veles-Groot, 100, 90, 8, SW, 578, 41°43'03.11"N, 21°44'05.34"E, 17.06.2005; **12**: Veles-Groot, 100, 95, 6, E, 583, 41°43'11.19"N, 21°44'04.19"E, 17.06.2005; **13-28:** Matevski et al. 2007: Tab. 1**;** **29**: Bešište-Pantelejmon, 100, 85, 30, W, 1050, 41° 8'16.35"N, 21°47'45.60"E, 13.06.1992; **30**: Bešište-Pantelejmon, 100, 80, 25, W, 1040, 41° 8'15.86"N, 21°47'42.43"E, 13.06.1992; **31**: Bešište-Pantelejmon, 100, 80, 30, W, 1090, 41° 8'13.86"N, 21°47'51.30"E, 13.06.1992; **32**: Bešište-Pantelejmon, 100, 70, 35, W, 1250, 41° 8'11.05"N, 21°48'6.30"E, 13.06.1992; **33**: Bešište-Pantelejmon, 100, 80, 30, NW, 1300, 41° 8'14.35"N, 21°48'12.91"E, 13.06.1992; **34**: Bešište-Pantelejmon, 100, 90, 35, W, 1280, 41° 8'13.83"N, 21°48'10.00"E, 13.06.1992; **35**: Bešište-Pantelejmon, 100, 70, 30, W, 1130, 41° 8'17.34"N, 21°47'57.92"E, 13.06.1992; **36**: Bešište-Pantelejmon, 100, 80, 35, W, 1210, 41° 8'17.05"N, 21°48'5.52"E, 13.06.1992; **37**: Vitolište-Gola Srka, 100, 60, 45, W, 1060, 41°10'28.88"N, 21°47'48.68"E, 12.06.1992; **38**: Vitolište-Gola Srka, 100, 50, 40, W, 1080, 41°10'31.15"N, 21°47'49.13"E, 12.06.1992; **39**: Vitolište-Gola Srka, 100, 65, 30, S, 1030, 41°10'25.29"N, 21°47'49.61"E, 12.06.1992; **40**: Vitolište-Gola Srka, 100, 65, 30, SE, 1080, 41°10'28.77"N, 21°47'52.37"E, 12.06.1992; **41**: Vitolište-Gola Srka, 100, 65, 40, S, 980, 41°10'21.66"N, 21°47'50.39"E, 12.06.1992; **42**: Vitolište-Gola Srka, 100, 70, 40, S, 910, 41°10'17.62"N, 21°47'46.75"E, 12.06.1992; **43**: Živovo, 100, 75, 30, N, 1220, 41°13'8.78"N, 21°47'53.50"E, 03.06.1993; **44**: Živovo, 100, 75, 30, N, 1220, 41°13'9.75"N, 21°47'54.95"E, 03.06.1993; **45**: Živovo, 100, 70, 25, N, 1220, 41°13'9.80"N, 21°47'58.84"E, 03.06.1993; **46**: Živovo, 100, 75, 25, N, 1220, 41°13'8.42"N, 21°47'50.95"E, 03.06.1993; **47**: Živovo, 100, 70, 25, N, 1220, 41°13'6.94"N, 21°47'48.99"E, 03.06.1993; **48**: Živovo, 100, 60, 15, NE, 1200, 41°13'6.95"N, 21°47'57.13"E, 03.06.1993; **49**: Živovo, 100, 55, 10, NE, 1210, 41°13'6.36"N, 21°47'58.70"E, 03.06.1993; **50**: Živovo, 100, 60, 10, E, 1210, 41°12'58.57"N, 21°47'48.40"E, 03.06.1993; **51**: Pantelejmon,above quarry, 100, 65, 30, SW, 1100, 41° 8'3.91"N, 21°47'47.46"E, 01.06.1993; **52**: Pantelejmon,above quarry, 100, 70, 30, SW, 1120, 41° 8'4.08"N, 21°47'50.60"E, 01.06.1993; **53**: Pantelejmon,-above quarry, 100, 65, 25, S, 1120, 41° 7'56.85"N, 21°48'6.63"E, 01.06.1993;**54**: Pantelejmon,above quarry, 100, 65, 25, S, 1130, 41° 7'56.64"N, 21°48'1.57"E, 01.06.1993; **55**: Pantelejmon,above quarry, 100, 65, 25, S, 1140, 41° 7'56.85"N, 21°47'56.46"E, 01.06.1993; **56**: Pantelejmon,above quarry, 100, 70, 30, S, 1080, 41° 7'39.05"N, 21°48'24.22"E, 01.06.1993;**57**: Pantelejmon,above quarry, 100, 75, 25, S, 1100, 41° 7'44.23"N, 21°48'16.24"E, 01.06.1993; **58**: Pantelejmon,above quarry, 100, 60, 30, SW, 1080, 41° 7'55.82"N, 21°47'46.83"E, 15.06.1992; **59**: Pantelejmon,above quarry, 100, 55, 30, SW, 1110, 41° 7'59.83"N, 21°47'49.11"E, 15.06.1992;**60**: Pantelejmon,above quarry, 100, 60, 30, SW, 1110, 41° 7'55.08"N, 21°47'49.76"E, 15.06.1992; **61**: Pantelejmon,above quarry, 100, 60, 30, SW, 1060, 41° 7'48.99"N, 21°47'46.97"E, 14.06.1992;**62**: Pantelejmon,above quarry, 100, 65, 30, SW, 1070, 41° 7'51.41"N,21°47'46.64"E, 14.06.1992; **63**: Mariovo-Sekulova Tumba, 100, 60, 40, S, 970, 41° 6'10.95"N, 21°47'58.57"E, 18.06.1995; **64**: Mariovo-Sekulova Tumba, 100, 65, 30, S, 960, 41° 6'9.65"N, 21°47'56.90"E, 18.06.1995; **65**: Mariovo-Labinica, 100, 60, 40, S, 1180, 41° 5'29.93"N, 21°48'47.87"E, 17.06.1995; **66**: Mariovo-Labinica, 100, 50, 40, S, 1170, 41° 5'35.00"N, 21°48'42.89"E, 17.06.1995; **67**: Mariovo-Labinica, 100, 60, 40, SW, 1180, 41° 5'31.94"N, 21°48'46.17"E, 17.06.1995;**68**: Mariovo-Labinica, 100, 65, 40, S, 1150, 41° 5'35.35"N, 21°48'37.43"E, 17.06.1995; **69**: Mariovo-Labinica, 100, 85, 45, SW, 1130, 41° 5'38.12"N, 21°48'28.80"E, 17.06.1995; **70**: Mariovo-Labinica, 100, 65, 40, S, 1160, 41° 5'36.06"N, 21°48'38.86"E, 17.06.1995; **71**: Mariovo-Labinica, 100, 80, 40, SW, 1160, 41° 5'35.19"N, 21°48'40.96"E, 17.06.1995; **72**: Mariovo-Labinica, 100, 85, 40, SW, 1140, 41° 5'39.19"N, 21°48'28.84"E, 17.06.1995; **73**: Mariovo-Toplik, 100, 85, 25, W, 1160, 41° 2'33.07"N, 21°48'34.13"E, 19.06.1995; **74**: Mariovo-Toplik, 100, 80, 35, W, 1140, 41° 2'32.66"N, 21°48'32.59"E, 19.06.1995;**75**: Mariovo-Toplik, 100, 85, 35, W, 1110, 41° 2'27.98"N, 21°48'28.72"E, 19.06.1995; **76**: Skopje-Kozjak, 100, 80, 15, SW, 1072, 41°53'21.02"N, 21°13'24.00"E, 08.07.2006; **77**: Skopje-Kozjak, 100, 85, 35, W, 1055, 41°53'20.19"N, 21°13'36.86"E, 08.07.2006; **78**: Skopje-Kozjak, 100, 83, 37, W, 1047, 41°53'22.19"N, 21°13'31.31"E, 08.07.2006; **79**: Skopje-Kozjak, 100, 83, 35, S, 896, 41°53'23.53"N, 21°13'30.15"E, 08.07.2006; **80**: M. Brod-Barbaras, 100, 93, 20, NW, 793, 41°30'26"N, 21°15'55"E, 10.06.2005; **81**: M. Brod-Barbaras, 100, 85, 18, W, 803, 41°30'34.04"N, 21°15'47.06"E, 10.06.2005; **82**: M. Brod-Barbaras, 100, 88, 20, W, 759, 41°30'29.79"N, 21°15'42.76"E, 10.06.2005;**83**: M. Brod-Barbaras, 100, 85, 25, S, 780, 41°30'31.93"N, 21°15'45.47"E, 10.06.2005; **84**: Prilep-Sivec, 100, 78, 25, E, 1051, 41°25'10.11"N, 21°35'23.03"E, 09.06.2005; **85**: Prilep-Sivec, 100, 70, 25, E, 1058, 41°25'4.36"N, 21°35'27.92"E, 09.06.2005; **86**: Prilep-Sivec, 100, 73, 8, W, 1063, 41°25'0.59"N, 21°35'22.52"E, 09.06.2005; **87**: Prilep-Sivec, 100, 70, 17, SW, 1010, 41°24'54.27"N, 21°35'35.41"E, 09.06.2005; **88**: Prilep-Sivec, 100, 70, 20, SW, 1031, 41°24'58.92"N, 21°35'27.48"E, 09.06.2005; **89**: Prilep-Sivec, 100, 65, 15, NW, 1014, 41°24'57.16"N, 21°35'23.90"E, 09.06.2005; **90**: Prilep-Sivec, 100, 68, 15, E, 964, 41°25'6.37"N, 21°35'27.54"E, 09.06.2005; **91**: Prilep-Kozjak, 100, 94, 7, E, 947, 41°23'19.77"N, 21°43'18.46"E, 20.06.2003; **92**: Prilep-Kozjak, 100, 95, 12, E, 991, 41°23'24.70"N, 21°42'52.39"E, 20.06.2003; **93**: Prilep-Kozjak, 100, 85, 12, E, 967, 41°23'25.13"N, 21°42'55.07"E, 20.06.2003; **94**: Prilep-Kozjak, 100, 7, 87, E, 999, 41°23'25.72"N, 21°42'51.58"E, 20.06.2003; **95**: Prilep-Kozjak, 100, 85, 17, E, 1066, 41°23'29.10"N, 21°42'44.04"E, 20.06.2003;**96**: Prilep-Kozjak, 100, 90, 15, E, 1193, 41°23'33.28"N, 21°42'29.17"E, 20.06.2003; **97**: Prilep-Kozjak, 100, 90, 12, E, 1205, 41°23'40.07"N, 21°42'50"E, 20.06.2003; **98**: 23, Prilep-Kozjak, 100, 85, 18, E, 1122, 41°23'33.34"N, 21°42'39.48"E, 20.06.2003; **99**: Prilep-Kozjak, 100, 88, 13, E, 1027, 41°23'27.14"N, 21°42'48.42"E, 20.06.2003; **100**: 55, Prilep-Pletvar, 100, 65, 40, SE, 1017, 41°22'19.21"N, 21°40'25.89"E, 16.06.2004;**101**: 54, Prilep-Pletvar, 100, 65, 40, SE, 1033, 41°22'17.75"N, 21°40'18.24"E, 16.06.2004;**102**: Prilep-Pletvar, 100, 65, 40, SE, 1031, 41°22'17.71"N, 21°40'18.62"E, 16.06.2004; **103**: Prilep-Pletvar, 100, 65, 30, S, 1083, 41°22'21.81"N, 21°39'21.14"E, 16.06.2004;**104**: Prilep-Pletvar, 100, 65, 35, S, 1073, 41°22'20.69"N, 21°39'8.67"E, 16.06.2004; **105**: Prilep-Pletvar, 100, 65, 27, S, 1152, 41°22'20.92"N, 21°39'35.41"E, 16.06.2004; **106**: Prilep-Pletvar, 100, 65, 30, S, 1194, 41°22'22.18"N, 21°39'12.33"E, 16.06.2004;**107**: Prilep-Pletvar, 100, 65, 25, S, 1131, 41°22'25.45"N, 21°39'9.50"E, 16.06.2004; **108**: Prilep-Pletvar, 100, 70, 25, S, 1077, 41°22'21.49"N, 21°39'5.74"E, 16.06.2004; **109**: Prilep-Pletvar, 100, 70, 20, S, 1063, 41°22'19.74"N, 21°39'8.98"E, 16.06.2004.

Other species:

Adonis flammea 2: +, 3: +, 4: +; *Aegilops comosa* subsp. *heldreichii* 9: +, 10: +, 11: +; *Aegilops triuncialis* 9: +, 10: +, 11: +; *Agrimonia eupatoria* 8: +; *Allium flavum* 101: +, 107: +; *Allium paniculatum* 3: +, 7: +, 12: +; *Althaea hirsuta* 5: +, 7: +; *Alyssum montanum* 80: +, 82: +, 83: +; *Amelanchier ovalis* 85: +, 89: +; *Anemone nemorosa* 1: +, 2: +, 3: +; *Anemone pavonina* 4: +, 5: +; *Anthemis arvensis* 12: +, 86: +; *Anthemis cretica* 73: +, 74: +, 90: +; *Anthemis ruthenica* 7: +; *Aphanes arvensis* 1: +, 4: +; *Arenaria leptoclados* 3: +, 4: +, 8: +; *Artemisia campestris* 90: +; *Asterolinon linum-stellatum* 2: +, 4: +, 5: +; *Bupleurum commutatum* 51: +, 55: +; *Bupleurum pachnospermum* 9: +, 12: +; *Buxus sempervirens* 76: +, 79: +; *Campanula lingulata* 77: +, 79: +; *Carex caryophyllea* 76: +, 77: 1, 80: +; *Carthamus lanatus* 7: +; *Centaurium erythraea* 76: +, 77: +, 79: +; *Cerastium rectum* subsp. *petricola* 50: +, 51: +, 57: +; *Chondrilla juncea* 7: +; *Colchicum doerfleri* 50: +, 58: +, 59: +; *Cotoneaster nebrodensis* 30: +, 36: +; *Crepis foetida* subsp. *rhoedifolia* 8: +, 9: +; *Crepis pulchra* 38: +; *Crupina crupinastrum* 6: +; *Cynodon dactylon* 3: +; *Cynosurus echinatus* 8: +; *Dianthus gracilis* subsp. *armeroides* 8: +; *Echinaria capitata* 6: +, 7: +, 8: +; *Echium vulgare* 8: +; *Edraianthus graminifolius* 73: +, 74: +, 75: +; *Epipactis atrorubens* 73: +, 74: +, 75: +; *Erodium cicutarium* 3: +, 9: +, 11: +; *Euphorbia falcata* 6: +, 7: +; *Euphorbia helioscopia* 7: +, 8: +; *Euphrasia salisburgensis* 107: +; *Ferulago sylvatica* 75: +; *Genista carinalis* 73: +; *Genista sagittalis* 95: +, 97: 1, 98: 1; *Genista sessilifolia* 30: +, 31: +, 51: +; *Geranium purpureum* 55: +; *Geranium sanguineum* 73: +, 75: +; *Goniolimon tataricum* 29: +, 32: +, 35: +; *Haplophyllum suaveolens* 8: +, 93: +, 95: +; *Hypericum perforatum* 8: +, 6: +, 7: +; *Iris reichenbachii* 11: +, 12: +; *Lathyrus cicera* 3: +, 11: +, 86: +; *Legousia hybrida* 11: +, 12: +; *Lomelosia argentea* 90: +; *Lomelosia brachiata* 5: +, 8: +, 88: +; *Melilotus neapolitana* 91: +; *Odontites luteus* 77: +, 83: +, 82: +; *Paliurus spina-christi* 9: +, 11: +; *Pinus nigra* subsp. *pallasiana* 73: +, 74: +, 75: +; *Pistacia terebinthus* 11: +, 41: +; *Plantago lanceolata* 3: +, 8: +; *Plantago subulata* 102: +; *Polygala major* 73: +, 75: +; *Polygala monspeliaca* 1: +, 8: +; *Polygala oxyptera* 103: +, 105: +; *Pontechium maculatum* 34: +, 33: +; *Pulsatilla halleri* subsp. *rhodopaea* 84: +, 85: +; *Ranunculus oreophilus* subsp. *balcanicus* 73: +, 75: +; *Salvia argentea* 100: +, 105: +, 106: +; *Scabiosa rotata* 10: +, 11: +, 60: +; *Scutellaria orientalis* subsp. *pinntifida* 31: +; *Senecio leucanthemifolius* subsp. *vernalis* 64: +, 65: +; *Sesleria rigida* 73: +, 74: +, 75: +; *Silene bupleuroides* subsp. *staticifolia* 58: +, 87: +, 90: +; *Silene saxifraga* 84: +, 86: +, 90: +; *Stachys recta* 77: +; *Taeniatherum caput-medusae* 9: +, 10: +; *Thymus sibthorpii* 86: +; *Torilis leptophylla* 8: +, 6: +; *Tragopogon pratensis* 9: +; *Trifolium angustifolium* 5: +; *Trifolium arvense* 12: +; *Viola kitaibeliana* 9: +, 11: +, 12: +.

ASSESSMENT OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI ASSEMBLAGES UNDER DIFFERENT REGIMES OF CROP ROTATION

MAGURNO, F*. – SASVÁRI, Z. – POSTA, K.

Microbiology and Environmental Toxicology Group, Plant Protection Institute,
Szent István University
Pater K. street 1, 2100, Gödöllő, Hungary
(phone: +36 28 522 910; fax: +36 28 410 804)

**Corresponding author*
e-mail: franco.magurno@gmail.com

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Abstract. Given the potential benefits of arbuscular mycorrhizal fungi (AMF) to agricultural productivity, the impact of agricultural practices on the AMF communities has been aim of an increasing number of studies. Using molecular markers on the large subunit rDNA, we investigated the influence of different long-term crop rotations on the structure and composition of the indigenous AMF community, at the experimental field in Martonvásár, Hungary. Only phylotypes belonging to the family Glomeraceae were detected. Comparing the root and soil AMF assemblages no significant correlations were found between the rotation systems and the composition of the communities. However the analysis of the diversity and the net relatedness index suggested an influence of the number and identity of the crops in rotation on the structure of the AMF communities.

Keywords: *arbuscular mycorrhizal fungi; crop rotation; maize*

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts which form symbiotic relationships with the majority of land plants including the ten most important human food crops (FAO, 2012). Among outputs of AMF symbiosis are considered of great interest an improved uptake of nutrients from soil in exchange for plant-assimilated carbon (Smith and Read, 2008) as well as a partial protection against root pathogens and drought stress (Pozo and Azcón-Aguilar, 2007; Augé, 2001). Given the potential benefits to agricultural productivity (Lekberg and Koide, 2005) AMF have received increasing attention for their possible utilization in a more sustainable agriculture without negative impacts on valuable natural resources. In the last few decades a significant effort has been dedicated to develop suitable formulations for AM fungal propagules and appropriate means for their application to the field (Gianinazzi and Vosatka, 2004). However the production and application of AMF inoculum on a large-scale can be difficult both for the high costs of propagation techniques and for the unpredictable outcome of the establishment among the native AMF community (Berruti et al., 2014). As in alternative or in concurrence with AMF inoculation, the manipulation of indigenous AMF communities through management of agricultural practice and edaphic factors has been reckoned to lead to a profitable capitalization of AMF at the field scale (Verbruggen et al., 2013). Beside stochastic-neutral processes (Dumbrell et al., 2010; Lekberg et al., 2012) several studies have revealed how niche-based mechanisms could be involved in the structuring of AMF communities. Among the edaphic factors, changes in soil nutrient availability, mainly nitrogen (N) and

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phosphate (P), soil pH and soil type (Balestrini et al., 2010; Oehl et al., 2010) were identified as major drivers of AMF community composition. Furthermore abundance and diversity of AMF have been found to be negatively associated with intensive agricultural practices such as tillage, high levels of mineral fertilization, herbicide application and frequent fallow periods with negative effects on fungal agroecosystem functions and services (Bardgett et al., 2005). Recently a new piece was added to the puzzle. Beside AMF diversity, the level of relatedness among AMF taxa co-occurring in the root has been shown to affect the outcome of the symbiosis (Roger et al., 2013). In the study, more phylogenetically related were the taxa, more high was the ability of symbionts to coexist consuming less resources in the competition and providing more benefits to the plant. Although the research was carried out using AMF isolates belonging to the same species, the level of relatedness among taxa offers a new perspective in the interpretation of the AMF community diversity.

Crop rotation is a widespread agronomic practice that exhibits general beneficial aspects associated with maintenance or improvement of soil fertility and decreased reliance on agricultural chemicals. Despite its importance, the effects of crop rotation on AMF communities are not widely investigated. Root colonization and spore abundance have been found to be affected by the presence of non-host crops in the rotation (Arihara and Karasawa, 2000). Monoculture was reported to host extremely low AMF diversity (Burrows and Pfleger, 2002; Oehl et al., 2003). Molecular studies regarding the diversity of AMF communities under different regimes of crop rotation are scarce and they have not yet given an unambiguous picture. In some cases the practice of crop rotation was shown to promote an increase of diversity in AMF communities (Hijri et al., 2006; Higo et al., 2013), while in others monocropping revealed an unexpected high level of AMF richness (Magurno et al., 2014a).

In the present work we propose to investigate root and soil AMF assemblages under different regimes of crop rotation, evaluating the differences in terms of diversity and relatedness of the taxa, and provide a more clear interpretation of the effect of crop rotation on the AMF community.

Materials and methods

Study site and sampling

The sampling site was located at Martonvásár (47°21’ N, 18°49’ E), Hungary, where an experimental field was established in 1958 by the Agricultural Research Institute of the Hungarian Research Academy of Sciences.

The crop rotation experiment was a two-factorial split-plot with four replications. The main plots were the crop sequences and the subplots were the fertilizer treatments, in a randomized design (Berzsenyi et al., 2000). The main plots consisted of seven crop sequences: 1) maize monoculture; 2) wheat monoculture; 3) 3 years alfalfa – 5 years maize; 4) 3 years alfalfa – 5 years wheat; 5) 2 years wheat – 2 years maize; 6) 3 years alfalfa – 3 years maize – 2 years wheat; 7) maize – spring barley – peas – wheat (Norfolk crop rotation). The main plots were split in five subplots (7 m × 7 m = 49 m^2) representing the fertilization treatments as follow: a) control; b) manure + NPK (60 t ha^{-1} manure every 4 years); c) crop residue + NPK; d) NPK; e) NPK (calculated for 15 t ha^{-1} maize yield and 10.5 t ha^{-1} wheat yield).

Crops were harvested in the end of October, followed one month later, by a conventional tillage of 20 cm depth. Weeds and insects were controlled by pesticide treatments in all plots as described in detail by Magurno et al. (2014a).

Four cropping systems were chosen for sampling: maize monoculture (CR 1) as a control, alfalfa-maize (CR 3), wheat-maize (CR 5) and Norfolk type rotation (CR 7). Two subplots (two replications), at least 40 m distant, were sampled per each cropping system choosing only the subplots without fertilization (treatment a). Three plants were randomly collected from each subplot on June 2012, when maize was present in all the trials except in Norfolk type with wheat. Twenty-four plants with a soil core were collected (4 rotation systems × 2 parcels × 3 plants). The roots were stored in separate plastic bags in a cooler box (~4°C) and then at the temperature of -20°C until processing. Chemical analysis of soil (*Table 1*) were performed separately on samples collected per each parcel by Soil and Plant Testing Laboratory of Faculty of Horticulture, Kecskemét College, accredited by the Hungarian Standard Institution (MSZ).

***Table 1**. The major physicochemical properties in the upper 20 cm soil layer of the experimental soils in different treatments*

Treatments	**Humus (%)**	**pH (KCl)**	**$CaCO_3$ (%)**	**P_2O_5 (mg kg^{-1})**	**K_2O (mg kg^{-1})**	**$(NO_3$-$+NO_2)$-N (mg kg^{-1})**
CR 1 P1	2.41_{ab}	5.95_{e}	0.29_{ab}	100.73_{b}	302.66_{c}	3.23_{a}
CR 1 P2	2.21_{a}	5.33	0.28_{ab}	70.96_{ab}	217.00_{a}	5.21_{a}
CR 3 P1	2.74_{bc}	5.32	0.28_{ab}	69.46_{ab}	271.00_{bc}	5.57_{a}
CR 3 P2	2.70_{bc}	4.93_{a}	0.29_{ab}	53.06_{a}	218.00_{a}	13.56_{b}
CR 5 P1	2.65_{bc}	5.75	0.13_{a}	64.06_{ab}	302.33_{c}	6.61_{ab}
CR 5 P2	2.81_{c}	5.60	0.17_{a}	55.56_{a}	248.33_{ab}	4.38_{a}
CR 7 P1	2.89_{c}	6.74_{f}	0.46_{b}	63.10_{a}	292.00_{bc}	4.08_{a}
CR 7 P2	2.60_{bc}	5.16	0.14_{a}	83.10_{ab}	282.33_{bc}	4.90_{a}

Shared lowercase letters indicate no statistical difference ($p>0.05$) between the treatments, as determined by the Tukey test.

Assessment of root colonization

Roots were washed accurately with tap water and used for DNA extraction and for measurement of mycorrhizal colonization. Five root fragments (corresponding to 1.5 g fresh weight) were collected from each plant, washed and stained following the ink and vinegar technique (Vierheilig et al., 1998). The level of the mycorrhizal colonization (M%) as well as arbuscule abundance (A%) were determined using MYCOCALC software (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) after examination of fungal structures inside the root according to Trouvelot et al. (1986).

Molecular analysis

The analysis of soil AMF assemblages was conducted using the sequences amplified from soil cores adjoining the roots sampled, published in Magurno et al., 2014b.

Root DNA extraction was performed from 600 mg of washed roots per plant, following the procedure described by Khan et al. (2007). After extraction the DNA concentration was measured with NanoDrop Spectrophotometer and the final concentration was standardized to 4 ng μl^{-1}.

Amplification of LSU fragments (large subunit rDNA) was achieved by means of a nested approach with Phusion High Fidelity DNA Polymerase (Thermo Scientific) using LR1 (5'-GCATATCAATAAGCGGAGGA-3') and NDL22 (5'-TGGTCCGTGTTTCAAGACG-3') as outer primers (van Tuinen et al., 1998) in combination with the inner primers 28G1 (5'-CATGGAGGGTGAGAATCCCG-3') and 28G2 (5'-CCATTACGTCAACATCCTTAACG-3'), specific for arbuscular mycorrhizal fungi (da Silva et al., 2006).

PCR was carried out in 40 µl final volume, in a reaction mix according to Phusion High Fidelity DNA Polymerase protocol. Annealing temperatures (62°C for the first PCR and 67°C for the nested PCR) were calculated using the Tm calculator and instructions on Thermo Scientific website (www.thermoscientific.com/pcrwebtools). PCR products of the expected size (about 585 bp) were purified from agarose gel with GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Fragments were cloned into CloneJet PCR Cloning kit (Thermo Scientific) and transformed into *Escherichia coli* DH5α. Positive transformants were checked by PCR with 28G1-28G2 primers for presence and size of the insert. Twenty positive clones per transformation (480 clones totally) were sent to Beckman Coulter Genomics (United Kingdom) for plasmid extraction and sequencing.

Phylogenetic analysis

Sequence similarities were determined using the blastn sequence similarity search tool provided by GenBank. Sequences were also checked for chimeras using the “chimera.slayer” command in Mothur v.1.23.0 (Schloss et al., 2009). Given a high number of chimera detected in the full length sequences, a hypervariable region (about 255 bp, from residue 160 to 415) was trimmed and tested for suitability in phylogenetic analysis. The trimmed sequences were verified again for similarities and presence of chimera. Only sequences belonging to Glomeromycota were selected for the subsequent analysis and the others were discarded. Sequence editing was conducted manually using MEGA 6.06 and Chromas Lite 2.01. The sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank with accession numbers KR536178 – KR536609. Sequences (KM879479 – KM879872) amplified from soil cores (Magurno et al., 2014b) were trimmed as described above and included in the subsequent analysis.

Before proceeding with the sequence clustering, a small subset of about 50 sequences from isolates representing the main morphospecies of Glomeraceae was used to define the most appropriate similarity level to distinguish AMF species on the basis of the molecular target considered. Morphospecies were chosen only from the family of Glomeraceae according to the blastn results of the sequences in study.

Clustering was performed using UCLUST (Edgar, 2010) from the pipeline of Qiime (quiime.org). A level of similarity of 94.7 % was chosen and applied to cluster root and soil sequences together.

Representative sequences for each MOTU were used in blastn to find reference sequences to build a phylogenetic tree. To ease the handling of data, we adopted the nomenclature proposed by Schüßler and Walker, 2010, including the modification of the genus *Rhizophagus* (Sieverding et al., 2014) and the introduction of two new genera (Błaszkowski et al., 2015). Reference sequences and MOTU representative sequences were aligned through the CIPRES web-portal with MAFFT on XSEDE (Miller et al., 2010). Phylogenetic tree inference, using a maximum likelihood/1000 rapid bootstrapping run, was computed through the CIPRES web-portal with RAxML ver. 7.3.0. To enforce the robustness of groups topology, a phylogenetic tree built with 30 LSU (560 bp long) reference sequences was used as constraint.

AMF communities' structure and diversity indices

We addressed the community structure by estimating several alfa-diversity indices per each parcel sampled. The communities were named according to the source (R: root; S: soil), the crop rotation type (1: monoculture; 3: alfalfa-maize rotation; 5: wheat-maize rotation; 7: Norfolk type) and the number of parcel. For example R12 was the root AMF assemblage of the parcel 2 in monoculture. Non-parametric richness indices (ACE and Chao1) and Shannon diversity index were estimated with Mothur. The standardized effect size of mean pair wise distance, SES-MPD (measure of the phylogenetic diversity considering the taxa abundances), was calculated using the picante package (Kembel et al., 2010) in R 3.0.2 (R Core Team, 2013) inputting a square distance-matrix produced by Mothur (calc=eachgap method) from the MOTU representative sequences, previously aligned with MAFFT on XSEDE. Furthermore the net relatedness index (NRI) value (measure of the clustering or segregation of the overall AMF community) was calculated using a null.model="sample.pool" and abundance.weighted=TRUE. The beta-diversity among AMF communities was evaluated as phylogenetic beta-diversity using the picante package, in order to include not only the "species" distribution but also the phylogenetic relationships among them.

Statistical analysis

Root colonization data normalized by arcsine transformation and soil data were subjected to one-way analysis of variance, using R software package version 3.0.2. Tukey's multiple comparison test was used to assess significant differences between pairs of mean values.

The data about MOTUs distribution were subjected to principal component analysis (PCA) and common components coefficients, eigenvalues and the proportion of the total variance expressed by each single MOTU were calculated. The analysis was implemented using the package FactoMineR (Lê et al., 2008) in R 3.0.2. Correlations between MOTUs and each principal component were calculated, and those having an absolute value >0.5 were considered relevant. A hierarchical clustering on principal components (HCPC) analysis was performed to define a set of clusters based on MOTUs identified. The cluster analysis was performed only on the most significant PCA components, with the remaining minor ones considered to represent noise.

A Principal Coordinate Analysis (PCoA) was computed, using the package ape in R 3.0.2, in order to assess the correlation between AMF assemblages on the basis of their pairwise phylogenetic distances, considering the effect of the soil variables after log-transformation (pH-H_2O, pH-KCl, $CaCO_3$, Humus, N, P, K).

Results and discussion

Root colonization

According to the Tukey's test there were not significant differences neither in the mycorrhizal colonization (M%) nor in the arbuscule abundance (A%) between samples collected from the four cropping systems. The lowest level of colonization (M%) and arbuscule abundance (A%) were observed in the roots of maize in monoculture (14.35% and 5.22%) and in the roots of wheat in Norfolk type rotation (14.49% and 2.90%). The highest values were detected in roots from the alfalfa-maize (25.09% and 6.72%) and wheat-maize rotation (22.71% and 8.25%). In a previous study (Magurno et al., 2014a) the root colonization of wheat in Norfolk type was significantly higher ($p<0.01$) than the colonization of maize roots in the other rotation systems. The difference in the results could be due to an overestimation of the colonization in wheat because of the grid-line intersect method used in the assay.

Molecular and phylogenetic analysis

DNA was extracted successfully from all the root samples with an average yield of 28 ng μl^{-1}. All samples gave positive PCR products after the nested amplification. Overall 435 good quality sequences were analyzed with blastn without detecting any non-AMF sequence, proving the high specificity of the primers 28G1-28G2 used in the amplification. According to the blastn results 100% of AMF sequences belonged to the family Glomeraceae (Schüßler and Walker, 2010; Redecker et al., 2013). The result is in agreement with several studies conducted on agro-ecosystem where land-use intensification through tillage, fertilizers, biocides and crop rotations negatively affects AMF richness promoting the occurrence of members of Glomeraceae due to their disturbance-tolerant and opportunistic behavior (Ijdo et al., 2010; Lumini et al., 2010; Verbruggen et al., 2012).

After trimming and chimera-checking, three sequences were discarded. The remaining 432 sequences (255 bp long) were pooled with 446 trimmed sequences from a previous analysis of the AMF community in the soil and then clustered by UCLUST in 29 MOTUs according to 94.7% similarity level. The representative sequences for each MOTU were used to build a phylogenetic tree (*Fig. 1*) with the reference sequences identified by blastn.

Considering the relative abundance of the MOTUs and their position in the phylogenetic tree, the genus *Rhizoglomus* (former *Rhizophagus,* Sieverding et al., 2014) resulted the most represented with 73.1% of root sequences and 28% of soil sequences. The genus *Funneliformis* was the second most represented with 8.1% of root sequences and 15.2% of soil sequences, followed by *Septoglomus* (7.6% of root sequences and 6.7% of soil sequences), *Dominikia* (3% and 5.2%), *Sclerocystis* (2.3% and 4%) and *Kamienskia* (0% and 2.5%). The remaining sequences were found in MOTUs related to *Glomus* species of uncertain affiliation clustering basal in the Glomeraceae. According to the phylogenetic tree these MOTUs were divided in four clades (*Glomus sensu lato* group 1-4).

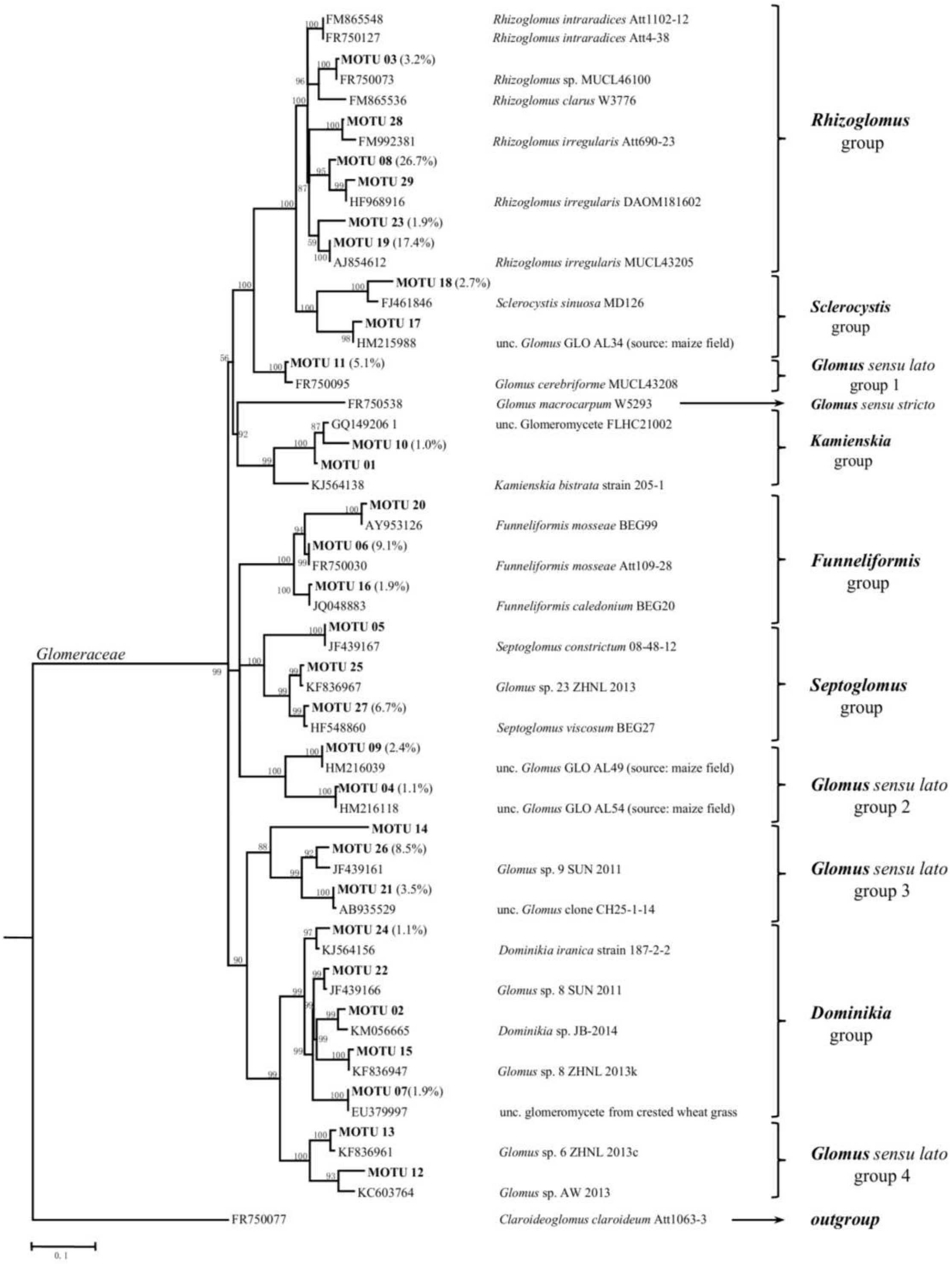

Figure 1. *Maximum likelihood phylogenetic analysis of arbuscular mycorrhizal fungal LSU rDNA sequences. The phylogenetic tree was built using representative sequences of 29 MOTUs, identified by UCLUST at 94.7% similarity level, and 31 reference sequences. In brackets the percentage (when >1) of sequences clustering in the MOTU is reported. Beside the nodes, bootstrap values >50 were reported. MOTUs were grouped according to the related genus when it was possible to determinate. Claroideoglomus claroideum FR750077 was included as outgroup*

AMF communities' structure, diversity and overlap

MOTUs distribution and relative abundance were analyzed for each parcel in each rotation system (*Fig. 2*). To simplify the interpretation of data the analysis was conducted considering the macro-clades illustrated in the phylogenetic tree (*Rhizoglomus* group, *Funneliformis* group...). In the root AMF communities, with the exception of the parcel R11, an overdominance of the *Rhizoglomus* group was observed. The relative abundance of *Rhizoglomus* MOTUs increased substantially from monoculture to Norfolk type root communities. Considering an average between the two parcels per rotation, a relative abundance of 49% was found in maize monoculture, 74% in alfalfa-maize rotation, 75% in wheat-maize rotation and 93.5% in Norfolk type. *Rhizoglomus* species are known to be widespread generalists, detected in a range of habitats, colonizing a number of different plant species (Öpik et al., 2006). Considering the influence of the host specificity in the plant-symbiont interaction (Helgason et al., 2007), the number of crops involved in a rotation could be correlated with a positive selection towards those taxa that, as generalists, represent an "optimal" symbiont for a high number of plant species (Magurno et al., 2014a).

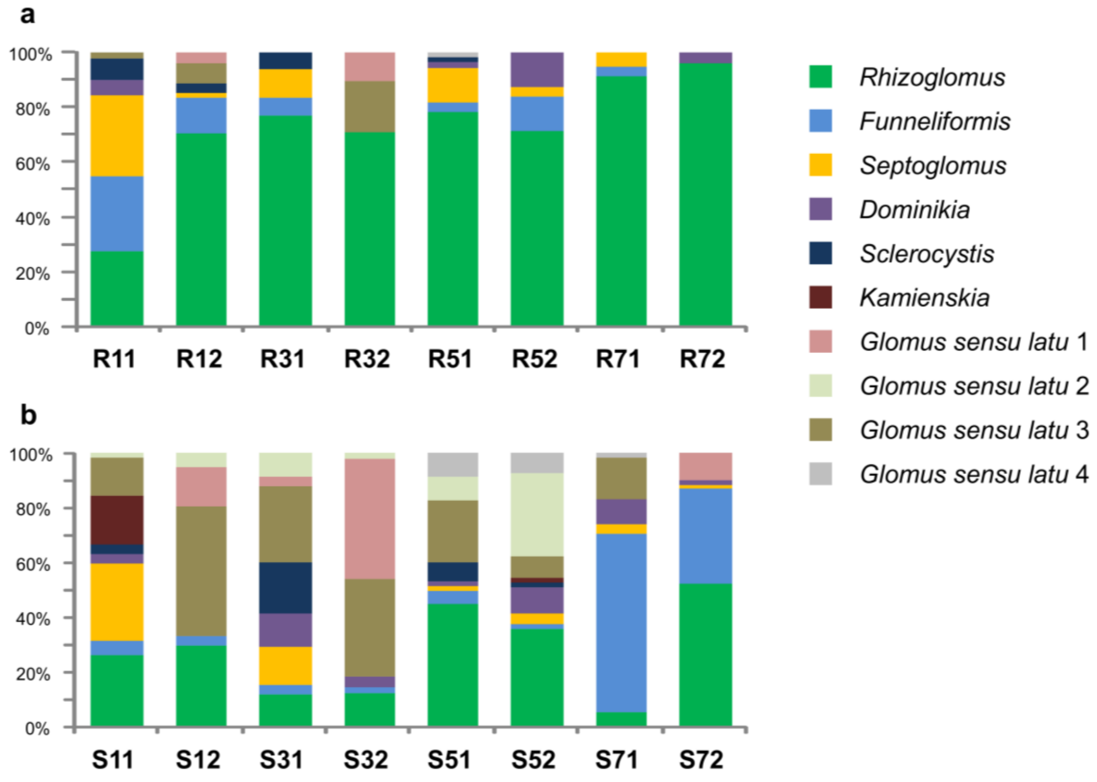

Figure 2. *Distribution of AMF phylotypes detected in the root samples (a) and in the soil samples (b). In the histograms AMF taxa were represented by groups, corresponding to the rank genus, as described in the phylogenetic tree. Crop rotations: maize monoculture (R1, S1), alfalfa-maize (R3, S3), wheat-maize (R5, S5), Norfolk type (R7, S7)*

In the soil AMF communities the distribution of MOTUs was more uniform with the exception of the parcel S71 where the group *Funneliformis* was overdominant. MOTUs belonging to the *Glomus sensu lato* group 4 were well represented in almost all the parcels while in the root AMF communities their presence was less significant.

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To evaluate a possible correlation between the crop rotations and the composition and structure of AMF communities the overlap among the root and soil AMF assemblages was computed by means of a Principal Component Analysis (PCA) (*Fig. 3*). Principal component 1 (35.02%) and 2 (17.86%) explained about 53% of the experimental variance, with a high correlation with the five most abundant MOTUs detected (MOTU08 and MOTU19, *Rhizoglomus* group; MOTU06, *Funneliformis* group; MOTU26, *Glomus sensu lato* group 4; MOTU11, *Glomus sensu lato* group 1). The PCA, supported by a hierarchical clustering on principal components analysis, showed six main clusters. All the root AMF assemblages were found in the same cluster with the exception of monoculture assemblages (R11 and R12) that clustered separately together with S11, S31 and S52. The remaining soil AMF assemblages did not show any correlation between crop rotation type and specific clusters.

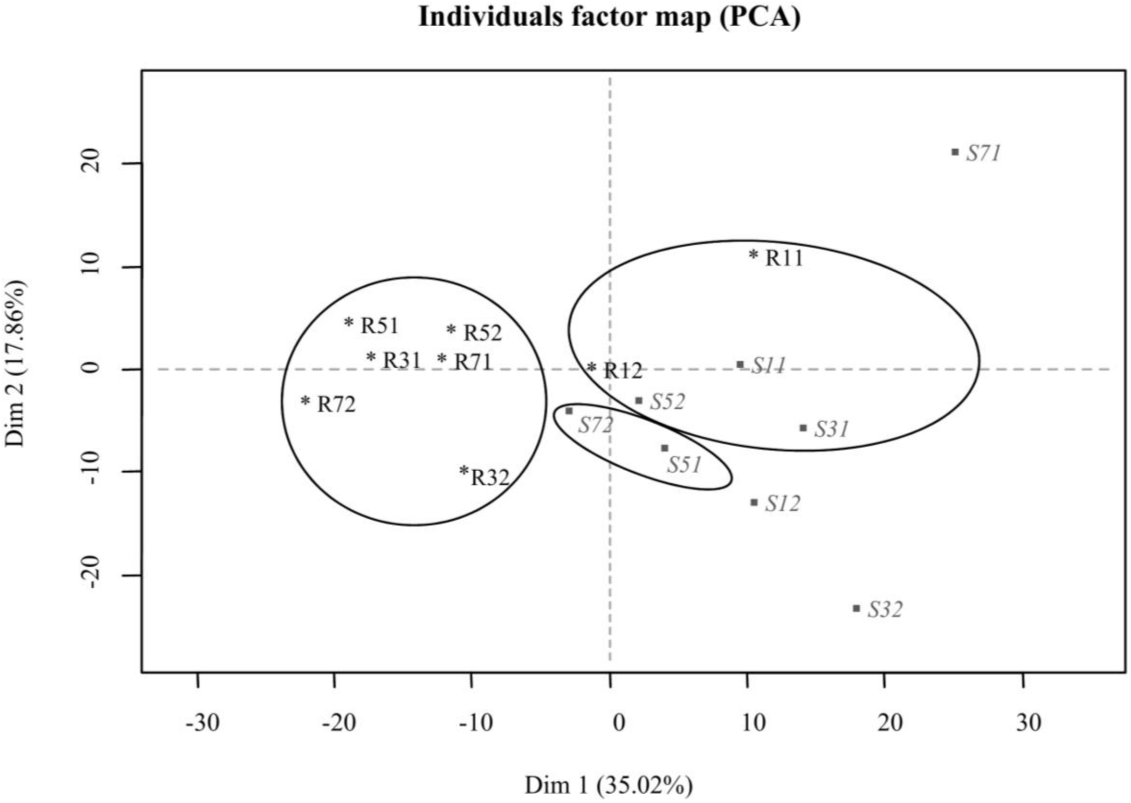

Figure 3. *PCA biplot of the AMF communities found in the soil (asterisk) and in the maize roots (square) for the four different crop rotations. The circles represent the clusters according to the HCPC*

Furthermore a Principal Coordinate Analysis (PCoA) (*Fig. 4*) was computed on the basis of pairwise phylogenetic distances of the assemblages and the soil variables. The analysis confirmed the results of the PCA, highlighting a possible influence of the nitrogen on the position of R11 and S32 on the opposite sides of the plot.

The lack of correlation between treatments and the taxa composition of AMF assemblages could be related to the findings of several studies where, beside niche-based mechanisms (Balestrini et al., 2010; Sasvári et al., 2011), the role of stochastic-neutral processes was highly involved in the shaping of AMF communities (Dumbrell et al., 2010; Lekberg et al., 2012). Analysis conducted in the same experimental field in 2008 have shown on the contrary an influence of the crop rotation on the composition of the AMF community (Magurno et al., 2014a). The discordance with the present results could be explained by the different gene target used and the lower number of clones sequenced in the previous study. With the primers AMV4.5F- AMV4.5F, for example, it was possible to detect one *Rhizoglomus* MOTU vs. the six found in the present analysis.

The PCA and PCoA did not highlight any correlation between the root and soil communities belonging to the same parcel. This result is actually misleading. Because the root AMF community is supposed to be a subgroup of the related soil AMF community, for each parcel the number of MOTUs shared between root and soil communities was divided per the total number of MOTU detected in the root assemblage. The resulting value was used as index of overlapping for the parcel. The percentages of overlapping ranged between 67% and 100%, confirming a high correlation between root and the related soil communities. The relative abundance of MOTUs was not considered because of the “distorted” abundances in the roots due to the overdominance of *Rhizoglomus* MOTUs.

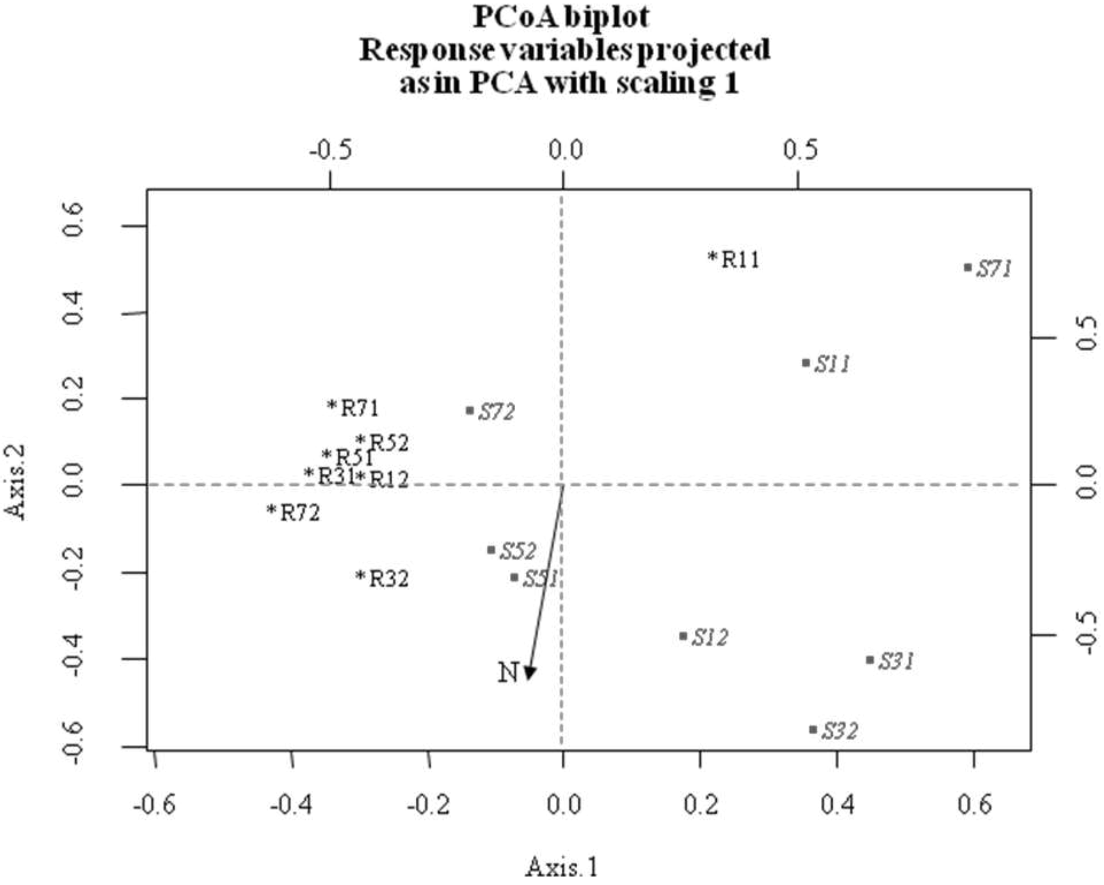

Figure 4. *Principal Coordinate Analysis (PCoA) conducted on the basis of pairwise phylogenetic distances among the AMF assemblages and including the log-transformed soil variables as explanatory variables (only N is shown)*

On the basis of the alfa-diversity predicted by ACE and Chao1 (*Table 2*) it was possible to estimate the level of richness collected by the sampling. On average 88% of the total richness predicted was represented by the MOTUs observed. The biodiversity, as number of MOTUs estimated and Shannon index, decreased strongly from the monoculture to Norfolk type in the root AMF assemblages. Even though less severe, an opposite trend was observed in the soil AMF assemblages, where the highest richness predicted was found in the wheat-maize rotation S5.

Table 2. *Observed versus estimated MOTUs, based on ACE and Chao1 non-parametric richness estimators, Shannon-Weaver diversity indices (H'), standardized effect size of mean pair wise distances (SES-MPD) and the net relatedness index (NRI). The values represent the mean of the two parcels per each rotation type in the root (R) and soil (S) compartments. Monoculture: R1, S1; alfalfa-maize: R3, S3; wheat-maize: R5, S5; Norfolk type: R7, S7*

Crop rotation	Observed MOTUs	SES-MPD	NRI	Estimated MOTUs			O/E[a]	Shannon Index H'
				ACE	Chao1	Average A-C		
R1	9.0	0.14	2.57	11.8	10.7	11.2	80.3	1.8
R3	5.5	0.10	2.74	5.8	5.5	5.6	97.6	1.3
R5	6.5	0.10	2.57	10.0	7.3	8.6	75.4	1.4
R7	5.0	0.06	3.82	5.5	5.0	5.3	95.2	1.1
S1	9.5	0.16	0.23	10.3	9.7	10.0	95.3	1.9
S3	8.0	0.16	-0.22	12.3	11.4	11.9	67.3	1.7
S5	13.5	0.17	0.24	18.1	16.6	17.4	77.8	2.2
S7	8.0	0.12	1.35	12.1	9.5	10.8	74.2	1.5

[a] O/E = % of the total richness sampled according to the MOTUs observed and the MOTUs estimated (average value between the two non-parametric richness estimators ACE and Chao1)

Regarding the standardized effect size of mean pairwise distance (SES-MPD) (*Table 2*), explaining the phylogenetic diversity of taxa inside an AMF community, for root AMF assemblages the index decreased from monoculture (R1, S1) to Norfolk type (R7, S7). In the soil community the values, as expected, were higher than the root communities because of wider range of MOTUs detected. Among the soil communities, a similar value of SES-MPD was found among all the treatments with the exception of the Norfolk type assemblages where it was lower.

The net relatedness index (NRI) gives a measure of the clustering or segregation of the AMF community. NRI was positive for all the root assemblages and the highest value was detected in the Norfolk type treatment. In the soil communities the values were lower compared to the former.

Monoculture (S1) and wheat-maize rotation (S5) shared a similar NRI value (0.23; 0.24) while a negative NRI was found for alfalfa-maize rotation (S3) (-0.22). On the other hand the Norfolk type rotation showed a high positive value (1.35).

The level of relatedness among taxa in the AMF assemblages and the indices of richness considered could provide a key to interpret the effect of different crop rotations on the AMF community. The maize monoculture was found to host in the soil and root compartment a high diversity both as number of taxa predicted and level of phylogenetic diversity SES-MPD. While neutral processes could have played a major role on the composition, a significant community clustering, as observed particularly in the root assemblages, might be evidence of positive ecological interactions (Vamosi et al., 2009), represented, in this case, by a long term stable relationship among host and AMF community. Compared to monoculture, the wheat-maize rotation root AMF community was characterized by a lower alfa-diversity (taxa predicted and SES-MPD) with a similar level of clustering. In the soil assemblages the number of taxa estimated was significantly higher than in monoculture while SES-MPD and NRI were similar.

Assuming preferential host-symbiont associations as an environmental filter, the two crops involved in the rotation, wheat and maize, could have selected, in the long term, their own AMF communities, inducing an overall increase of the richness in the soil. As a consequence at root level the “host preferential” AMF community would have taken advantage in the colonization causing a reduction in the diversity. It would be expected to find, at soil level, a community overdispersion (assemblages made up of distantly related organisms) but the NRI value was similar to that calculated in monoculture. A possible explanation could rely upon the fact that maize and wheat belong to the same family (Poaceae) and thus they could have similar “criteria” in the selection of preferential AMF communities. This hypothesis could explain the alfalfa-maize rotation outcomes where a lowly diverse but clustered community was detected at root level, while a richer and slightly overdispersed community was present in the soil. In this case alfalfa and maize, given their long permanence in the field (3 years alfalfa, 5 years maize), would represent a reduced niche overlap for the related AMF communities with a negative effect on the overall soil community clustering (Cavender-Bares et al., 2009). In Norfolk type treatment, wheat was present instead of maize at the sampling times. Even considering the possible influence of a different host on the structure of the AMF community, the data about root and soil assemblages were fitting with the “preferential associations” model proposed. In the root assemblages the richness and phylogenetic diversity were indeed extremely low while the level of community clustering was the highest among all the assemblages due to strong relatedness among the taxa detected. In the soil (S7) the richness predicted was slightly higher compared to the monoculture but the SES-MPD value was the lowest among all the soil assemblages. Unlike the alfalfa-maize rotation, in the Norfolk type permanence in the field of the four crops in rotation was limited to one year per crop. Instead of an overdispersion for reduced niche overlap, the fast succession of crops promoted the selection of a clustered AMF generalist community, characterized by a number of predicted taxa comparable with the monoculture but with the highest relatedness among the taxa. Indeed the dominant MOTUs found in S7 were related to the generalist taxa *Funneliformis mosseae* and *Rhizoglomus irregularis* (Oehl et al., 2010).

Conclusions

In conclusion the analysis of the AMF communities associated to different crop rotations did not reveal any influence of the treatments on the composition of the communities, where stochastic processes play probably a major role. At the same time the results were in agreement with Magurno et al. (2014a), about a gradient of diversity in the root communities, decreasing from monoculture to Norfolk type rotation.

Furthermore, considering root and soil communities, the analysis of the phylogenetic diversity and the index of relatedness, shined a light on a possible model to interpret the correlation between rotation system and structure of AMF communities.

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