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CONTENTS

ASSESSMENT OF SOIL EROSION ON HILLSLOPES (A CASE STUDY CARRIED OUT IN THE ASHAN DRAINAGE BASIN, IRAN)

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ABSTRACT

The objective of this study is to determine the rate of soil erosion on slopes of differing steepness and its effects on agricultural land and pastures in the drainage basin around Ashan. Exogenous factors like water and wind and endogenous elements such as erodibility of the soil have key roles in erosion and the results of this study will help in the management of soil and soil conservation programs. Soil erosion in the drainage basin around Ashan has accelerated and because of this it is important to determine the erodibility of the soil. In this study, the soil on four different hill slopes was sampled and after drying, soil size distribution and soil texture and the organic content of forty samples, and the k-factor (erodibility) using the USLE equation, were determined. According to the results of the ANOVA test there are strong relationships between the variables, which is illustrated by box plots. The results indicate that erodibility is significantly ($p <$ 0.05) associated with the type of land use and landforms. The highest levels of erosion were recorded on the back-slope and the least at the summit and on the toe-slope. Discriminant function analysis was used to determine the discriminatory power of the erodibility factor associated with the different uses of land and landform components. According to the DFA results, the K factors indicate the use of the land and landforms were the most significant factors, with significances of 0.000 and 0.002, respectively.

Keywords: erodibility, k-factor, Ashan drainage basin, Universal Soil Loss Equation

Introduction

Erosion is closely associated with an irreversible reduction in the amount of soil and its productivity and results in irreparable damage to the environment. In Iran, 8 to 16 tons of agricultural soil are annually made useless due to erosion. Erosion rates reached 800 percent between 1951–2001 (Nosrati 2011). The accumulation of sediment behind dams equals about 120 million hectares per year, indicating a decrease in the lifetime of dams of about 1 to 2% (IWRM 2009). For the prevention of soil erosion, therefore, it is necessary to reduce the rate of erosion as it is considered to be one of the most critical factors in terms of protecting and managing natural resources (Agassi 1996). This requires the recognition of the factors that contribute to erosion, prediction of the degree of soil erosion and the provision of proper solutions.

Many models can predict soil erosion, among which the Universal Soil Loss Equation (USLE) is the most common and widely used to estimate the amount of erosion by water. According to this model, one of the six factors affecting this kind of erosion is the erodibility of soil. This model includes: soil loss (A), a rainfall erosivity factor (R), soil erodibility factor (K), length of the hillslope (L), gradient (S), vegetation (C) and conservation practices (P) (Renard et al. 1997).

According to Veihe et al. (2003), erodibility of soil is the inherent sensitivity of soil to erosion and the separating of soil particles due to the kinetic energy of rain and their transport by rain water. Based on the results of extensive studies in various parts of the world, soil erodibility is determined by five soil characteristics, namely the percentage of sand, total percentage of very fine silt and sand, organic matter content and permeability of the soil profile (Veihe et al. 2003). In the vegetated areas of the planet, geomorphic and hydrologic processes, which involve the transport of hillslope sediment, are very dependent on soil properties. Such effects are recognized in the theoretical paper of Chorley (Chorley 1959), but in practice, the dynamic and complex effects of the properties of shallow soil on the removal of sediments by water from hillslopes are neglected by geomorphologists. Although the hydraulic conditions determine the surface flow of erosive forces working on the soil, soil properties change these conditions and finally they all contribute to the uneven terrains (hillslopes) (Bryan 1991). The K-factor reflects the soil separability during rainfall or surface flow and soil shift per unit of soil caused by external forces. This factor is related to the combined effect of rainfall, runoff and soil permeability and is a consequence of the effect of soil properties on soil loss due to rain and is derived by using the USLE nomograph (Wischmeier 1978). However, the effect of soil properties on erodibility in different parts of a hillslope with various aspects and different usages in different parts of the world is unknown and therefore more research is required in this field.

There are abundant deposits behind the Alavian Dam in the Ashan drainage basin north of the city of Maragheh (Fig. 1), which are the result of soil erosion. However, conservation management needs to know what the resistance of soil is to erosion, that is, the erodibility of soil, which in this region is unknown; given the above facts, identifying the erodibility (k) in different parts of hillslopes subject to various kinds of land use is one of the main goals of this study that aims to reduce the accumulation of sediment in the reservoir and detect the spatial variations in soil erosion in the Ashan drainage basin.

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Bennett (1926) started research on soil properties related to erosive resistance in Cuba and Middleton (1903) formulated the concepts of erodibility and provided two erodibility indexes, which incorporated runoff characteristics and soil particle separability, which are dependent on the behaviour of soils in California (Middleton 1903; Bennett 1926), but from 1960 to the present, numerous studies (Smith and Wischmeier 1962; Bryan 1968; De Ploey 1985; Romkens 1985; Lal 1990; Bryan 1991) have tested, developed, replaced or corrected these indexes as universal indexes of erodibility. In the research done by Kirkby and Morgan (1980), the effect of mineral particles on soil erosion is demonstrated (Kirkby and Morgan 1980). Young et al. (1990) review the data from US Agricultural Stations and conclude that the K-factor is at a maximum following snow melt and decreases markedly at the end of the growing season. According to their results, the effects of the physical and chemical characteristics of soil and other parameters such as soil depth and vegetation on the rate of soil erosion is dependent on the relationship between these elements. Ghoddousi and Ghaderi (2005) in their study on the Telvarchay drainage basin in Kurdistan conclude that soil erosion increases with increase in the clay to sand and silt ratio (Ghaderi and Ghoddosi 2005). On the other hand, studies in Iran indicate a strong relationship between soil erosion and soil management (Bahrami et al. 2005), soil texture and organic matter and organic matter content and lime content (Nosrati et al. 2011).

Fig. 1 Sediments behind the Alavian Dam at the exit of the Ashan drainage basin.

Materials and Methods

Study area

The present research was conducted in the Ashan drainage basin, which covers an area of 320 square kilometers in the County of Maragheh in South East Azerbaijan (Fig. 1). According to the Demarton climate classification, this region has a cold and dry climate (climatology map of Iran, 2014). The region has an average annual rainfall of 360 mm and average annual temperature of 7.8–12.5 °C. The city covers an area of approximately 840 square kilometers and only accounts for 8.1%

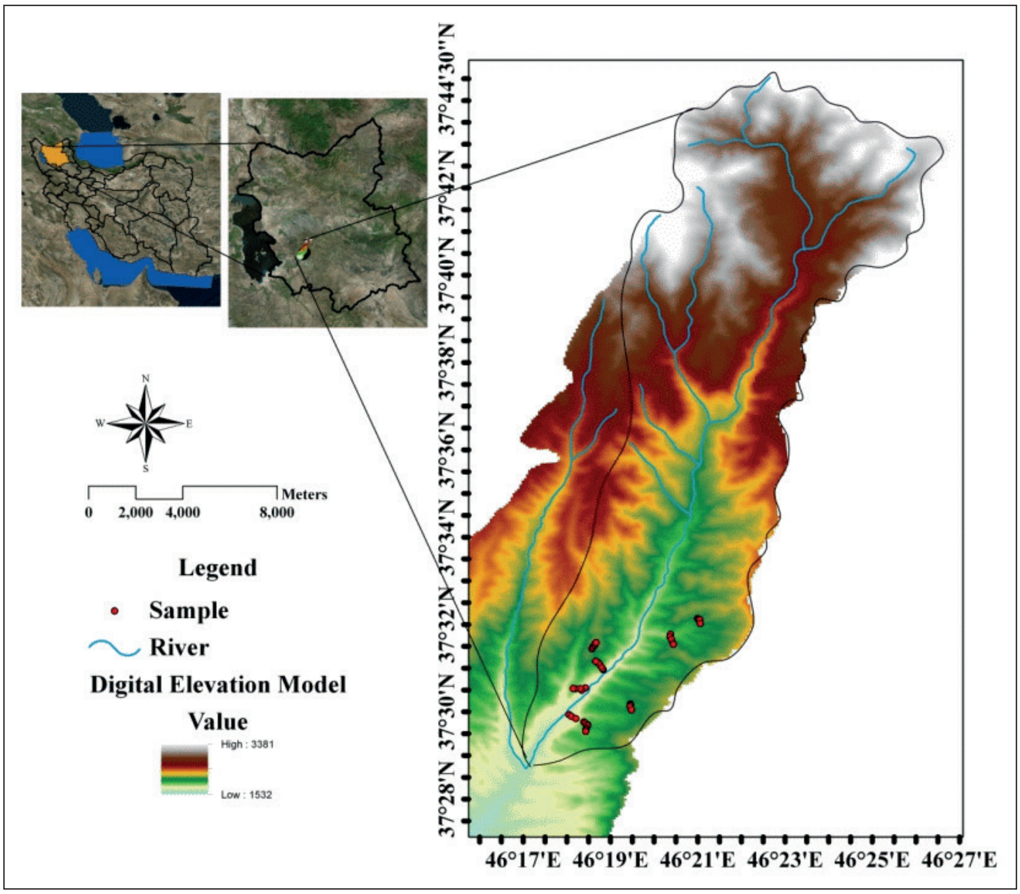

Fig. 2 Geographical location of Ashan drainage basin (National Cartography Organization of Iran 2011).

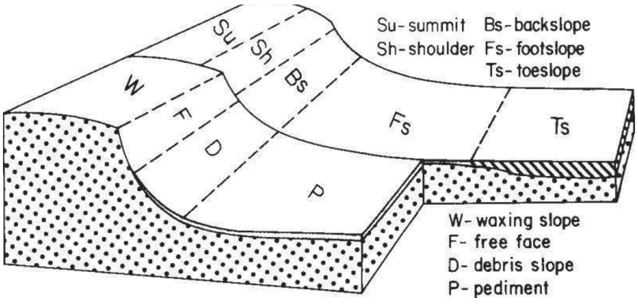

Fig. 3 Landform positions of a hill-slope (Ruhe 1960).

of the total area of the province. The altitude in the region is 1290 m above sea level in the west and up to 2000 m in the north and most of the region is at an altitude of less than 1700 m. Total area of cultivated land in the study area is 60 km^2. Dry farming is the dominant agriculture method in this region. In terms of agrology, soils in the region fall within the range of Loam-Clay Loam with an electrical conductivity of 2–16 decisiemens.

Sampling and laboratory analysis of soil samples

Maps of the geographical location, topography and land use were prepared using Geographical Organization maps with a scale of 1: 50,000 (Fig. 2). Since soil erosion is affected by land use and erodibility is not independent of the land use and is influenced by the amount of organic matter, soil structure and permeability (Nosrati et al. 2011), 40 soil samples were collected in a random way. Soils were sampled at a depth of 15 cm and collected from agricultural and pastoral land on four hillslopes. Five positions on the hillslopes with four different aspects based on the classification of Ruhe (1960) (Fig. 3) were sampled (Table 1). The positions were the summit, shoulder, back slope, foot slope and toe slope. The erodibility of the soil was determined as mentioned in treatments. The sampling at the five positions on the hillslopes started on October 30, 2014 and ended on November 6, 2014. In the sampling process GPS was used to determine the exact location at which a sample was collected (Fig. 4). The parameters recorded during sampling were: latitude and longitude in degrees, minutes and seconds, altitude above sea level, land use, hillslope aspect, position on hillslope, local name of the location and date sampled. The samples were taken to the laboratory.

In the laboratory, samples were first dried in an oven at 120 °C for 24 hours. After drying, they were pressed and then weighed. In order to determine the content of very fine sand, one of the parameters required for the calculation of the K-factor, a sieve-shaker device with a 2 mm mesh size was used. The soil texture (clay (< 0.002 mm), silt (0.002–0.05) and sand (0.05–2 mm)) were determined

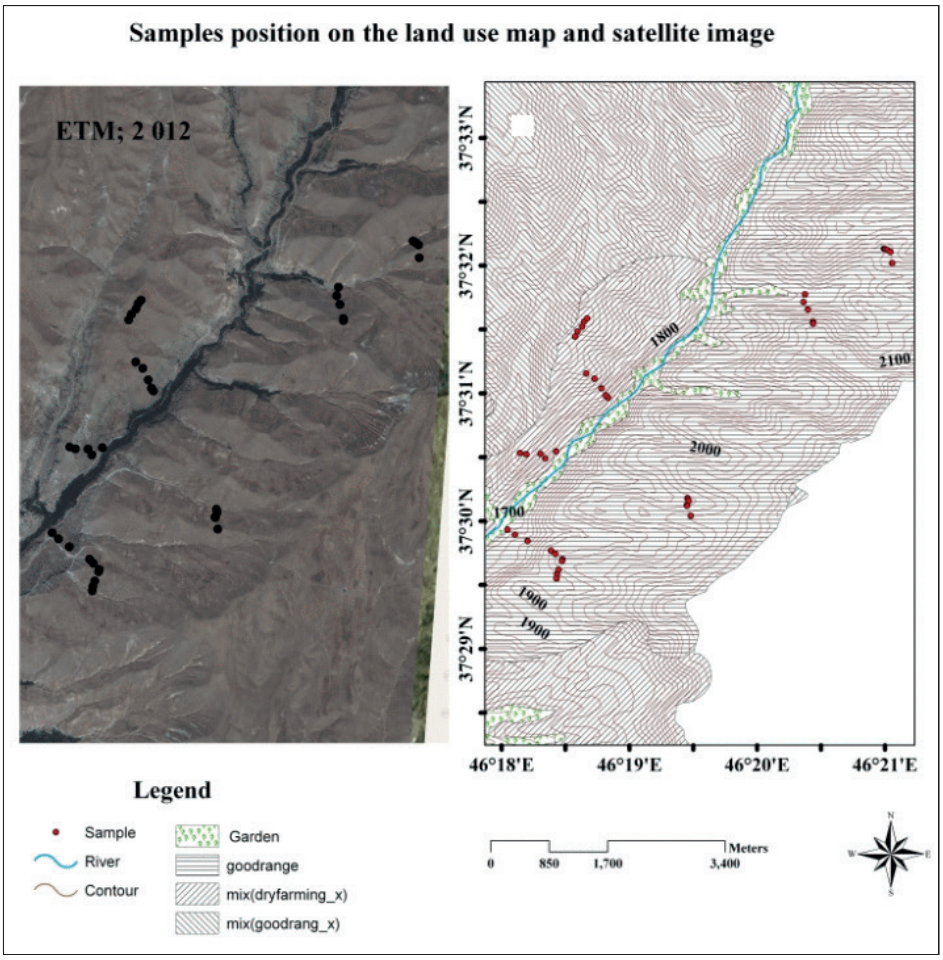

Fig. 4 Locations for the samples collected from the Ashan drainage basin.

Tab. 1 The laboratory analysis of the soil samples collected from different positions on hillslopes subject to different land uses in the Ashan drainage basin.

Land use	Direction	Hill slope position	Local name	Sampling date	Clay %	Silt %	Sand %	Very fine sand %	Structure code	Organic mater %	Texture code	K factor
Agriculture	North	summit	Ashan	06/11/2014	9.28	35.28	55.44	16.63	1	1.99	2	0.29
Agriculture	North	shoulder	Ashan	06/11/2014	10.56	42.72	46.72	14.02	1	1.1	4	0.35
Agriculture	North	Back slope	Ashan	06/11/2014	25.28	38	36.72	11.02	4	0.01	6	0.36
Agriculture	north	Foot slope	Ashan	06/11/2014	8.16	27.44	64.4	9.32	2	0.63	3	0.25
Agriculture	North	Toe slope	Ashan	06/11/2014	27.28	41.64	31.08	9.32	2	0.85	2	0.27
Agriculture	South	summit	Ashan	03/11/2014	15.28	18	66.72	20.02	3	1.915	3	0.24
Agriculture	South	shoulder	Ashan	03/11/2014	24.72	18	57.28	17.18	4	0.432	3	0.26
Agriculture	South	Back slope	Ashan	03/11/2014	35.28	24	40.72	22.22	4	0.011	6	0.30
Agriculture	South	Foot slope	Ashan	03/11/2014	39.28	30	30.72	13.22	3	0.735	4	0.22
Agriculture	South	Toe slope	Ashan	03/11/2014	17.44	36.56	46	13.80	2	1.5	3	0.29
Agriculture	East	summit	Ashan	01/11/2014	15.28	24	60.72	18.22	2	0.975	2	0.26
Agriculture	East	shoulder	Ashan	01/11/2014	20.72	12.72	66.56	32.97	4	0.495	4	0.34
Agriculture	East	Back slope	Ashan	01/11/2014	20.72	14	65.28	35.58	4	0.275	5	0.37
Agriculture	East	Foot slope	Ashan	01/11/2014	11.28	12	76.72	23.02	2	0.625	4	0.23
Agriculture	East	Toe slope	Ashan	01/11/2014	16.56	11.84	71.6	21.48	3	0.825	3	0.23
Agriculture	West	summit	Ashan	01/11/2014	23.44	24.56	52	15.60	3	0.755	3	0.26
Agriculture	West	shoulder	Ashan	01/11/2014	16.16	30	53.84	16.15	4	0.59	4	0.36
Agriculture	West	Back slope	Ashan	01/11/2014	18	18.72	63.28	28.98	4	0.19	6	0.37
Agriculture	West	Foot slope	Ashan	01/11/2014	18.56	9.84	71.6	21.48	3	0.645	3	0.26
Agriculture	West	Toe slope	Ashan	01/11/2014	19.28	10	70.72	21.22	3	1.875	2	0.19
Range	North	summit	Heris	30/10/2014	18.2	19.64	62.16	18.65	1	1.99	2	0.17
Range	North	shoulder	Heris	30/10/2014	14.16	24	61.84	16.55	2	1.58	3	0.24
Range	North	Back slope	Heris	30/10/2014	18.56	16.16	65.28	27.58	3	0.64	4	0.30
Range	North	Foot slope	Heris	30/10/2014	16.4	24.32	59.28	17.78	2	1.3	3	0.25
Range	North	Toe slope	Heris	30/10/2014	14.4	13.44	72.16	18.65	2	1.79	2	0.18
Range	South	summit	Ashan	01/11/2014	23.44	12.72	63.84	19.15	2	1.7	2	0.16
Range	South	shoulder	Ashan	01/11/2014	11.44	28.72	59.84	6.95	3	1.5	3	0.25
Range	South	Back slope	Ashan	01/11/2014	8.16	34.72	57.12	7.14	2	1.325	4	0.27
Range	South	Foot slope	Ashan	01/11/2014	29.84	18.56	51.6	25.48	2	1.525	3	0.21
Range	South	Toe slope	Ashan	01/11/2014	20	33.44	46.56	8.97	1	1.815	2	0.19
Range	East	summit	Ashan	03/11/2014	15.28	24	60.72	18.22	1	2.05	2	0.20
Range	East	shoulder	Ashan	03/11/2014	8	22.56	69.44	20.83	1	1.9	5	0.24
Range	East	Back slope	Ashan	03/11/2014	49.44	21.28	29.28	28.78	3	0.415	4	0.22
Range	East	Foot slope	Ashan	03/11/2014	46.72	21.28	32	25.60	2	1.45	3	0.17
Range	East	Toe slope	Ashan	03/11/2014	19.44	30.16	50.4	5.12	2	2.815	2	0.17
Range	West	summit	Heris	02/11/2014	26.72	18	55.28	16.58	2	1.998	3	0.16
Range	West	shoulder	Heris	02/11/2014	35.44	5.28	59.28	27.78	4	1.65	3	0.20
Range	West	Back slope	Heris	02/11/2014	49.44	14	36.56	39.97	3	0.44195	4	0.23
Range	West	Foot slope	Heris	02/11/2014	19.44	32	48.56	10.57	2	2.585	3	0.21
Range	West	Toe slope	Heris	02/11/2014	45.28	24.72	30	29.00	1	1.905	2	0.16

in the laboratory using the hydrometer method (Bouyoucos 1962), which is very accurate (Hank and Ashcroft 1970). Another parameter required for determining soil erosion, is organic carbon, which was obtained using the Walkie-Black method (Skjemstad and Baldock 2007). In this method, particles smaller than 2 mm were used for determining the organic carbon in soil and soil particles larger than 2 mm were weighed, divided by the total weight of the soil and then multiplied by 0.3 in order to determine the quantity of very fine sand in each sample. The erodibility values were analyzed using ANOVA and SPSS software and map processing was done using ArcGIS software.

Soil erodibility factor (K)

The measurement of erodibility is a sophisticated process because sensitivity of soil to erosion can vary depending on environmental conditions and many other factors such as physical, chemical, mineral, and biological properties, vegetation and depth of the soil. On the other hand, direct measurement of erodibility requires long term data, which is time-consuming but there are many ways of determining erodibility using more easily available soil properties (Zhang et al. 2008). The properties of soil included in the erodibility factor (K-factor) are organic material, stability of soil particles, soil permeability and chemical composition, which can be measured and are indicators of soil erosion (Zhang et al. 2007). One of the most common experimental models that is widely used to investigate the amount of erosion by water is the USLE Model; this model is used to estimate soil loss from farmland due to surface and rill erosion (Schiettecatte et al. 2008). In this model, the erodibility is estimated based on the coarse sand, silt and very fine sand, organic matter, structure and permeability of soil (Wischmeier et al. 1978).

Soil erodibility is determined using the K-factor and intrinsic properties of the soil.

K-factor formula is expressed in equations (1) and (2) below (Wischmeier et al. 1978):

$$k = (0.00021 \times M^{1.14} \times (12 - a) + 3.25 \times (b - 2) + \\ + 3.3 \times 10^{-3} (c - 3))/100 \tag{1}$$

$$M = (\%\ \text{Silt} + \%\ \text{very fine sand}) \times (100 - \%\ \text{clay}) \tag{2}$$

where: M is particle size, a is percentage of organic matter, b is soil structure code where sand (1 = <5%; 2 = 5–15%; 3 = 15–50%; 4 = >50%), c is permeability of soil profile in saturated hydraulic conductivity. In terms of permeability, 1 = fast (150 mm per hour), 2 = moderate or fast (50 to 150 mm per hour), 3 = moderate (12 to 50 mm per hour), 4 = low to moderate (5 to 15 mm per hour), 5 = low (1 to 5 mm per hour), 6 very low (1 mm per hour). The soil particles are graded based on their size: for fine sand particles 0.05–0.10 mm, for silt 0.002–0.05 mm, and for clay 0.002 mm, organic matter content is calculated using the organic carbon content and a constant of 1.72. Following the measurement of all these parameters in the laboratory, the relevant values are placed in equation 1 and soil erodibility is obtained.

Results and Discussion

Two-way ANOVA statistical test was used to determine the significant differences among groups shown in Table 1. Given what was described earlier, these tests have a high power in showing within group and among group differences in erodibility. With respect to Table 2, the two-way ANOVA test revealed a significant difference in the data.

In Table 2, the results of the analysis confirm that there is erosion associated with both land use and landform both are less than 0.000, which indicate a significant difference. According to Fig. 5a, mean quantity of silt on agricultural land is higher than on pastures. Very fine sand on agricultural land is the same as on pastures. But percentage of very fine sand is higher on the back slopes. Fig. 6 indicates the percentage of organic matter recorded in soil from areas with different land uses. There is little organic matter (%) on the back slopes subject to both land uses (Fig. 6). Organic matter (%) in pastures is higher than in cultivated land. Silt (%) is higher in agricultural land than in pastures with low erodibility. The results of the discriminant function analysis (DFA) recorded in Table 3 indicate the discriminatory power of land use and landform components in terms of erodibility. According to Table 3, the K factor is able to discriminate between differences in land use (Agriculture, Pasture) and landform components with a significances of 0.000 and 0.002, respectively. Aspect as a treatment was not discriminated by the DFA and erodibility did not separate different aspects from each other.

Table 2 Two-way ANOVA of soil erodibility factors (K) associated with land use and landform.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Mxxodel	0.119[a]	9	0.013	14.071	0.000	0.808
Intercept	2.440	1	2.440	2596.128	0.000	0.989
Land use	0.058	1	0.058	61.447	0.000	0.672
Landform	0.055	4	0.014	14.665	0.000	0.662
Land use * landform	0.006	4	0.002	1.633	0.192	0.179
Error	0.028	30	0.001			
Total	2.588	40				
Corrected Total	0.147	39				

[a] R Squared = 0.808 (Adjusted R Squared = 0.751)

Table 3 Discriminant function analysis of soil erodibility factors (K) associated with land use, aspect and landform.

Test of functions	Wilks lambda	Chi square	Sig
Land use	0.608	18.67	0.000
Aspect	0.96	1.23	0.74
Landform components	0.62	16.89	0.002

Soil properties

Texture and structure of soils

Soil texture is determined by the percentage by weight of sand, silt and clay particles in the soil. Size of sand particles are about 0.05–2 mm, silt particles about

0.05–0.002 mm, clay particles are smaller than 0.002. Soils with medium textures (loams) are highly erodible due to the large amount of silt and very fine sand and small particles (silts and clays) they contain, which are easily transported by water (O'Geen and Schwankl 2006). When measuring soil erodibility, silt and clay content are the main elements in the distribution of particle size. With increasing clay, the viscosity and resistance of soil particles are enhanced and the soil becomes more resistant to erosion. Soils with a high sand content are also more resistant to erosion because sand increases soil porosity and improves soil permeability. A soil with a high volume of silt has little resistance to erosion and is therefore susceptible to erosion (Thang 2002). According to Fig. 5b, there is less very fine sand at the summits than at other positions on the hillslopes; on the other hand, average percentage of very fine sand in foot-slope soils is higher than in soils at other positions on hillslopes. In Fig. 5b, a particular trend is seen in the amount of very fine sand, which occurs in both agricultural and pastoral land. The parent material in this region is divided into two parts a lower and upper: the lower part contains a sequence of tuffs and altered tuffs, sand and compressed sand and the upper part is much thicker and contains tuff marl, tuff, siltstone, volcanic stones, conglomerate and river sediments and rounded rubble and cross bedding. Standard deviations for both shoulder landforms is the lowest; while in the foot-slope landform, both average and standard deviations are higher than for other landforms. According to Fig. 7, the erodibility of back slope landform is higher than other landforms and this trend is repeated in both agricultural and pastoral land. According to Fig. 5b, the percentage of very fine sand in back slope soils under both land uses is the highest and this is regarded as a good indicator of the relationship between erodibility and very fine sand. Fig. 5 reveals that the average for all pastoral land is less than for agriculture land. The conditions on hillslopes can differ considerably over short distances, which reflects the complexity of the relations among factors.

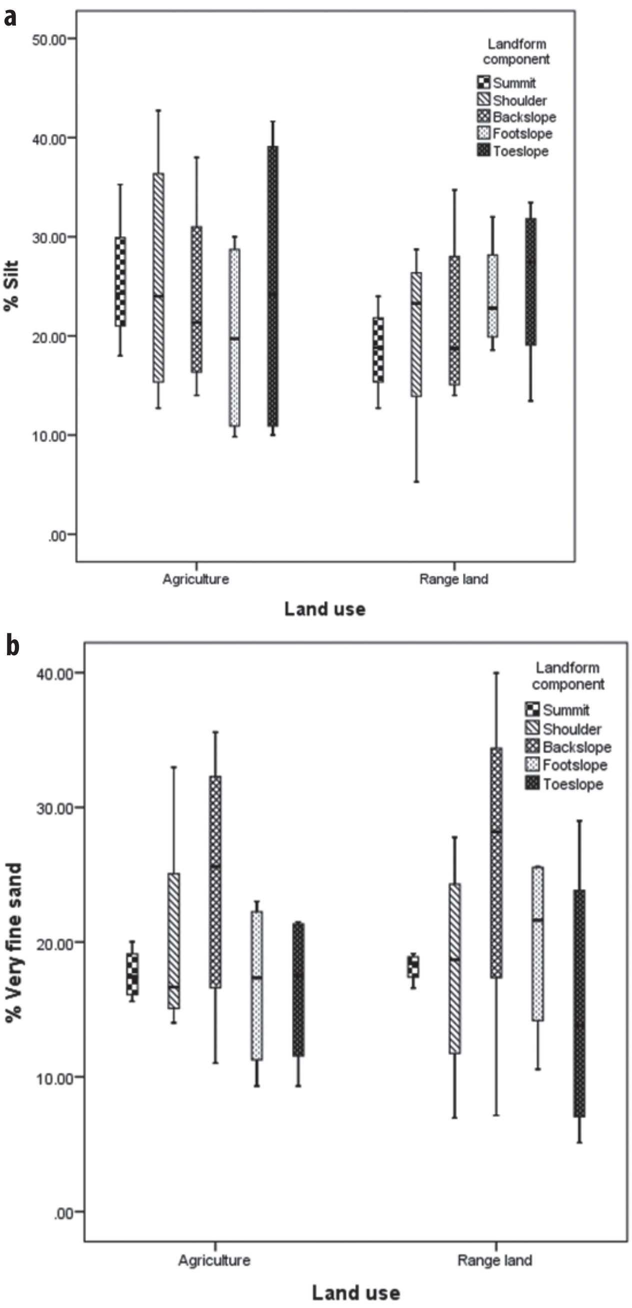

Fig. 5 Box plot of silt in soil (a) and very fine sand (b) collected at different positions on hillslopes.

Soil permeability

The terms permeability and permeation have many different definitions. Permeation describes the diffusion of water into soil while permeability is the ease with which water or any liquid move inside soils (O'Geen and Schwankl 2006). According to the studies of Wischmeier and Mannering (1969), there is a simple linear regression between permeability, runoff, amount of soil in runoff and loss of soil for soils with various properties; therefore, permeability has a critical role in the erosion of soil (Wischmeier and Mannering 1969). In Fig. 5a, the silt content of soils from agriculture land in all 5 landforms was higher than in pastoral land; so the rate of erosion of agriculture land is estimated to be higher than that of pastoral land. Foot slope and toe slope soils used for agriculture have the least and the highest content of silt, and summit and toe slope soils used for pasture have the least and highest silt content, respectively (Fig. 5a). Permeability of soil with silt is less than soil with very fine sand and therefore subject to greater erosion. Meanwhile, according to Figs. 5a and 7a soils with a high silt content, such as toe slope and back slope soils, are at high risk of erosion. Scientifically speaking, soils that have a high silt and low organic matter content, in contrast, have high erodibility. The percentage of silt, clay and sand reflects the current status of the physical and chemical properties of the soils and very fine sand particles (0.1–0.05) have a similar behaviour to silt during erosion (Wischmeier and Mannering 1969). Experimental analysis of the soil samples revealed that the soils in the drainage basin have a clay-loamy, silty-loamy and silty-loamy-clay texture

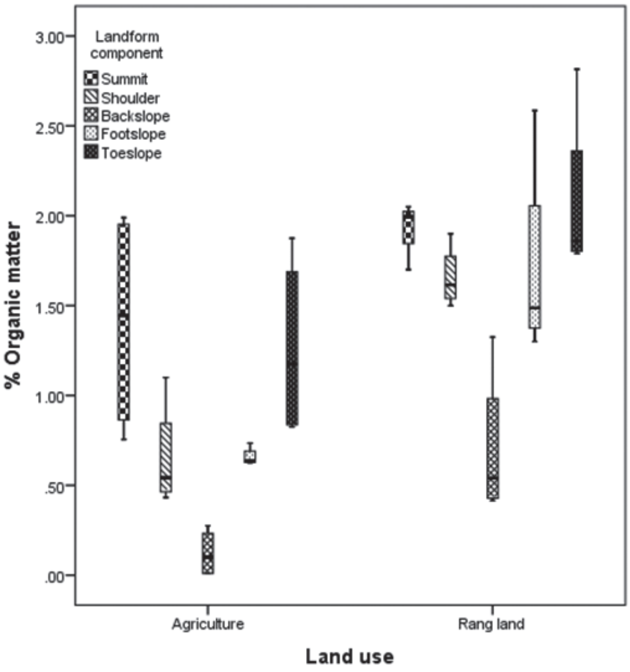

Fig. 6 Box plot of the organic matter in soils collected at different positions on hillslopes.

generally (Table 1). Consequently, the highest permeability was recorded for foot and toe slope soils, which is consistent with the erodibility results in Fig. 7a.

Soil organic material

Organic carbon storage is one of the valuable natural resources that determines the physical, chemical and biological processes in the soil and improves soil quality (Carter 2007). The depletion of soil organic carbon results in deterioration of the soil, increase in erosion and decrease in production. Organic matter in the soil prevents the breakdown of soil aggregates, decreases soil erodibility, increases water-holding capacity, increases soil permeability and improves soil structure. Dynamics of soil organic carbon is associated with the distribution of nutrients and transport of sediments both temporally and spatially (Schiettecatte et al. 2008). Nutrients such as phosphorous can be leached from a drainage basin together with sediments by river processes, and plant material and humus can affect soil erosion by providing a protective layer against the erosive effects of rain drops and the drainage processes; thus, understanding the spatial and temporal dynamics of soil organic carbon and dynamics and sedimentation of soil organic carbon is important (Van Oost et al. 2005). On the other hand, the location and properties of the land with respect to the curvature and slope of the hillslopes is a critical factor in the variability of soil organic carbon (Li et al. 2007). As in Fig. 6, the maximum amount of organic matter was recorded in the soils at the summit and in toe slopes, and the lowest value in back slope soils; these results are consistent with the findings of Li and Luo 2006 and Li et al. 2007. The carbon concentration in the topography, therefore, can be modelled and the results of this study confirms the above fact. According to the results, the K factor has a polynomial relationship with organic matter. The equation extracted from this relationship is given below and has an R^2 of 0.87.

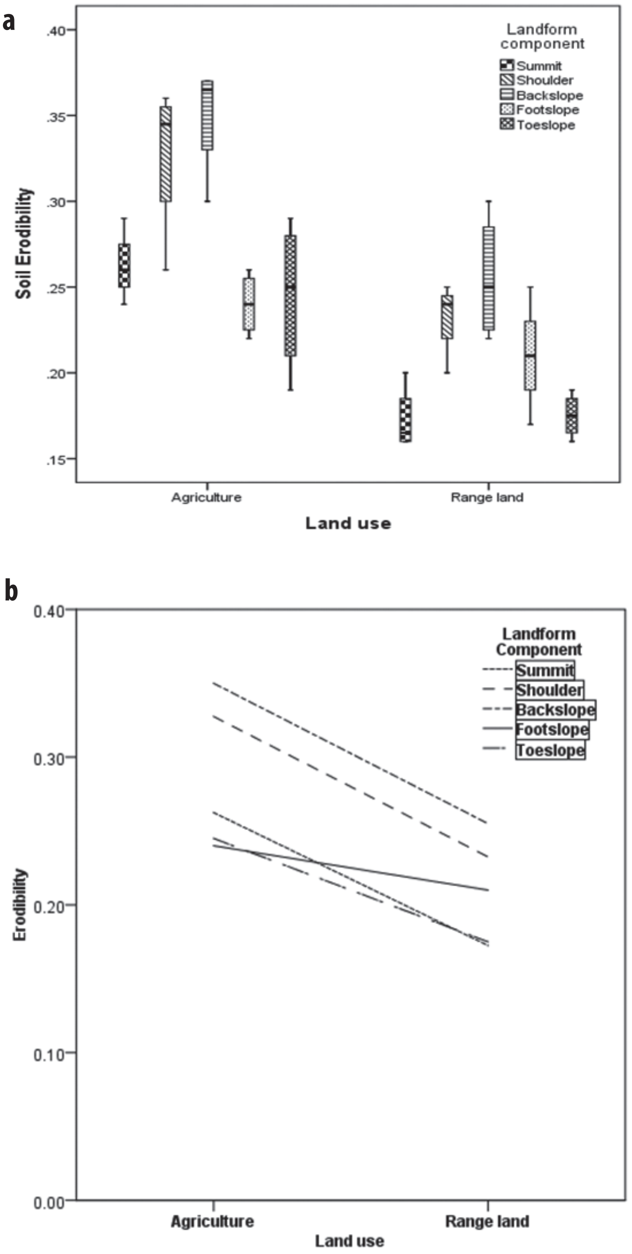

Fig. 7 Box plot of k factor with land use and position on hillslopes (a) and (b).

$$y = -0.0576x + 0.3174 \quad (3)$$

In the above equation, x is organic carbon and y is erodibility. In this equation, coefficient x is negative; this means that increasing organic matter reduces erodibility of soils. Equation (3) is based on samples from a particular area and the results may not be relevant to other regions. The photograph of this region support the results of this research (Figs. 8a and 8b).

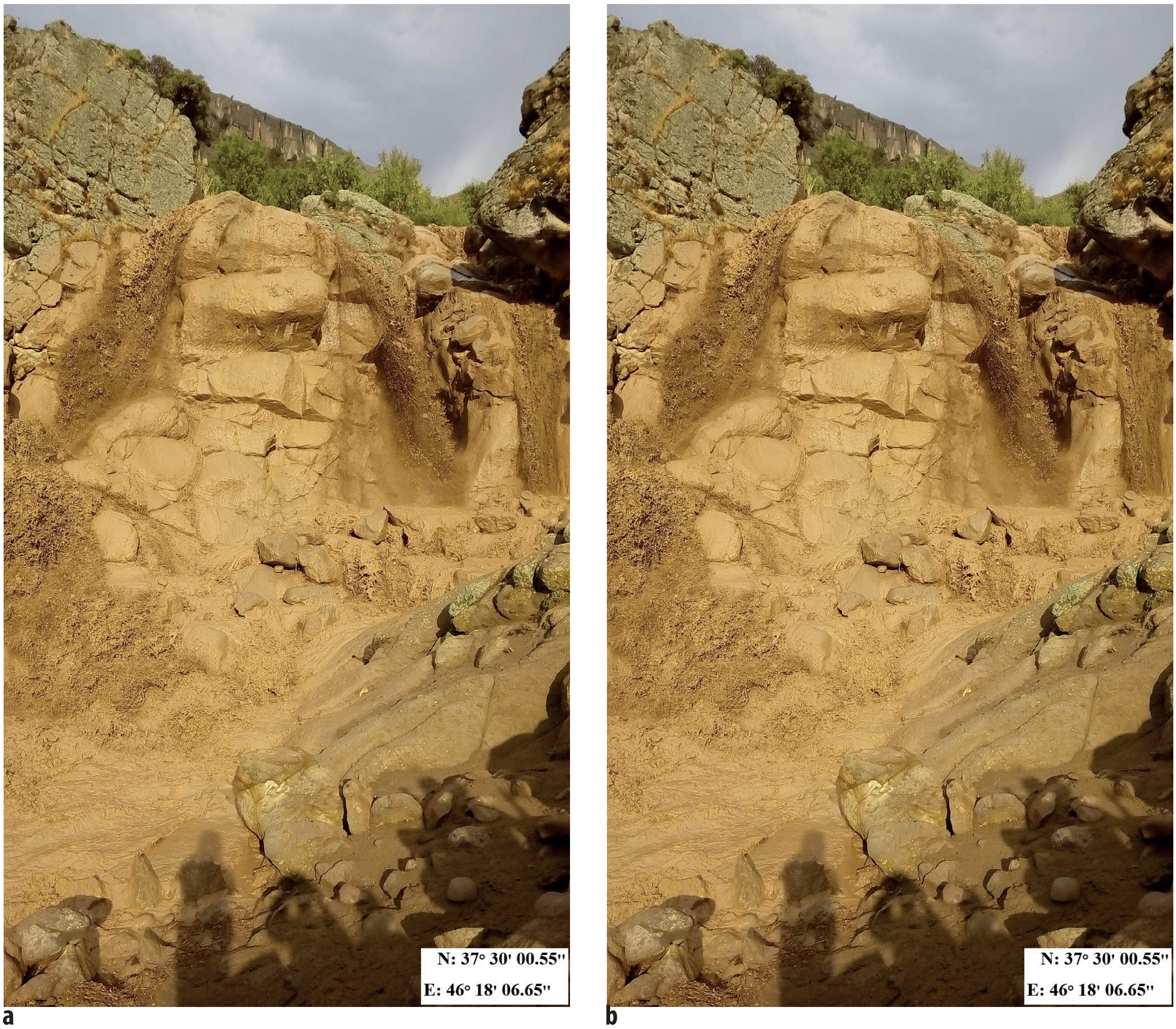

Fig. 8 Photographs of the Ashan drainage basin with geographical coordinates.

The relationship between the erodibility variables

Hillslope positions 2 and 3, i.e. shoulder and back slope agricultural and pastoral soils , are subject to the greatest erosion and those at the summit the least erosion. Foot slope and toe slope soils are subject to the least erosion according to Figs. 7a and 7b. Rate of erosion decreases from land use 1, agriculture, to land use 2, pasture. In the studies conducted by Wischmeier and Mannering (1969) on 55 soil samples, soil permeability decreases with decrease in the amount of organic matter, sand, aggregate index, density and increase in the percentage of sand and silt, percentage of suspended solids and pH, while in the studies of Middleton (1903), the percentage of suspended solids is closely related to only erodibility (Middleton 1903; Wischmeier and Mannering 1969). In this study, the rate of erodibility is significantly associated with organic matter content in both land uses and the minimum content of organic matter was recorded in soils of back slopes subject to both landform uses (Fig. 6 and Equation (3)). There is a significant relationship between erosion and very fine sand (Figs. 5b, 7a and 7b), so toe slope soils subject to both landform uses have a high very fine sand content, whereas the least erodibility is associated with toe slope soils used for agriculture and summit soils used for pasture. Ashan drainage basin is mountainous and slopes are very steep in most areas (Fig. 4) and because erodibility can be affected by topography; according to Table 1, erosion occurs at all the positions on the hillslopes under both forms of land use, therefore, other factors such as topography (slope and aspect) also have an effect on erodibility.

Conclusion

The results of this research indicate that there is a strong relationship between land use, soil properties and landform components, and erodibility of hillslopes. Silt content of soil is strongly positively associated with its erodibility. According to the results, erodibility is higher in agricultural land than pastures. On the other hand, the back slopes of hillslopes, are subject to greater levels of erosion than the other positions on hillslopes. In back slope soils, the organic matter is less than at other positions on hillslopes and the maximum amount of organic matter in back slope soils is similar to that recorded at the summit. Therefore, soils with a high amount of organic matter are less subject to erosion than soils with a low organic matter content. There was no relationship between erodibility and the aspect of a hillslope.

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ANALYSING CHANGES IN LAND COVER IN RELATION TO ENVIRONMENTAL FACTORS IN THE DISTRICTS OF ZNOJMO AND TŘEBÍČ (CZECH REPUBLIC)

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ABSTRACT

The aim of this study is to determine the influence of selected environmental factors on the dynamic changes in the landscape in the Czech Republic: 1) to detect land use changes between 1986–2013 along altitudinal gradients in two neighbouring Czech districts (Třebíč and Znojmo), 2) to test if there is a relationship between the spatial distribution of the main changes and selected environmental factors, 3) to identify differences in the sizes of agricultural fields between 1953 and 2013, and whether they are associated with changes in agricultural land use. Satellite Landsat TM/ETM scenes for 1986, 1994, 2002 and 2013 were used to define land cover categories (arable land, grassland, coniferous forest, deciduous forest, mixed forest, urban areas and inland water). The association between the distribution of changes in land-cover with environmental factors such as gradient, aspect, altitude, topographic wetness index (TWI), less-favoured areas (LFA), main soil units and climate zones was determined. Only a limited proportion of landscape changes were dependent on environmental factors in the study area. Over the period 1994–2013 there was a decrease in arable land and increase in grassland, mainly in the LFA zone. Slope of the terrain was a dominating factor in landscape changes. The association with TWI values was most significant in permanent arable land and in grassland in transition to arable land. There was an increasing trend in the annual average temperature and sum of solar radiation in both the districts, Třebíč and Znojmo. A change from small fields (1953) to large fields (2013) was recorded in the study area. Distribution of field sizes was different in LULC classes for different climatic zones and the main soil units.

Keywords: land use, satellite data, climatic parameters, altitudinal gradient, slope of terrain, field size

Introduction

Changes in land use / land cover (LULC) influence climate and weather conditions at local and global scales (Pielke et al. 2002). Local land use studies provide an historical perspective and the current land use, which helps to learn changes in global scale landscapes and to forecast future trends. Understanding the causes and consequences of changes in land use has been one of the main research topics in the last decade (Turner and Robbins 2008).

Recent studies reveal that changes in the landscape are determined by a complex set of interactions between environmental and socio-economic factors (Mottet et al. 2006; Serra et al. 2008). Human activity is a major force affecting spatial and temporal changes in land use (Olsson et al. 2000; Krausmann et al. 2003; Bucala 2014). Olsson et al. (2000) report a significant increase in area of grassland areas at the expense of that of forest mainly as a result of human activity in the period 1960–1993 in Mid-Norway. A direct effect of socio-economic changes in Austria to changes in agricultural land use between 1950 and 1995 is reported in Krausmann et al. (2003). Bucala (2014) agricultural abandonment and an increase in forest area explored over the period of 40 years (1954–2004) in the Western Polish Carpathians, with a total decrease in the acreage of arable land of around 80% due to the human activity. Several studies indicate that the magnitude of the LULC change differs depending on the time period considered (Weng 2002), geographical location (Rindfuss et al. 2004) and slope and altitude (Poyatos et al. 2003; Kindu et al. 2013). Serra et al. (2008) analysed the factors determining LULC by combining biophysical (mean temperature, solar radiation, precipitation, altitude, slope) and human variables (agricultural area in use, agricultural holding by size, number of fields, total population, hotel capacities and others) for 1977–1997 in the north-east of Spain. These authors found, that landscape homogeneity depends on irrigated agricultural intensification on the coastal plain, and erosion and landscape degradation due to permanent abandonment of cropping in the transitional subregion and mountainous area. In Pyrenean mountains changes in land use is supported by an analysis of socio-economic drivers (various issues at village and farm level, land-tenure) and natural drivers (slope, altitude, type of soil, distance from cultivation and grazing) from 1980s to 2003 (Mottet et al. 2005). Verburg and Chen (2000) report that the spatial distribution of all land-use types in China is best described by an integrated set of biophysical and socioeconomic factors.

Changes in the landscape over the past 150 years (before 1999) in the Czech Republic, in particular the social forces, were analyzed recently (Bičík et al. 2001; Opršal et al. 2013), which revealed that social forces had a great effect on land-use changes during the period of the study. Opršal et al. (2013) selected three case studies as representative of the diversity of natural conditions and explained the association between land-cover and environmental factors. However, the time period (1938–2009) encompassed significant variations in the socio-eco-

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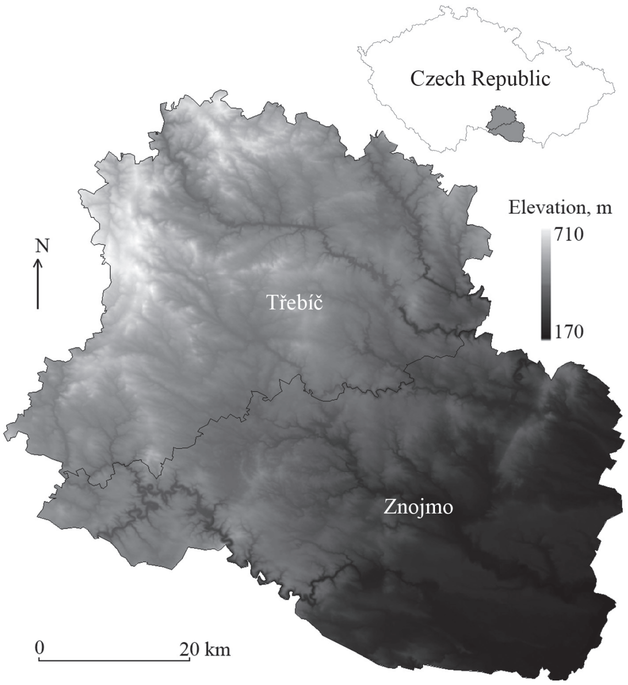

Fig. 1 Location of the study areas: Znojmo and Třebíč districts.

nomic conditions in Czech society. These authors show that highly dynamic landscape processes associated with political and socio-economic changes reduced the role of natural factors in the process of landscape changes. Changes in agricultural land are associated with changes in field sizes, which indicate the degree of agricultural capital investment, mechanization and labour intensity (Rodriguez and Wiegand 2009; Janovska et al. 2017).

This study aims to improve our understanding of the influence of particular environmental factors on the recent dynamic changes in landscape and potentially that of future landscape planning in the Czech Republic. It has a three objectives: 1) to detect land use changes that occurred between 1986–2013 along altitudinal gradients and geographically defined by two neighbouring Czech districts, 2) to test if there is a relationship between the spatial distribution of the main changes in land use and particular environmental factors, 3) to identify differences in the sizes of agricultural fields between 1953 and 2013, and determine the association between changes in agricultural land use and the size of agricultural fields.

Materials and Methods

Study area

The districts of Třebíč and Znojmo cover an area of 3.150 km^2 and are situated in South Moravian and Vysočina regions of the Czech Republic (Fig. 1). These districts vary in soil conditions, from very fertile soils in the South (Znojmo) to the less fertile in the Czech-Moravian Highlands (Třebíč) and hence also in the intensity of agriculture. The area studied includes 6 agricultural climatic zones from very warm to slightly cold (Culek et al. 2013). The drainage systems built in 1960–70, occupy 17% and 8% in the districts of Třebíč and Znojmo, respectively.

Data

Satellite data

Satellite Landsat TM/ETM scenes of vegetation during the period from April to October of 1986, 1994, 2002 and 2013 when cloud cover was less than 20%, were downloaded in GeoTIFF format from the Geological Survey web-page (http://earthexplorer.usgs.gov). The correction of atmospheric satellite data was carried out using ENVI 5.1 software in FLAASH (Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes) module containing an MODTRAN atmospheric radiative transfer code (Kaufman et al. 1997; Adler-Golden at al. 1999). Satellite data were used to classify the types of land-cover in the study area.

Airborne historical data

Airborne panchromatic digitized images from 1953 were used for obtaining the size of the fields in the agricultural areas studied.

Digital altitudinal model

Digital altitudinal model (DEM) with a spatial resolution of 25 m (State Administration of Land Surveying and Cadastre of Czech Republic, www.cuzk.cz) was used to obtain the topographic parameters of the area studied.

Less-favoured areas map

Less-favoured areas (LFA) are areas where farming is handicapped by geography, topography or climate and in which farmers are eligible for compensation for the extra costs incurred or income foregone (Glossary of Agricultural Policy Terms, OECD). There are three existing categories of LFA: 1) mountain/hill areas, 2) areas in danger of abandonment of a particular land-use, and 3) areas affected by specific handicaps. Each category has two grades – partial and total (source: Agricultural and rural development, http://ec.europa.eu/agriculture/rurdev/lfa/index_en.htm). The LFA map was used as an environmental factor in the Canonical correspondence analysis (CCA).

Main soil unit classes

Main soil unit (MSU) classes from BPEJ (bonitovaná půdně ekologická jednotka, in Czech, Němec 2001) taking into account factors such as climatic zones, soil depth, granularity and texture were represented by 14 MSU types from 6 climate zones (Table 1). MSU and climate zones were introduced into a further CCA analyses and an analysis of the distribution of the sizes of agricultural fields to investigate their association with changes in land-cover.

Table 1 Characteristics of the main soil unit classes (MSU) in the study area.

MSU	Morphological soil type	Texture	Depth of soil	Climate zone
01	Haplic Chernozem	Moderate	Deep to very deep	2
08	Haplic Chernozem	Light moderate to moderate	Deep to very deep	2
10	Orthic Luvisol	Moderate to heavy	Very deep	1, 8
12	Orthic Luvisol	Moderate to heavy	Deep to very deep	4, 5
28	Eutric Cambisol	Moderate to heavy	Medium to deep	4
29	Eutric Cambisol	Moderate	Medium to deep	2, 5, 7
32	Eutric Cambisol	Light	Medium to deep	5, 7
37	Ranker	Light to moderate	Shallow	4, 5, 7, 8
47	Dystric Planosol	Moderate to heavy	Deep to very deep	5, 7
55	Eutric Fluvisol	Light	Very deep	5
68	Dystric Gleysol	Moderate to heavy and very heavy	Deep to very deep	5, 7, 8
64	Dystric Planosol	Heavy to very heavy	Deep to very deep	7

Climate zones are: 1 – warm, dry; 2 – warm, slightly dry; 4 – slightly warm, dry; 5 – slightly warm, slightly humid; 7 – slightly warm; 8 – slightly cold, wet.

Agricultural parcel identification system

The Czech agricultural parcel identification system (LPIS) is a geographic informational system for agricultural parcel identification, which includes recent data on the agricultural land use in the Czech Republic (www.lpis.eu). Shape files with borders of agricultural fields from 2015 were downloaded from LPIS (www.lpis.eu) for the area studied. Data were used for determining the changes in the sizes of agricultural fields between 1953 and 2015.

Extraction of land-cover types

Object-oriented classification of satellite data was done in eCognition Developer software (Trimble) (Walter 2004). Segmentation procedure, object features (vegetation indices, simple spectral band ratios, mean and standard deviation values of spectral bands) and a nearest neighbour algorithm, were used to classify 7 land-cover types from satellite data, namely: arable land, grassland, coniferous forest, broadleaved forest, mixed forest, inland water and settlements. The training areas for the classification were derived from a field survey conducted at the time of the project CzechCarbo, from LPIS and archival aerial photographs.

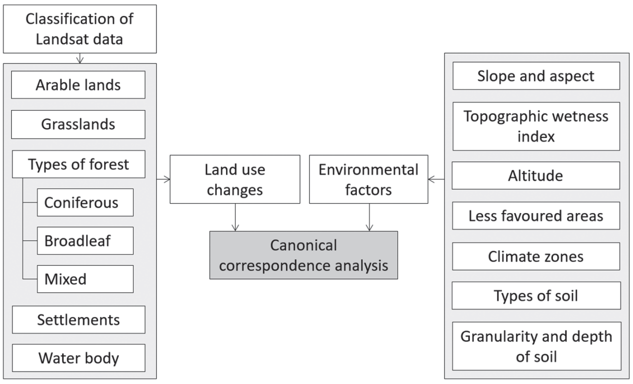

Fig. 2 Data input for canonical correspondence analysis (CCA).

Extraction of topographic parameters

Topographic wetness index (TWI) was used as one of the quantitative parameters in this study to characterize the spatial distribution of potential soil moisture depending on orographic conditions (Burt and Butcher 1986). TWI was calculated based on DEM with a spatial resolution of 30 × 30 m using equation (1) (Beven and Kirkby 1979):

$$TWI = \ln(a/\tan\beta), \quad (1)$$

where a is a specific catchment area calculated from accumulation image, $\tan\beta$ is a slope of the pixel, calculated with reference to its neighbouring pixels.

Slope, aspect and altitude were calculated in the geographic information system (ArcGIS) based on DEM.

Canonical correspondence analysis

Canonical correspondence analysis (CCA) was used to explore the distribution of changes in land-cover along slope gradient, aspect, altitude, TWI, LFA, main soil unit classes, soil granularity with soil depth and climatic zone (Table 1) (Fig. 2). The analysis was done for the districts of Znojmo and Třebíč over three time periods: 1986–1994, 1994–2002 and 2002–2013.

Analysis of climatic parameters

Climatic parameters (precipitation, temperature and solar radiation) were processed for the period of 1961–2014. Data were interpolated into grid layers with a daily step and a resolution of 500 × 500 m using an interpolation method based on the regression-Kriging technique. Altitude from DEM was a main predictor in the regression. It was smoothed for precipitation and solar radiation and without smoothing for air temperature. Auxiliary predictors (terrain roughness, slope and exposure) were smoothed for processing of all climatic parameters. Precipitation, temperature and solar radiation were analyzed based on the altitudinal gradient at the study sites. The trend in annual precipitation, temperature and solar radiation in determining land cover changes was taken into consideration.

Mean values of climatic parameters were calculated for LULC classes (permanent arable, permanent grassland, arable to grassland and grassland to arable) in each MSU class for the period 1986–2013.

Field size distribution and changes

Object-oriented image analysis was done in eCognition software to extract agricultural field borders from historical airborne orthophoto images. We clipped out current fields using LPIS polygons. The standard multiresolution segmentation procedure of eCognition was used in order to divide these polygons into individual homogenous areas of historical fields. The quantification of agricultural field size changes was performed in ArcGIS using a comparative analysis of the shape file from LPIS 2015 and shape file from object-oriented classification of airborne historical data for 1953. A frequency of occurrence of a specific field size was estimated separately for the Znojmo and Třebíč districts in 1953 and 2015. The distribution of field sizes associated with agricultural land use changes was estimated according to MSU using ArcGIS tools.

Results

Types of land-cover, distribution and changes

The classification of satellite data showed that arable land was the dominant type of land-cover during the period of 27 years covered by this study in both districts (Fig. 3).

Land-cover change matrix (Table 2) indicates the changes between each of land cover classes between 1986 and 2013 (in %). The values in each of the cells are the percentages of land that was converted from one type of land

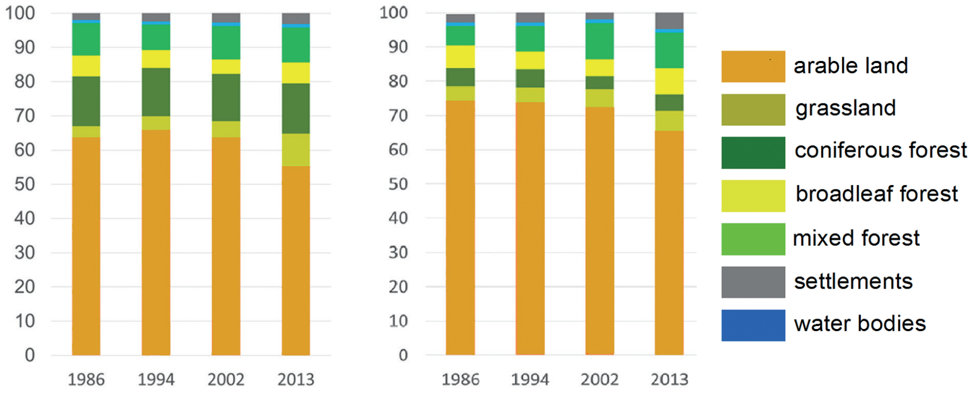

Fig. 3 Types of land-cover, distribution and changes in Třebíč (left) and Znojmo (right) in 1986, 1994, 2002 and 2013 (Summary of changes is in Table 2).

Table 2 Land use change matrix [%]. Summary of land-cover changes from 1986 to 2013 for the Třebíč and Znojmo districts.

Třebíč	Arable land	Grassland	Broadl. forest	Conif. forest	Mixed forest	Water bodies	Settlements	Total 2013
Arable land	**46.7**	0.4	1.3	2.8	1.6	0.2	0.1	53.1
Grassland	4.8	**3.2**	0.9	0.7	0	0	0.6	10.2
Broadl. forest	3.4	0.2	**2.2**	0.2	0.1	0	0.01	6.1
Conifer. forest	4.3	0.3	0.8	**8.0**	2.7	0	0	16.1
Mixed forest	3.3	0	1.0	2.2	**4.8**	0.02	0.3	11.6
Water bodies	0.1	0	0	0	0.2	**0.7**	0	1.0
Settlements	1.1	0.003	0	0	0	0	**0.8**	1.9
Total 1986	63.7	4.1	6.2	13.9	9.4	0.9	1.8	100

Znojmo	Arable land	Grassland	Broadl. forest	Conif. forest	Mixed forest	Water bodies	Settlements	Total 2013
Arable land	**57.8**	1.2	3.1	1.2	1.8	0.004	0.2	65.3
Grassland	2.4	**2.5**	0.2	0.1	0.6	0.01	0.1	6.0
Broadl. forest	4.7	0.2	**2.5**	0.2	0.6	0.1	0.01	8.3
Conifer. forest	1.6	0.1	0.6	**1.3**	1.5	0	0	5.1
Mixed forest	5.8	0.2	0.02	2.9	**1.5**	0	0	10.4
Water bodies	0.09	0	0.01	0	0	**0.9**	0	1.0
Settlements	1.7	0.1	0	0.1	0	0	**2**	3.9
Total 1986	74.1	4.3	6.4	5.8	6.0	1.0	2.4	100

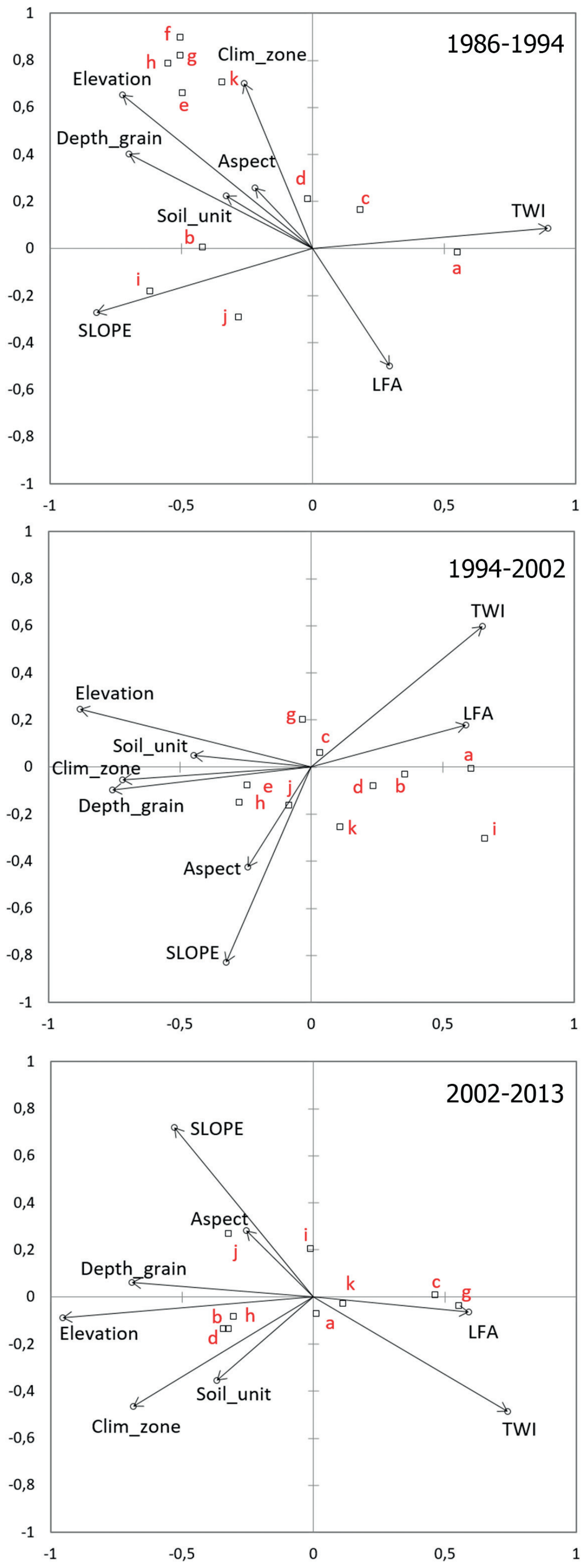

Fig. 4 CCA ordination of land-cover transitions in three time periods. Selected classes of land-cover: a – permanent arable land, b – permanent grassland, c – grassland to arable land, d – arable land to grassland, e – coniferous forest to broadleaved forest, g – broadleaved forest to arable land, h – arable land to broadleaved forest, i – permanent broadleaved forest, j – permanent coniferous forest, k – broadleaved forest to grassland.

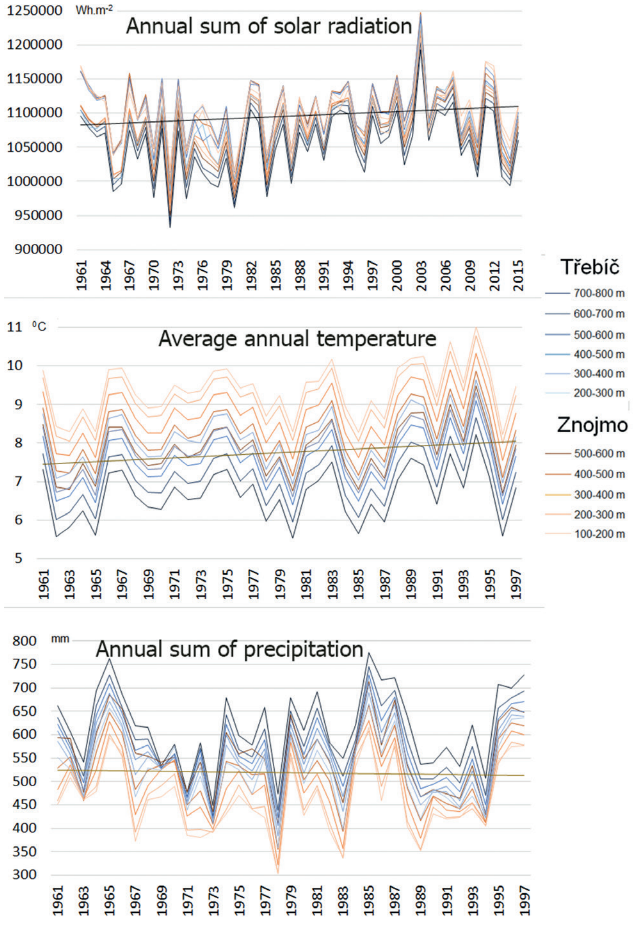

Fig. 5 Changes in climatic parameters in the study area during the last 40–50 years: Annual sum of solar radiation, average annual temperature and annual sum of precipitation.

cover to another. The bold diagonal of the matrix is the percentage of the area of each class that remained unchanged.

Canonical correspondence analysis

CCA resulted in an ordination diagram. Arrows represent environmental factors. The length of an arrow is the correlation of a given variable with the extracted ordination axis on the diagram. The length of the arrow is proportional to the rate of change. Points represent the transformation processes in land cover. The position of points indicates a relationship with the corresponding variable (Fig. 4).

Analysis of climatic parameters

Analysis of selected climatic parameters showed that they fluctuated over the past 40–50 years (Fig. 5). Distribution of climatic parameters in LULC classes were estimated for the periods: before 1986, 1986–1994, 1994–2002 and 2002–2013 and illustrated for the study area using a box and whisker chart (Fig. 6).

Distribution and changes in field sizes

Agricultural fields extracted from a classification of airborne historical data (Fig. 7a) and fields from LPIS 2015 (Fig. 7b) were compared to determine the frequency of occurrence of specific field sizes in 1960 and 2015

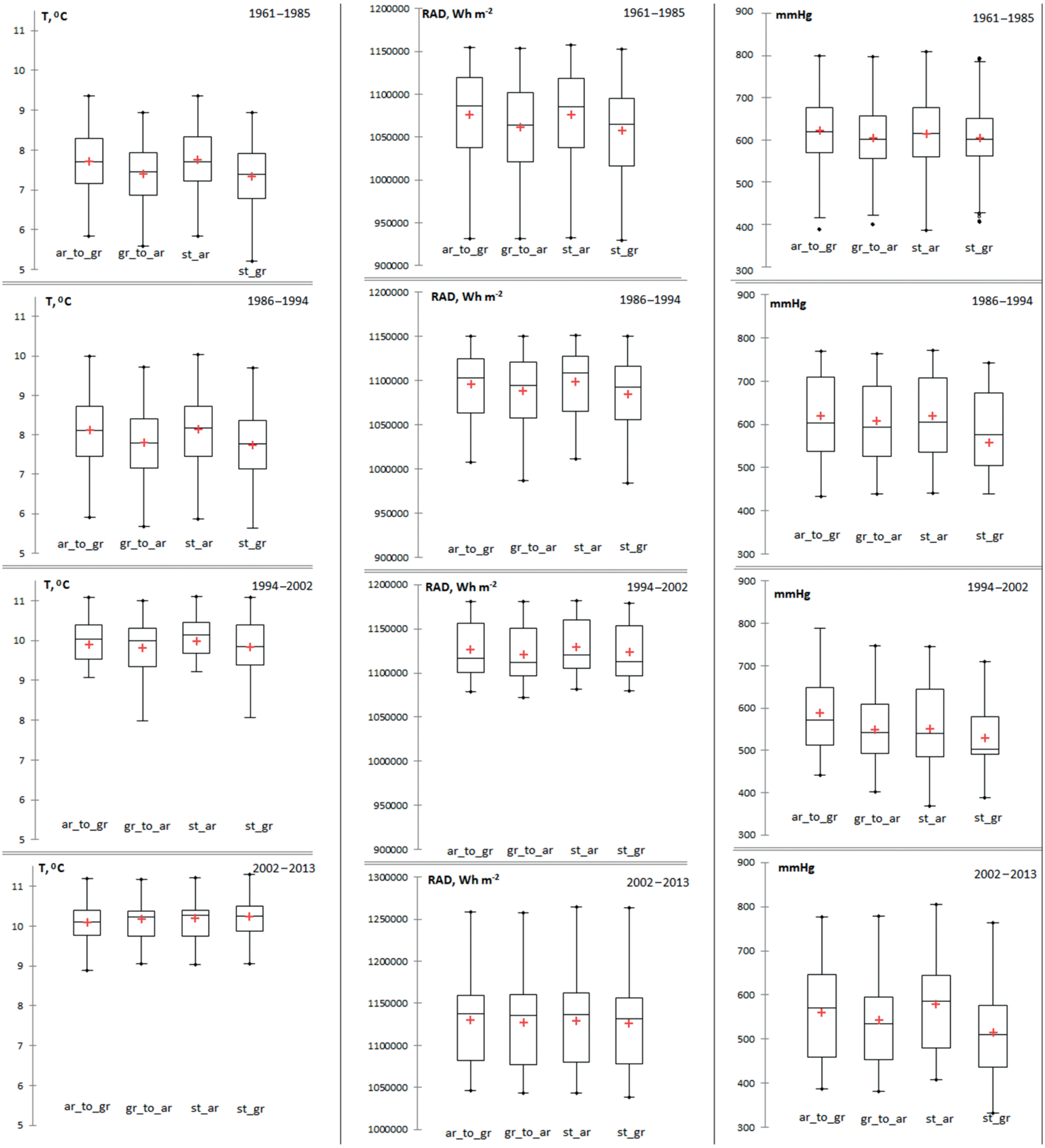

Fig. 6 Means (the red crosses) and medians (the central horizontal bars) of climatic parameters (Temperature, Radiation and Precipitation) calculated for LULC classes (st_ar – stable (permanent) arable, st_gr – stable (permanent) grassland, ar_to_gr – arable to grassland and gr_to_ar – grassland to arable) for the periods: before 1986, 1986–1994, 1994–2002, 2002–2013

(Fig. 8), and by the distribution of field sizes according to MSU classes (Figs 9, 10).

Discussion

Types, distribution and changes in land-cover

The classification of satellite data revealed that arable land was the dominant type of land-cover accounting for from 63.7% to 53.1% in the Třebíč landscape and from 74.1% to 65.3% in the Znojmo landscape over the 27 year period of this study (Fig. 3). Forest occupied around 20% and 30% of the Znojmo and Třebíč sites, respectively. Distribution of land-cover types along a slope gradient indicated, that arable land was dominant on gentle slopes at both sites. On moderate slopes, forest was the dominant cover: broadleaved in Znojmo and coniferous in Třebíč. Mixed forests were mainly typical of moderate,

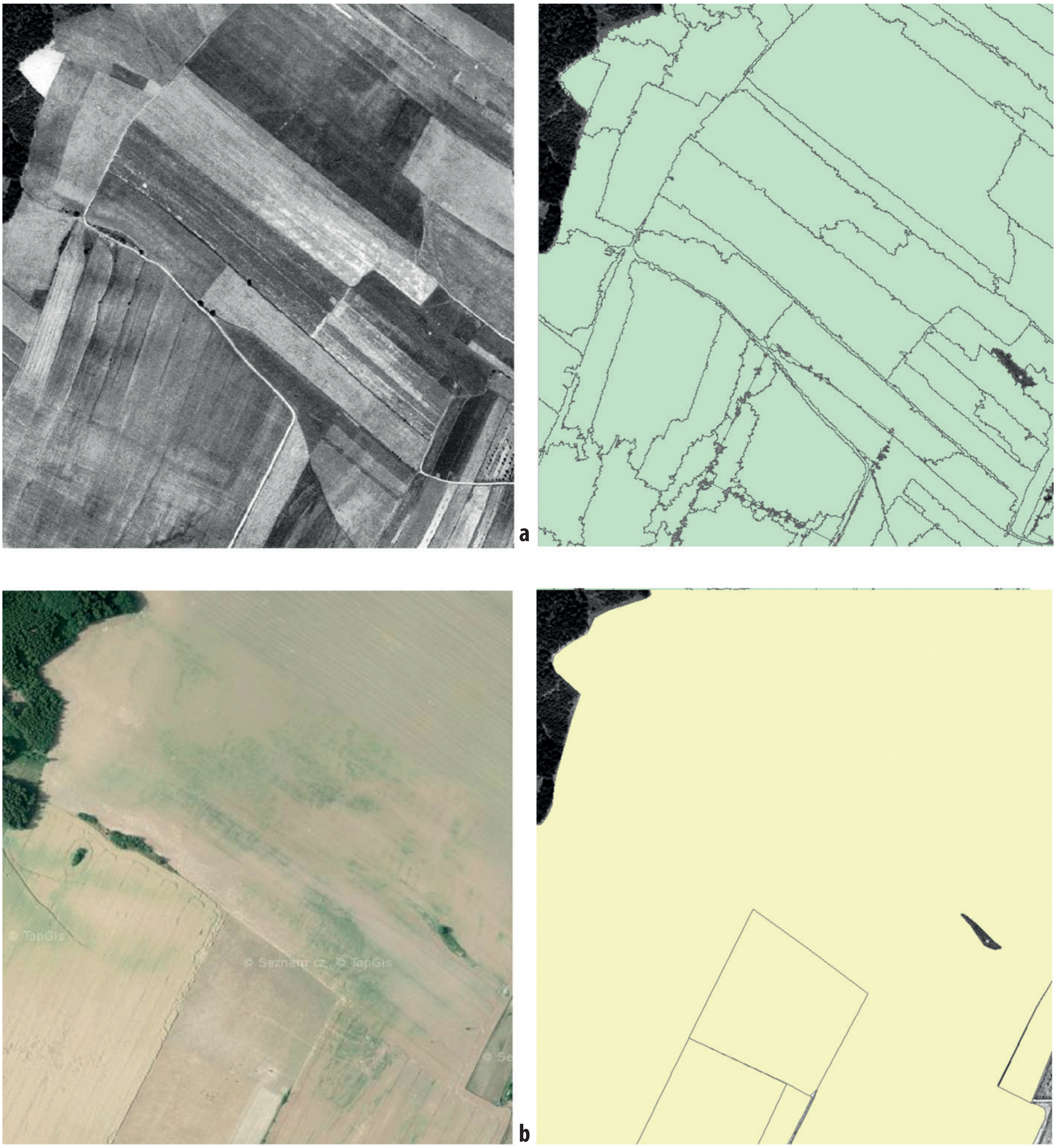

Fig. 7 Fragments of image and shape file with agricultural fields: a – 1953, airborne image (left) and agricultural fields extracted from object-oriented classification (right); b – 2015, airborne image (TopGIS, www.topgis.cz) (left) and agricultural fields downloaded from LPIS (agricultural parcel identification system) (right)

steep and very steep slopes. Distribution of the different land-covers was associated with wetness (TWI index). Forest dominated in dry areas at both sites: coniferous forest prevailed at Znojmo, and mixed and coniferous forest at Třebíč. Arable lands occurred mainly in average to very wet areas (high TWI).

The use of about 66% of the land in Třebíč and 69% in Znojmo remained unchanged during the study period (Table 2). Most of changes were from arable land to grassland in both districts. Almost 16% and 13% of arable land was converted to grassland in the Znojmo and Třebíč districts, respectively, in the different time periods. Mixed and broadleaved forests increased by 25% in Znojmo on account of an increase in coniferous forest. An increase in conifers by 7% was recorded in the Třebíč district. The increase in mixed forest resulted not only from the conversion of one type of forest to another, but also from afforestation of arable land and grassland. About 5% of all changes in Třebíč and 3% in Znojmo districts occurred on drained soils. Bičík et al. (2001) analyzed

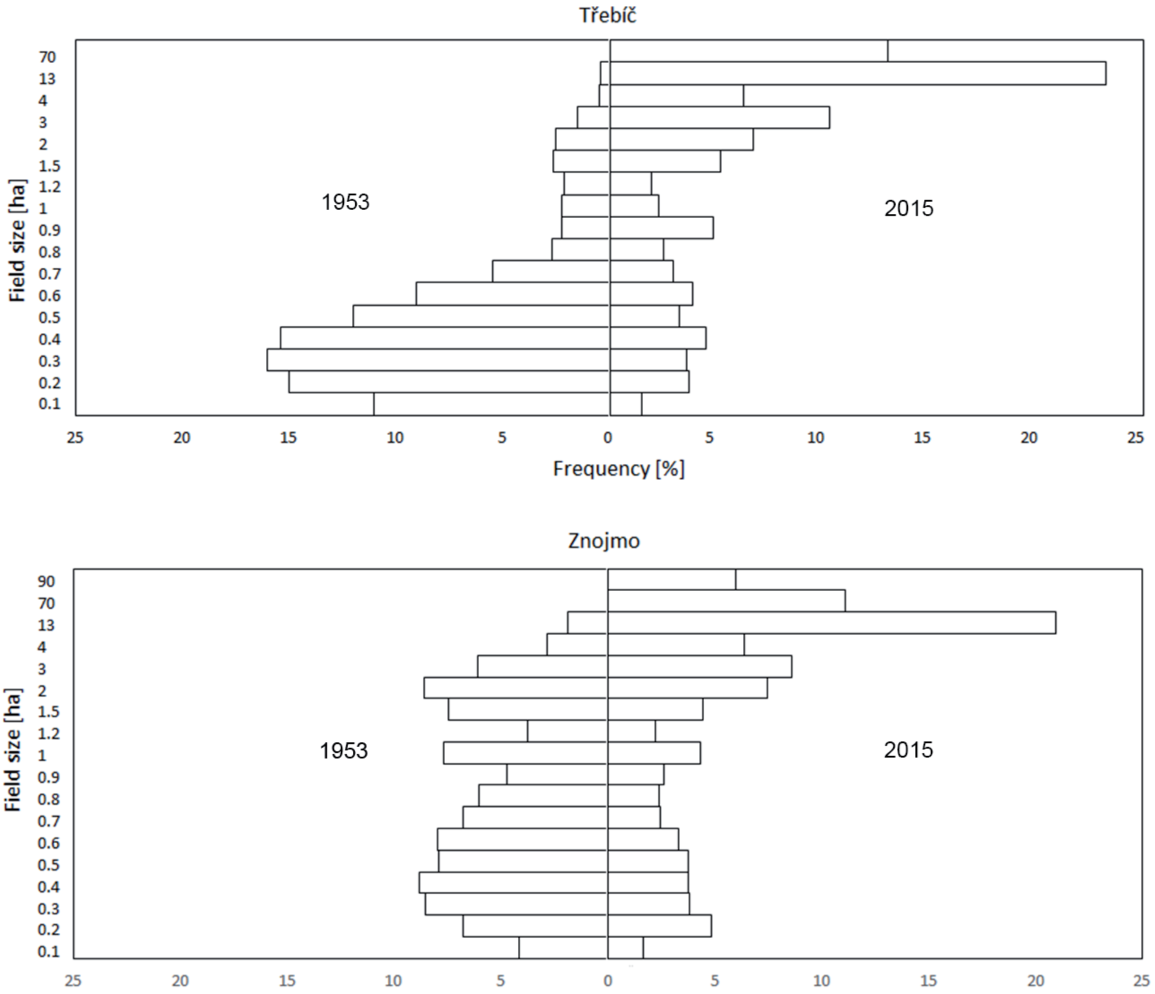

Fig. 8 Back to back histograms of field size frequency in Třebíč and Znojmo in 1953 and 2015

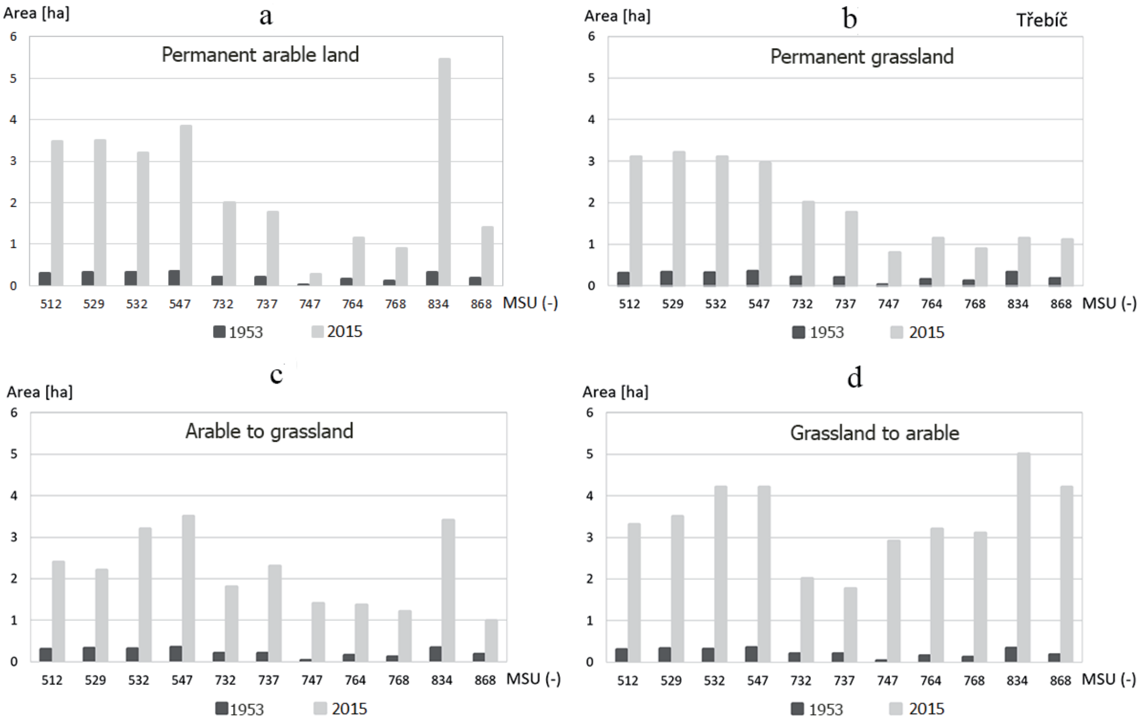

Fig. 9 Distribution of field sizes in LULC classes according to MSU (Main Soil Unit) and climate zone (from Table 1) in Třebíč; a – permanent arable, b – permanent grassland, c – arable to grassland, d – grassland to arable.

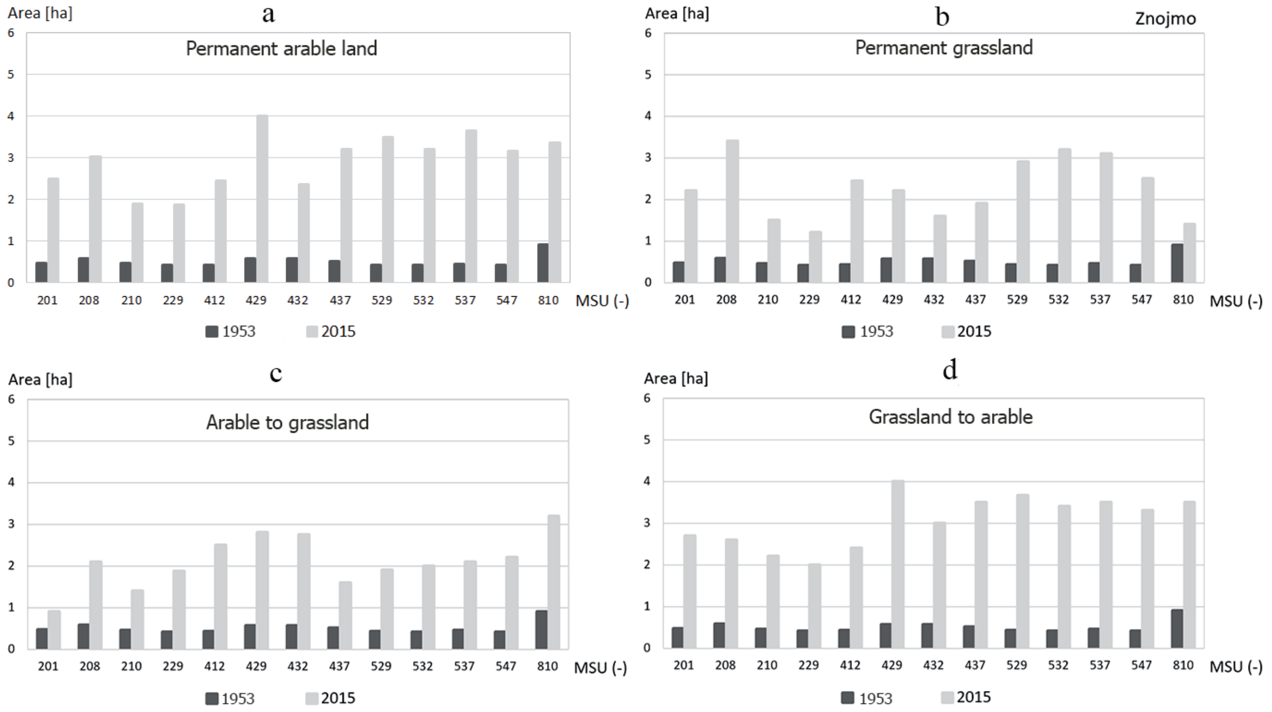

Fig. 10 Distribution of field sizes in LULC classes according to MSU (Main Soil Unit) and climate zone (from Table 1) in Znojmo; a – permanent arable, b – permanent grassland, c – arable to grassland, d – grassland to arable.

LULC changes for the whole Czech Republic and report, that arable and grassland areas decreased over the period 1970–1990, whereas we recorded a slightly increase in arable at the Třebíč site and no change in arable and grassland at Znojmo and Třebíč over the period 1986–1994. The key tendencies post-1990 according to Bičík et al. (2001) is a decrease in arable land, great increase in grassland (pastures) and slight increase in forests, which is in agreement with our results for the Znojmo and Třebíč sites for the period 1994–2002.

Canonical correspondence analysis

The environmental variables in the ordination diagrams are significantly correlated with CCA axes at $p < 0.01$ according to the Monte Carlo permutation test. The CCA results indicate that only a limited proportion of the changes in landscape were dependent on environmental variables (Fig. 4). Transition of arable land to grassland was connected with climate zones in 1986–1994 and 2002–2013, and with LFA classes in the period 1994–2002. Transition of grassland to arable land and broadleaved forest to arable land was associated with moderate TWI values in 1994–2002. Permanent arable land was also associated with TWI in 1994–2002. Permanent broadleaved and coniferous forests were associated with slope during the whole time period. The landscape changes were independent of the main soil unit classes. Transition of coniferous forest to broadleaved forest was associated with soil parameters (depth and granularity) in 1994–2002. Opršal et al. (2013) state, that the effect of slope was the greatest of the environmental variables considered (altitude, aspect, area, distance) in land transformation in Moravia. Studies of Chen et al. (2001) and Hietel et al. (2004) support the association between slope and land-use in small catchments in China and the Lahn-Dill Highlands in Germany, correspondently. As in the LFA, agro-ecological zones (AEZ) were used as a conditional factor for LULC changes by Kindu et al. (2013), where the transition of grassland to cropland was typical for AEZ in sub-humid highlands with slight slopes (0–5%).

Analysis of climatic parameters

Changes in the climate in the area studied over the past 40–50 years were characterized by an increase in annual average temperature and sum of solar radiation (Fig. 5). Annual sum of precipitation did not change. These trends indicate the possible causes of the drying of the Central European landscape (Trnka et al. 2015). Analysis of the distribution of climatic parameters in LULC classes indicate, that radiation and temperature values were higher in permanent arable areas than in other land use classes during the study period (Fig. 6). Permanent grassland was located in areas with the lowest temperature and precipitation. The highest precipitation was recorded in areas with arable to grassland type land use except in 2002–2013, when the highest precipitation was recorded in permanent arable areas. Temperature, radiation and precipitation medians were the same in arable to grassland and permanent grassland classes in the period before 1986 and in 1986–1994.

Distribution and types of change in field sizes

The transformation from small to bigger fields was recorded in the Třebíč and Znojmo districts with the average field size of 0.5 ha (Třebíč) and 1 ha (Znojmo) in 1953, and around 40 ha (Třebíč and Znojmo) in 2015 (Fig. 8). Land consolidation, unemployment rate and soil fertility were main factors, which are significantly associated the size of agricultural holdings in the Czech Republic (Cay et al. 2010; Janovska et al. 2017).

The largest field sizes were recorded in the Třebíč (70 ha) and Znojmo districts (up to 90 ha) with 13% frequency in 2015. The magnitudes of these values are close to those reported by Janovska et al. (2017), who record a maximum field size of 91.4 ha in the Czech Republic. There were small fields (about 0.1 ha) in both districts with a 5–10% frequency in 1953 and 2% frequency in 2015. The field sizes of 0.2–2 ha and 0.1–0.5 ha were more frequently recorded in the Znojmo (up to 8%) and Třebíč (10–17%) districts in 1953. The sizes of 13–70 ha were the most common (20% frequency) field sizes in both districts in 2015.

Analysis of the distribution of field sizes in LULC classes according to MSU revealed the largest field sizes were recorded on cambisol soils with a slightly cold and wet climate zone in permanent arable class and grassland to arable class at Třebíč (Fig. 9). The smallest field sizes were recorded in permanent grassland and arable to grassland LULC classes on glues with a slightly warm climate in Třebíč. Znojmo district had the largest fields on modal cambisol soils in slightly cold and wet areas and in slightly warm climate zones in permanent arable, arable to grassland and grassland to arable LULC classes (Fig. 10). Smallest fields occurred only on modal cambisol and brown soils with warm and slightly dry climates for all LULC classes in Znojmo.

Conclusion

The distribution of changes in land-cover revealed a decrease in arable land and increase in grassland, mainly in the less-favoured areas (LFA) during the period of 1994–2013. The CCA results indicate a strong correlation of landscape changes with particular environmental factors in the area studied. The slope of the terrain was a dominant factor in landscape changes. The influence of TWI values was most significant in permanent arable land and also in the transition of grassland to arable land. Increase in the average annual temperature and solar radiation was recorded in the Třebíč and Znojmo districts. A transformation of small fields to bigger fields was recorded in the study area. Distribution of field sizes was different in LULC classes in different climatic regions and main soil units. This study revealed a moderate association of particular environmental factors with dynamic changes in the landscape. The degree of association may be affected by socio-economic driving forces operating in the area studied. These results should prove useful in future landscape planning in the Třebíč and Znojmo districts of the Czech Republic.

Acknowledgements

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OPTIMAL DISINFECTION TIMES FOR SEEDS OF MEDITERRANEAN ORCHIDS PROPAGATED ON NUTRIENT MEDIA

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ABSTRACT

A time-consuming yet mandatory step during *in vitro* sexual propagation of orchids is the treatment of seeds with a disinfecting solution that also serves to scarify the seeds. If the seeds are not properly disinfected, microorganisms grow within the culture vessel, thus reducing the efficacy of the process and burdening the operation with unnecessary materials and labour. On the other hand, a long period of disinfection may damage the seed. The literature is inconclusive with respect to the proper combination of solution strength and duration of the treatment, especially with respect to Mediterranean orchids. The objective of this research is to determine optimal disinfection/scarification times for two species with thin and thick seed coats, respectively. Seeds of *Anacamptis laxiflora* (Lam.) and *Himantoglossum robertianum* (Loisel.) were treated in 1% NaClO solution for 5, 15, 25, 35 and 45 minutes and sown in modified organic Malmgren medium. Logistic regression models were fitted to the results. Due to the small number of observations per treatment, regression models of infection rates on treatment duration had no more predictive ability than the mean infection rate. On the other hand, regression models of germination rates on treatment duration proved statistically significant or nearly so. Treatment of only a few minutes in 1% NaClO seems to be optimal for seeds with relatively permeable seed coats such as those of *A. laxiflora* (Lam.). Conversely, treatment of 45 minutes in 1% NaClO may be inadequate for seeds with relatively impermeable seed coats such as those of *H. robertianum* (Loisel.).

Keywords: *Anacamptis laxiflora* (Lam.), Cephalonia, *Himantoglossum robertianum* (Loisel.), *in vitro* propagation, Orchidaceae, scarification

Introduction

Greece hosts more than 200 species and subspecies of terrestrial orchids (Petrou et al. 2011). This remarkable biodiversity is threatened by diverse environmental pressures such as overgrazing, cultivation, urbanization, tourism, illicit collection and climate change. Meanwhile, the horticultural, nutritional and medicinal value of native orchids remains relatively unexploited. *In vitro* propagation of native orchids would yield multiple benefits: protection and conservation of valuable genetic resources, the development of an innovative floricultural product for export, manufacture of salep and the possibility of restoring disturbed ecosystems. It is desirable to propose a protocol for *in vitro* propagation suitable for Greek native orchids that can be adopted by plant propagation laboratories and professionals for the mass production of seedlings for use in restoration programs.

A time-consuming yet vital step during the *in vitro* sexual propagation of orchids is the treatment of seeds with a disinfecting solution. This treatment serves a dual purpose: First, to disinfect the seed. Second, to break embryo dormancy and initiate germination, a process known as chemical scarification (Rasmussen 1995). If seeds are not properly disinfected, within a few days' moulds, yeasts and (less frequently) bacteria grow within the culture vessel, reducing the efficacy of the process and thus burdening the operation with extra materials and labour. Too short a treatment may result in unduly high infection rates; too long a treatment may damage the embryo. The decision of how long to disinfect seeds is further complicated by the fact that different species have seed coats (testas) of different thickness, necessitating different treatment times. If Mediterranean orchid propagation is ever attempted at a mass scale for commercial or conservation purposes, optimal treatment times must be determined.

The literature is contradictory as to the recommended duration of disinfection/scarification. Svante Malmgren, a veteran practitioner who has propagated more than 200 terrestrial species of orchids and hybrids *in vitro*, recommends soaking the seeds in 0.3–1% NaClO solution for 5–45 minutes (Malmgren and Nyström 2017). According to Malmgren, the appropriate combination of solution concentration and treatment duration must be determined for each species. In principle, the calibration method is straightforward: A suspension of seeds in NaClO solution is observed under magnification, until most seeds are bleached. Despite the simplicity of the technique, the cut-off point is subjective. In our experience, the maturity of the seed affects the outcome; we have determined the cut-off times for seeds at different stages of maturation. Overall, we found it difficult to replicate Malmgren's technique consistently.

At the other end of the spectrum is Rasmussen (1995). In the general section of his monograph, he suggests a stronger NaClO solution (5%) and much longer treatment times, up to several hours. In the special section

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of his monograph, Rasmussen details the requirements for *in vitro* propagation of several N. European and N. American species. For the related genera *Orchis/Anacamptis/Aceras* he recommends treatment durations several times longer than those proposed by Malmgren. Another example is *Ophrys sphegodes* Mill., a species with a wide European distribution: Rasmussen proposes a treatment of 45 minutes in 0.3% NaClO, whereas Malmgren proposes only 4–8 minutes in a similar concentration of NaClO. For *O. sphegodes*, Rasmussen reports germination times of up to seven months, whereas Malmgren reports germination times of at most four weeks. Overall, Rasmussen considers *Ophrys* as one of the most difficult genera to germinate, whereas Malmgren considers *Ophrys* as one of the easiest.

These contradictions mean one of two things: First, Rassmussen consulted only a limited body of research that was available at the time. We have already questioned his broad assertion that orchids grow on poor soils (Katsalirou et al. 2016). Second, results from different experiments are not comparable; unreported factors related to laboratory materials, technique, or seed maturity confound the effects of treatment duration and concentration of the disinfecting solution. For instance, the number of seeds treated relative to the volume of solution is rarely reported in the literature, although generally a surplus of disinfectant is assumed (Rasmussen 1995). Researchers may not report certain factors if they consider them self-evident or if they do not comprehend their significance. Propagation of Mediterranean orchids in particular is poorly documented. There are just a few reports from Greece, although Greece is the richest country in Europe based on the number of taxa per unit area. The few reports that exist lack detail about the laboratory technique used (e.g., Kitsaki et al. 2004).

To identify possible factors that affect infection rates of culture vessels, we sowed 30 batches consisting of 24 culture vessels each, using mature and immature seed from 27 taxa (species and subspecies) collected in Cephalonia, Greece, plus seeds of five European taxa from the collection of Svante Malmgren. Factors such as size, geometry and material in the culture vessels varied inadvertently; we had to use a variety of culture vessels due to shortage of laboratory supplies. Nevertheless, we arrived at some tentative conclusions:

a) Treatment in 0.5–1% NaClO solution for less than 20 minutes for mature seed and less than 45 minutes for immature capsules may not provide adequate disinfection.
b) Immature capsules are more difficult to disinfect than mature seeds.
c) Mature seed must be free of foreign matter such as capsule fragments.
d) Operator speed counts.

The second conclusion is surprising, given that the inside of immature capsules is considered sterile. Possibly, the disinfection protocol that we followed (Koirala et al. 2013) is better suited for tropical than temperate orchids. Also, capsules of tropical orchids are robust enough to withstand blazing, but not necessarily suitable for the delicate capsules of temperate orchids.

Having acquired general experience of *in vitro* culture of Mediterranean orchids, we initiated a formal experiment to determine optimal seed disinfection times. The objective was to determine treatment times that minimize infection of culture vessels and maximize seed germination without unduly delaying the *in vitro* procedure.

Materials and Methods

Preparation of disinfecting solution

As a stock solution we used a commercial formulation of household bleach (Klinex®, Unilever) with a nominal NaClO concentration of 4.8% w/w. To verify the strength of the commercial formulation, we titrated three different volumes (5, 10 and 20 ml) with $AgNO_3$ solution following Mohr's method (Harris 2010). The manufacturer's reported concentration was found to be accurate. The stock solution was diluted with deionised water to a final concentration of 1% w/w. Four drops of washing up detergent per litre were added to reduce surface tension.

Experimental treatments

We opted to use mature seed for ease of storage and handling, as opposed to seed from immature capsules that must be sown shortly after collection. Combinations of five treatment durations (5, 15, 25, 35 and 45 minutes) and two orchid species were tested. We decided against longer treatment times or stronger disinfecting solutions because of the effect such treatments might have on seed viability; Dowling and Jusaitis (2012) report some embryo damage after 1 or 2 hours of soaking in 5% NaClO. The two species of orchids were selected based on the permeability of their seed coats; based on preliminary tests, we opted for a species with a relatively permeable seed coat, *Anacamptis laxiflora* (Lam.), and a species with a relatively impermeable seed coat, *Himantoglossum robertianum* (Loisel.).

In a proper factorial experiment, combinations of treatments are assigned to culture vessels at random. However, this means we would have to keep track of five different treatment durations during each experimental run, unduly increasing the complication of the experimental setup and the possibility of operator error. Instead, we tested one treatment duration per experimental run, in reverse order; the longest duration was tested first to minimize the effect of operator learning. If we proceeded from the shortest duration to the longest, we would give undue credit to the longest duration as a result of the operator improving his or her technique. Although operator experience can be an important factor in specialized work, such as *in vitro* propagation, this factor should best be tested in a separate experiment.

Each experimental run consisted of eight replicates for each species plus eight "blanks," i.e., culture vessels without seed. The blanks were subject to the same treatment as the vessels with seed, including simulated sowing on a nutrient medium. The blanks would reveal possible shortcomings in our laboratory technique that are not due to seed-borne contaminants.

Field method

The seeds were collected on the island of Cephalonia, Greece. At flowering, healthy and robust individuals of *Himantoglossum robertianum* (Loisel.) and *Anacamptis laxiflora* (Lam.) were identified in the field, photographed, labelled and recorded. At senescence, seed was collected from mature capsules. Both species are abundant enough so that seed collection did not jeopardise the natural populations. The seed was sieved to remove foreign matter such as capsule fragments, dried in a desiccator with silica gel, sealed in glass vials and stored at −20 *°C* until sowing.

Laboratory method

In nature, germinating orchid seeds develop an association with symbiotic fungi that supply them with nutrients, including C. The seeds have almost no nutrient reserves of their own, so that effective germination depends on a fungus-orchid association called mycorrhiza (Garbaye 2013). It is possible to reproduce the mycorrhizal association in the laboratory. Although this approach most closely mimic nature, it is complicated because the operator must manage two organisms instead of one. Instead, many practitioners opt for a sterile nutrient solution that supplies the seed with the nutrients needed for germination and early development.

Preparation of nutrient medium

There exist numerous recipes for nutrient media for propagating terrestrial orchids (Rasmussen 1995). A few base ingredients are shared by most recipes, which makes sense, as most terrestrial plants require the same 16 nutrients for growth and development (Marschner 1995). Some recipes add elements such as Al and I whose benefit in plant nutrition has not been unequivocally demonstrated; others add tropical fruit juices or tree sap, whose role has yet to be explained. We opted for a modified version of "SM-organic" (Rasmussen 1995), a time-tested formula of the veteran practitioner Svante Malmgren. The medium is termed "organic" on account of its N source, an amino acid mixture sold under the trade name Vaminolac®. However, in Greece Vaminolac® is considered a medicine and as such cannot be sold without prescription. Therefore, we substituted Amina-Fe (Humofert), a liquid fertilizer containing amino acids and chelate Fe. Because the addition of Amina-Fe more than covers the Fe requirement in Malmgren's recipe, we omitted the Fe salt in the original formula. Further, we substituted $CaHPO_4$ for $Ca_3(PO_4)_2$. Bottled water was substituted for tap water, on account of the high salinity of the municipal water supply (electrical conductivity at 25 °C > 1 mS cm^{-1}). The formula is in Table 1.

Table 1 Formula for modified "SM-organic" nutrient medium.

Ingredient	**Quantity**
Bottled water to make	1 l
$CaHPO_4$	99 mg[a]
KH_2PO_4	75 mg
$MgSO_4–7H_2O$	75 mg
Soluvit (water soluble vitamins)	10 ml
Amina-Fe	0.92 ml[b]
Kinetin	5 mg
Saccharose (sucrose)	10 g
Activated charcoal, powdered	1 g
Agar	8 g
Pineapple (*Ananas comosus*) juice	25 ml
NH_3 or HCl for pH adjustment	1–2 drops
Potato (Solanum tuberosum) tuber	1 cm^3 per culture vessel

[a] Equivalent to 75 mg $Ca_3(PO_4)_2$ in the original formula.

[b] Equivalent to 0.5 ml Vaminolac® plus 10 mg $FeSO_4$ in the original formula.

The ingredients were mixed in a 1 l Erlenmeyer flask placed on a hot plate with a magnetic stirrer. After the solution cooled to ambient temperature, pH was adjusted to 5.5–6.0 with either NH_3 or HCl.

Preparation of materials

The nutrient medium was distributed to 250 ml Erlenmeyer flasks for autoclaving. Glassware, steel tools, filter paper, potato cubes and deionised water were also sterilized in an autoclave, a Tuttnauer 2340 programmed on a 30 minute cycle (for cold departure) or a 25 minute cycle (for hot departure) at a temperature of 121 °C and pressure of 1.2 bar.

The sowings were done inside an Esco EQU/04-EBC-2A laminar flow cabinet. The interior surfaces were wiped with cotton soaked in 70% ethanol. The culture vessels were 100 mL urine samplers made of polypropylene, individually wrapped in sterile packaging. All non-autoclavable materials were placed in the laminar flow cabinet and exposed to a germicidal UV lamp for 50 minutes. The workspace outside the laminar flow cabinet was also disinfected with a germicidal UV lamp for 50 minutes. The airflow in the cabinet was turned on for 15 minutes to purge airborne contaminants as per manufacturer's instructions[1]. The sterile seal of the culture vessels was broken. The Erlenmeyer flasks with the nutrient medium were transferred from the autoclave onto a hotplate inside the laminar flow cabinet with the thermostat set to 50 °C.

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[1] Esco®. Class II Type A/B3 Biohazard Safety Cabinets. User Operation Manual.

Seed disinfection

The seed was disinfected in plastic test tubes sealed with a cap. Thirty mg of seed was scooped up with a spatula and placed in each tube, except those assigned as blanks. The test tubes were filled nearly to the top with disinfecting solution, capped, and shaken vigorously to remove any air bubbles in contact with the seed. The shaking was repeated every 15 minutes thereafter till the end of the disinfection period.

At the end of the disinfection period, the suspensions were decanted into Erlenmeyer flasks fitted with a polypropylene funnel lined with filter paper to recover the seed. The filter paper was soaked in the same disinfecting solution as the seed. After filtering the suspensions, each filter paper was rinsed five times with approximately 7 ml of sterile deionised water.

Sowing

Each culture vessel was filled with approximately 17 ml of sterile nutrient medium and a potato cube. Once the nutrient medium cooled down to about 35 °C, the seeds were scraped with a spatula from the filter paper and distributed in the culture vessels. The tips of the steel tools were heat sterilized between sowing.

The culture vessels were incubated in a dark cabinet at an ambient temperature of 28 °C. At 41 days after sowing, the vessels were visually examined for the development of fungus, yeast and bacterial colonies. The infected vessels were counted and the non-infected vessels were returned to the cabinet. At 189 days after sowing, vessels with germinated seed were counted.

Statistical methods

Because the response variables are nominal (infected vs. non-infected, germinated vs. non-germinated), one way to analyse the responses is to use logistic regressions (SAS Institute 2003). The level of significance for statistical tests was set *a priori* at $\alpha = 0.05$.

Results and Discussion

The infection rate for the 40 "blank" vessels was nil, which proves that our technique is valid and that all contamination comes from the seed. The infection rates for the two species are compared in Fig. 1. Logistic regression models for both species were not statistically significant, probably owing to the small number of observations per treatment ($n = 8$). In other words, the models did not have a better predictive ability than the mean infection rate, 7.5% for *A. laxiflora* (Lam.) and 25% for *H. robertianum* (Loisel.).

At 189 days after sowing, the seed in several of the vessels had germinated (Fig. 2). The germination rate for the 40 "blank" vessels was nil. Germination rates for the two species are compared in Fig. 3. Despite the small number of observations per treatment ($n = 8$) the logistic regression model for *A. laxiflora* (Lam.) was significant, and the logistic regression model for *H. robertianum* (Loisel.) nearly significant ($p = 0.06$). Model results are presented in Table 2.

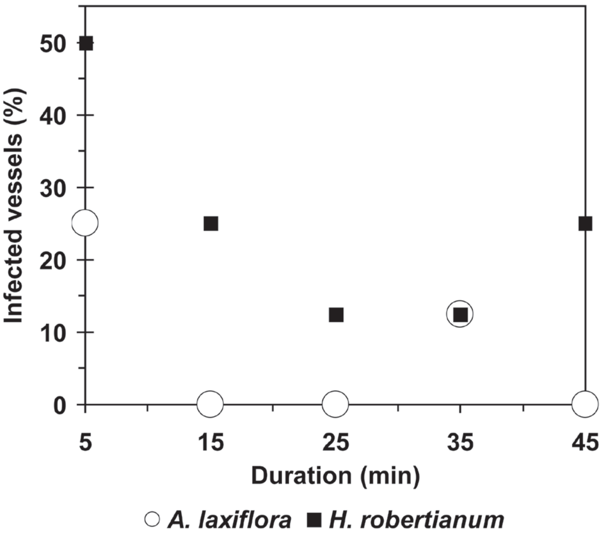

Fig. 1 Effect of treatment duration on infection rates of culture vessels sown with either *Anacamptis laxiflora* (Lam.) or *Himantoglossum robertianum* (Loisel.), 41 days after sowing (n = 8).

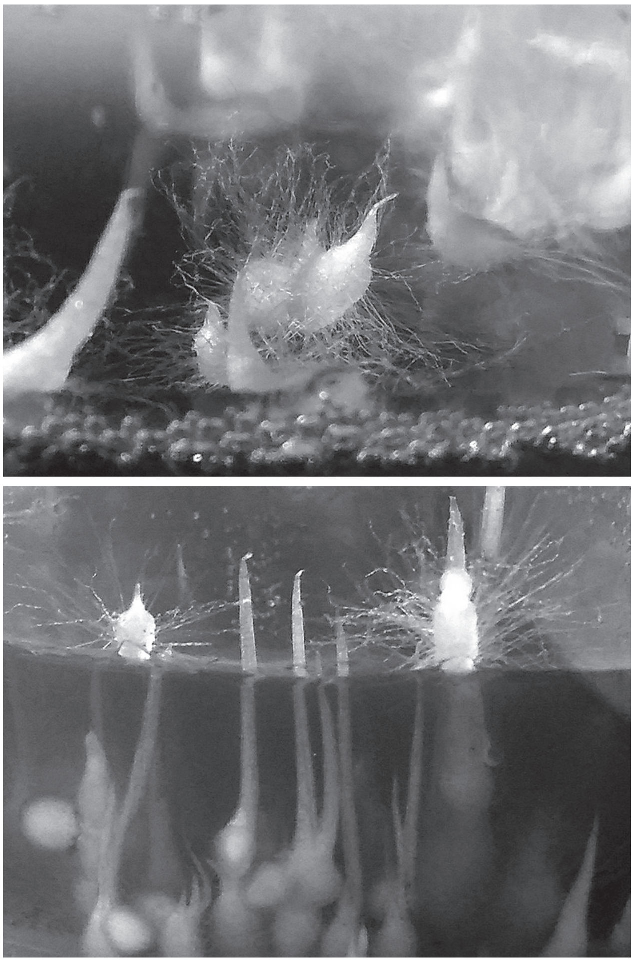

Fig. 2 Photographs of germinated seed: (a) *Anacamptis laxiflora* (Lam.) and (b) *Himantoglossum robertianum* (Loisel.).

Table 2 Model test and parameter estimates for logistic regression of germination rates on treatment duration: LL = log likelihood, DF = degrees of freedom, χ^2 = chi-square, p = probability, R^2 = ratio of "Difference" to "Reduced" LL.

Whole Model Test								
	Anacamptis laxiflora (Lam.)				*Himantoglossum robertianum* (Loisel.)			
Model	–LL	DF	χ^2	$p > \chi^2$	–LL	DF	χ^2	$p > \chi^2$
Difference	4.88	1	9.77	0.002	1.75	1	3.51	0.061
Full	21.58				24.71			
Reduced	26.46				26.46			
R^2	0.18				0.07			
Observations	40				40			
Parameter Estimates for Log Odds of P(germinated)/P(non-germinated)								
Term	Estimate	SE	χ^2	$p > \chi^2$	Estimate	SE	χ^2	$p > \chi^2$
Intercept	1.3827	0.74	3.51	0.061	–0.5680	0.67	0.72	0.395
Duration	–0.0821	0.03	7.56	0.006	0.0452	0.03	3.22	0.073

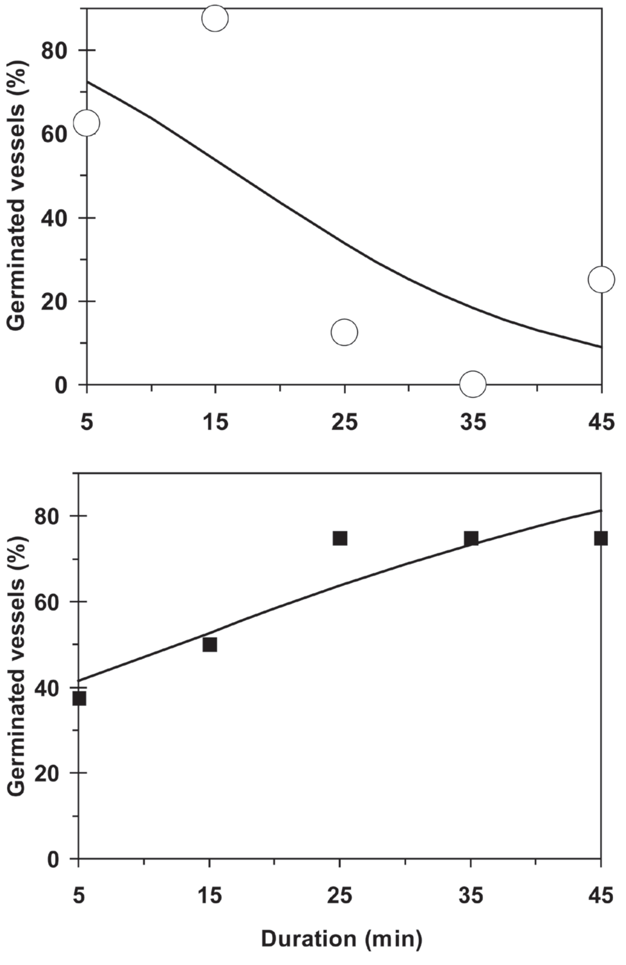

Fig. 3 Effect of treatment duration on germination rates of (a) *Anacamptis laxiflora* (Lam.) and (b) *Himantoglossum robertianum* (Loisel.), 189 days after sowing. Markers are measured points (n = 8) and lines fitted models.

At the end of the day what matters most is germination rates rather than infection rates, because germination rates determine how many useful seedlings are produced. In that sense, the regression models are useful for illustrating the difference in disinfection times required for germination between various species. The germination rate of a species with a thin testa, such as *A. laxiflora* (Lam.), decline with disinfection time, whereas the germination rate of a species with a thick testa, such as *H. robertianum* (Loisel.), increase with disinfection time, within the range of times tested, 5 to 45 minutes. It is important that models are not extrapolated outside the interval of the times tested in order to avoid illogical predictions such as negative times.

Conclusions

Chemical scarification of orchid seeds can be a critical cost in the mass production of orchid seedlings. On one hand, too short a disinfection time may increase infection rates, which translates into a waste of labour and material resources. On the other hand, unduly long disinfection times slow down the procedure and may decrease seed viability. At the end of the day what matters most is to be able to predict germination rates, because these determine how many useful seedlings are produced. Scarification times of only a few minutes in 1% NaClO seem optimal for germination of seeds with relatively permeable seed coats such as those of *Anacamptis laxiflora* (Lam.). Conversely, scarification times of as long as 45 minutes in 1% NaClO may not be long enough for seeds with relatively impermeable seed coats such as those of *Himantoglossum robertianum* (Loisel.). Of course, there is a practical lower limit to the duration of the treatment, which is the time it takes to fill up, agitate and empty the test tubes containing the disinfectant solution. It follows that, for seeds with relatively permeable walls, a weaker NaClO solution such as 0.5% w/w will enable a more precise control of disinfection times.

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MEASUREMENTS OF GROUND-LEVEL OZONE IN CZECH MOUNTAIN FORESTS: WHAT WE HAVE LEARNT FROM USING DIFFUSIVE SAMPLERS

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ABSTRACT

Ground-level ozone (O_3) is a key atmospheric constituent participating in many important atmospheric reactions, and has many negative effects both on human health and the environment. We have measured O_3 ambient concentrations in four Czech rural areas of great natural value: the Orlické hory Mts., Novohradské hory Mts., České Švýcarsko and Jizerské hory Mts. For our measurement we used Ogawa diffusive samplers. The measurements were carried out during the vegetations seasons of 2004–2010, the samplers were exposed for two-weeks periods and the filters analyzed in the CHMI laboratory. The aim of this paper is to summarize the experience we have of using the diffusive sampling method in the field.

Keywords: ground-level ozone, diffusive samplers, exposure

Introduction

Ground-level ozone (O_3) is a key component of the atmosphere. As a potent oxidant and a source of the OH radical, it plays an important role in atmospheric chemistry, removing many air pollutants from the atmosphere by oxidation reactions (Seinfeltd and Pandis 1998). Moreover, due to its radiation-absorbtion properties, O_3 is a potent green-house gas, contributing substantially to climate change (IPCC 2007). At high concentrations, O_3 has negative effects both on human health, ecosystems and the environment (Royal Society 2008). For forests, it is still considered to be the most important air pollutant (Paoletti et al. 2010).

Ozone is a criteria pollutant and there are legal limit values for human health, vegetation and ecosystem protection (Hůnová 2003), and regular monitoring of concentrations of ambient O_3 is therefore required. In Europe, the limit values for O_3 are extensively exceeded (EEA 2016), and with regard to ambient air pollution, O_3 is considered, together with aerosol, as the major threat.

In the Czech Republic O_3 measurements started in 1993, and currently there are *ca* 50 stations, in different environments (urban, rural, mountain), within the nation-wide monitoring network continuously measuring O_3 (ČHMÚ 2016). The data are gathered as 1-hour mean concentrations in a nation-wide database ISKO (Information System of Air Pollution) run by the Czech Hydrometeorological Institute (CHMI).

The results are published regularly in annual reports in a bilingual Czech/ English version (ČHMÚ 2016) free to download at http://portal.chmi.cz/files/portal/docs/uoco/isko/tab_roc/tab_roc_CZ.html, and there are papers on the effects on human health and ecosystems (Hůnová et al. 2003; Hůnová and Schreiberová 2012; Hůnová et al. 2013).

With respect to a nation-wide O_3 analysis, the current monitoring network is suitable. However, if we need a finer spatial resolution, for example, at the scale of a mountain range, the current network is not sufficient. To get a more detailed insight into the spatial variability in concentrations of O_3 we need additional measurements. Passive or diffusive samplers, which provide such a complementary system, are used in many regions throughout the world (e.g. Grosjean et al. 1995; Helaleh et al. 2002; Sanz et al. 2007; Adon et al. 2010), and in particular are recommended for environmental studies (Krupa and Legge 2000). These low-cost devices do not need a power supply and are very easy to manipulate, which makes them very handy for use at locations that are difficult to access, such as mountain forests. Diffusive samplers might be useful for diverse tasks in atmospheric chemistry (WMO GAW 1997) and are widely used for determining the levels of both indoor and outdoor ambient air pollution. Nevertheless, there are still many uncertainties in application of diffusive samplers to be accounted for (Cox 2003). For example, in the field, it is necessary to be aware of the effect of environmental conditions (such as wind, air temperature and relative humidity) and what abrupt changes in these conditions may have on the performance of diffusive samplers (Vardoulakis et al. 2009).

To enlarge the existing nation-wide monitoring network we used diffusive samplers in four rural areas of a great natural value: the Orlické hory Mts., Novohradské hory Mts., České Švýcarsko and the Jizerské hory Mts. The measurements were made by students during the vegetations seasons in 2004–2010 as part of their diploma and Ph.D. theses. The aim of this paper is to summarize the experience we obtained using diffusive samplers, which might be useful for researchers using these measurements.

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Methods

Diffusive samplers

Diffusive samplers are devices for measuring concentrations of ambient air pollutants. As indicated by their name, the sampling procedure is based exclusively on the process of diffusion, in which the pollutant is transported to a filter impregnated with a suitable collecting medium by free flow, according to Fick's first law of diffusion (1).

$$J_1 = -D_{12} \times (dc_1/dz) \quad (1)$$

where

J_1 is the diffusion flux of gas [mol cm^{-2} s^{-1}],
D_{12} is the diffusion coefficient of gas 1 in gas 2 [cm^2 s^{-1}],
c_1 is the concentration of gas 1 in gas 2 [mol cm^{-3}],
z is the length of diffusion [cm].

A diffusive sampler is defined by the European Committee for Standardization as "a device capable of taking samples of gases or vapours from the atmosphere at a rate controlled by a physical process such as gaseous diffusion through a static air layer or a porous material and/or permeation through a membrane, but which does not involve an active movement of air through the device". This is determined by the difference between the pollutant ambient concentration and the concentration at the sorbent, which should be negligible compared to the ambient level.

Diffusive samplers provide time-integrated values for periods ranging from days to several weeks.

The diffusive samplers are simple, inexpensive, and do not require an electricity supply. The major disadvantages are: (1) they do not provide continuous values, (2) and are sensitive to meteorological conditions. Careful design of the sampler, however, can substantially reduce the uncertainty (Hofschreuder et al. 1999).

Currently, diverse commercial diffusive samplers are available. Gerboles et al. (2006) in their laboratory and field comparison of measurements obtained using all the available diffusive samplers for ozone (Ogawa, Analyst, Radiello, IVL badge, Palmes tubes, Passam tubes, Passam badge) found that most of the samplers are suitable for measurements over period of a week and meet the requirement of 30% accuracy required by European legislation (EC 2002).

Diffusive sampler Ogawa

For our measurements, we used the commercial Ogawa sampler (Ogawa and Co., Pompano Beach Florida), which is widely and successfully used for measuring O_3 in U.S. National Parks (e.g. Manning et al. 1996; Cooper and Peterson 2000). Koutrakis et al. (1993), who validated the Ogawa sampler, report that a relative humidity ranging from 10 to 80% and temperature ranging from 0 to 40 °C do not influence the performance of the sampler at typical O_3 levels (40–100 ppb). Fig. 1 shows the Ogawa badge sampler in a shelter provided by the manufacturer, which protects it from unfavourable meteorological conditions.

Fig. 1 Diffusive sampler Ogawa.

The measuring technique was described in detail by Koutrakis et al. (1993). The principle is based on oxidation of nitrite ions on the filter to nitrate by ambient O_3, and analysis of nitrate by ion chromatography. The amount of nitrate ion is proportional to the concentration of ambient O_3. The filters coated with a nitrite-based solution were supplied by the manufacturer. The samplers were prepared and the exposed filters were analyzed for nitrate by ion chromatography in the CHMI laboratory in Ostrava. All laboratory procedures were done according to the Protocol for ozone measurement by Ogawa passive badges (Ogawa Company 2001). The time-weighted average O_3 concentrations for two-week measurements were calculated based on the collected amount of nitrate on the filter, the uptake rate of the sampler and the exposure time. For each campaign, mean nitrate concentration obtained from field blanks was subtracted from the exposed filter values in order to determine the net nitrate concentration. The calculation of the O_3 concentrations in our case, however, in contrast to the Ogawa Protocol (Ogawa Company 2001), was based on the empirical uptake rate obtained from the collocated measurements using a real-time analyser (Thermo Environmental Instruments TEI, M49), based on UV-absorbance as required in the EU (EC 2008). Detailed description of our approach is presented in Hůnová (2006).

Study areas and organizational aspects of monitoring

Ground-level ozone measurements were carried out by students, within their diploma and Ph.D. theses, in the Orlické hory Mts. (Šikýřová 2005), Novohradské hory Mts. (Běláková 2004; Matoušková 2005), České Švýcarsko (Habermann 2006; Holečková 2006) and Jizerské hory Mts. (Matoušková 2011), (Table 1, Fig. 2).

The samplers were placed *ca* 2 m above ground level in forest clearings, at a reasonable distance from vegetation (trees and bushes) in order to avoid the O_3 concentration reduction due to deposition and uptake by vegetation.

Table 1 Areas studied.

	Areas			
	Orlické hory Mts.	**Novohradské hory Mts.**	**České Švýcarsko**	**Jizerské hory Mts.**
Level of wildlife protection	Protected Landscape Area	Natural Park	National Park	Protected Landscape Area
Altitude [m a.s.l.]	940–1011	770–1032	210–420	760–1100
Measurement period	2004, 2005 vegetation season	2004, 2005 vegetation season	2004, 2005 vegetation season	2006–2010 vegetation season
References	Šikýřová (2005)	Matoušková (2005)	Habermann (2006), Holečková (2006)	Matoušková (2011), Hůnová et al. (2011, 2016)

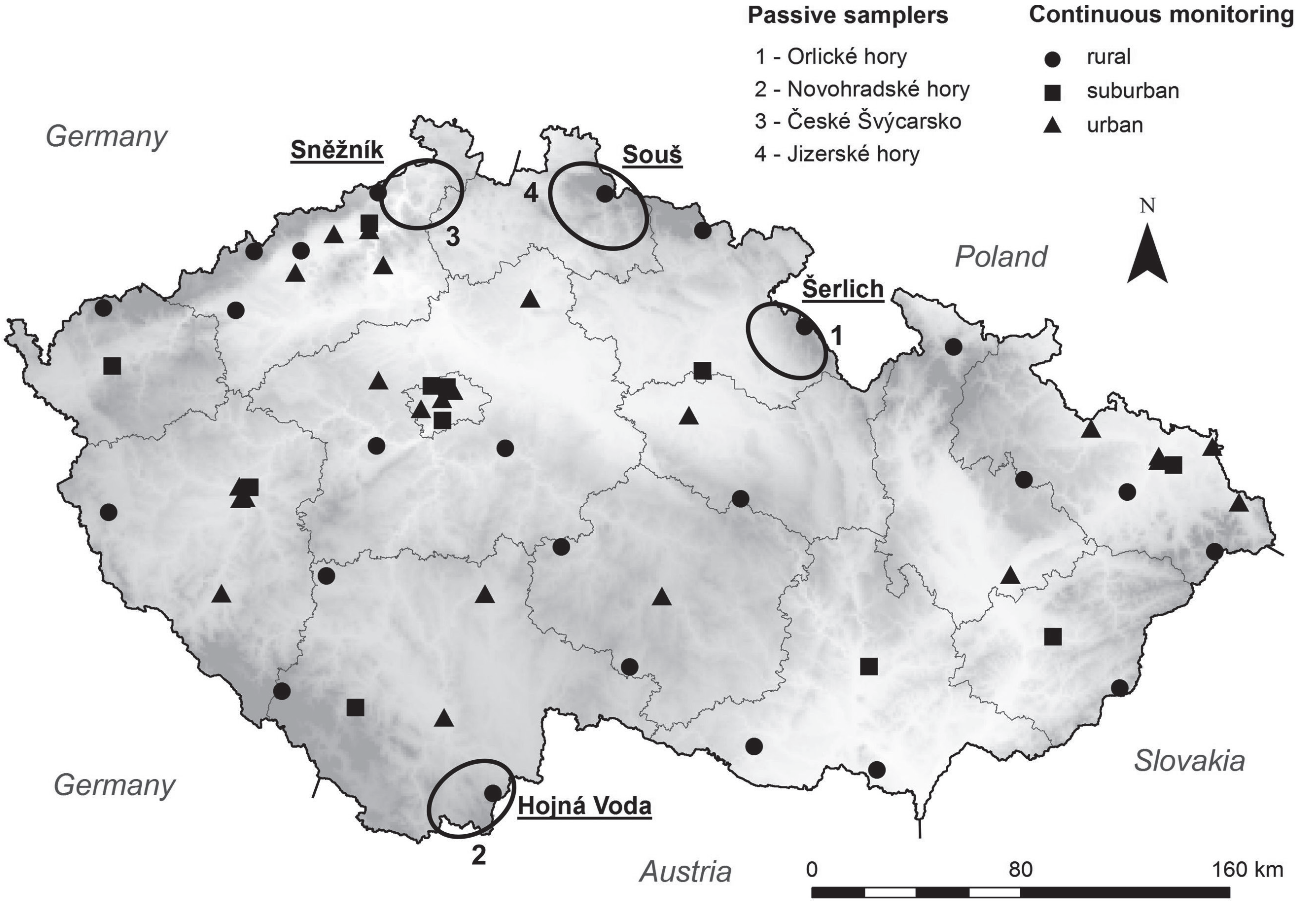

Fig. 2 Ground-level ozone monitoring. The sites at which there was continuous monitoring run by CHMI are depicted by point marks, areas with diffusive samplers are encircled. The underlined sites were used for collocated sampling.

We used a two-week exposures during the vegetation seasons. The first exposure of samplers was highly dependent on the meteorological conditions, as snow cover at the measuring sites prevented access to these areas.

Results and Discussion

Overall functioning of the Ogawa sampler

Meteorological shelter

The Ogawa sampler performed very well, it was simple to use and very convenient for measuring in mountain areas. The sampler itself was protected by a white shelter, specifically designed and provided by the producer. The purpose of this shelter is to protect the sampler from direct sunshine, which might affect O_3 formation, and from wind and rain. Generally, the shelter provided sufficient protection, though during heavy rain accompanied by strong wind, the filters became wet. In such cases, the filter could not perform as expected and the reaction resulting in the oxidation of NO_2^- to NO_3^- was disrupted. Nevertheless, such events rarely occurred. The protective shelter was made of white propylene to reflect solar radiation. The white colour, however, made them very visible in the field and we lost several shelters due to vandalism.

Empirical uptake rate

To estimate the correct uptake rate is critical for obtaining reliable ambient concentrations of O_3. Diffusion driving forces depend on the concentration gradient between the trapped pollutant and its ambient concentration (e.g. Namiesnik et al. 2005). Fick's law holds, however, under ideal, steady state conditions assuming that the sorbent functions as a perfect sink. Nevertheless, in real sampling there are deviations from ideal conditions and thus the theoretical uptake rate can differ from actual rate (Yu et al. 2008). Moreover, Koutrakis et al. (1993), who validated the Ogawa sampler, explicitly declare that its function is not influenced by temperatures between 0 to 40 °C, and relative humidity between 10 and 80%. While the temperature range was met in the field in the areas studied, the relative humidity frequently exceeded 80%.

In contrast to some other studies (e.g. Gerboles et al. 2006; Gibson et al. 2009), the theoretical uptake rate of 21.8 $cm^3 min^{-1}$ stated by the producer (Ogawa Company 2001) did not prove realistic in this study. Therefore, we developed an empirical uptake rate based on collocated measurements of diffusive samplers and continuous monitoring, similar to Bytnerowicz et al. (2008). The O_3 levels, calculated using the theoretical or empirical uptake rate, differed by a factor of 2.3–3.9. This resulted in substantial underestimates of the concentration of O_3 by a factor of 2–4 if the theoretical uptake was used. We assume that in the field, the laboratory uptake rate declared by the manufacturer is not applicable. Namiesnik et al. (2005) in their thorough review of the use of passive samplers in environmental analyses indicated the possible effects of environmental conditions on analyte uptake as the most difficult problem to cope with. Nevertheless, the authors who used Ogawa badge samplers in their studies mostly reported that they used the uptake rate declared by the manufacturer and did not expanded on the issue. The only exceptions are the papers presenting results from the U.S. (e.g. Bytnerowicz et al. 2008). Recently, Malmquist et al. (2014) reported an uptake rate of 27.3 $cm^3 min^{-1}$ for an Ogawa sampler in a Swedish urban environment. We can reasonably assume, however, that in mountain conditions the uptake rate would differ much more from the theoretical uptake rate, as occurred in our mountain forest measurements.

Precision and accuracy

The precision of our measurements was very high (R^2 equalled 0.98). We could reduce the number of parallel filters from four, used earlier, to two. The accuracy was somewhat worse (R^2 equalled 0.82) but still acceptable. Similar studies with Ogawa samplers report analogous precision and accuracy (Manning et al. 1996).

Macrosetting

Macrosetting of the measuring site is critical for obtaining spatially representative results, and so careful attention should be paid to the selection of relevant sites. When measuring O_3 at several sites, it is necessary to consider the aspect of the mountain slope. We found that records from sites with northern aspects to be consistently and significantly lower compared to the sites at similar altitudes but with southern aspects (Hůnová et al. 2016). This fact should be kept in mind in particular when constructing the map of O_3 concentrations based on point measurements. To get comparable results, the measuring sites should have the same aspect.

Regarding the macrosetting of measuring devices, it is extremely important to place them at a sufficient distance from vegetation. Our results from the Jizerské hory Mts. clearly indicated that measuring at the forest edge consistently gave significantly lower O_3 concentrations than that recorded at the same site but in a forest clearing at a distance of ca 20 m from the forest edge (Hůnová et al. 2016). This finding was foreseeable and is in agreement with other similar studies, such as e.g. Karlsson et al. (2006).

Conclusions

The diffusive samplers Ogawa proved to be a very good device for environmental studies in rural areas with a complex terrain during vegetation seasons. They are light to carry, easy to operate and do not need a power supply. They can be used very effectively for indicative measurements in complex terrain of mountain forests, to study both the spatial and temporal variability.

Based on our results, we cannot, however, recommend the use of the theoretical uptake rate stated by the producer for measurements in the field. Rather we would recommend the use of the empirical uptake rate obtained from the collocated measurement of a diffusive sampler and a continuous analyser located in the same area. This empirical uptake rate reflects field conditions of changing meteorological variables much better than a constant uptake rate derived under laboratory conditions. The commercially provided meteorological shelter for protection of exposed filters against extreme meteorological phenomena (such as direct sunshine, heavy rain, strong wind) proved effective under usual conditions. In exceptional weather, such as heavy rain accompanied by strong wind, it failed, however, to provide the required protection, and subsequently the filters became wet and did not perform satisfactorily.

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PREFERENCE OF THE APHIDOPHAGOUS LADYBIRD PROPYLEA DISSECTA FOR TWO SPECIES OF APHIDS REARED ON TOXIC HOST PLANTS

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ABSTRACT

We investigated prey preference of adult male and female *Propylea dissecta* (Mulsant) when fed on the aphids, *Aphis craccivora* and *Lipaphis erysimi*, which sequester toxic allelochemicals from their host plants. Both the male and female adults of *P. dissecta* prefer to consume *L. erysimi* in most mixed diet treatments (cafeteria experiment). This was well supported by significantly high values of the β and C prey preference indices. However, when provided with these aphids separately, the adults showed no significant difference in aphid consumption, regardless of the species of aphid and sex of the adult ladybird. We conclude that host plant allelochemicals/toxicants have a direct effect on prey preference of ladybirds. Host plant toxic constituents can alter the biochemical composition of the most preferred prey and make them the least preferred. Mixing two toxic similar diets can make one diet more suitable than the other.

Keywords: Coccinellidae, *Aphis craccivora*, *Aphis nerii*, *Ranunculus sceleratus*, *Brassica*, diet

Introduction

Dietary habits of predaceous ladybirds (Coleoptera: Coccinellidae) have fascinated many workers (Evans and Gunther 2005; Michaud 2005; Provost et al. 2006; Honek et al. 2008a, b; Sloggett 2008a, b; Hodek and Evans 2012). Ladybirds' food is classified as either essential (supports development and reproduction) or alternative (supports only survival) (Hodek and Evans 2012). Much focus has been given recently to diets that are nutritious or toxic for ladybirds (Guroo et al. 2017). Toxic prey can become more nutritious if ladybirds are fed on them for several generations (Rana et al. 2002). Mixing toxic and nutritious diets could be more beneficial than providing a suitable monotypic diet (Soares et al. 2004; Ferrer et al. 2008; Nedved and Salvucci 2008). Toxicity of aphids is directly dependent on host plant toxicants or allelochemicals, which are sequestered by aphids (Pratt et al. 2008) as a means of defence against predators and may reduce their growth rates (Noriyuki et al. 2012). Hence, prey suitability is directly related to the host plants and the same prey could be either nutritious or toxic depending on its host plant. Wu et al. (2010) found that the aphid, *Aphis gossypii* (Glover) reared on five different host plants have different effects on the population parameters of its predator, *Hippodamia variegata* (Goeze).

Aphids are the primary food of aphidophagous ladybirds, however, their suitability is dependent on various factors (Rana et al. 2002; Soares et al. 2004). Hodek and Evans (2012) consider the black bean aphid, *Aphis craccivora* (Koch) to be toxic due to allelochemicals and/or toxicants, *viz.* amines canavanine and ethanolamine (Obatake and Suzuki 1985) that it sequesters from its host plant (Hukusima and Kamei 1970). The buttercup, *Ranunculus sceleratus* L. is a toxic plant with global wide distribution and contains toxic allelochemicals that may be easily sequestered by aphids, particularly *A. craccivora* (Gupta and Singh 1983; Aslam et al. 2012). It is likely that *A. craccivora* raised on this host plant will be toxic for predators that prefer this aphid when feeding on other hosts. For instance, *A. craccivora*, infesting *Dolichos lablab*, is palatable for the predatory ladybird, *Propylea dissecta* (Mulsant) and is preferred over other aphids (Omkar and Pervez 2001; Chaudhary et al. 2016) and is highly nutritious (Pervez and Omkar 2004; Omkar and Mishra 2005).

Mustard aphid, *Lipaphis erysimi* (Kaltenbach) infesting *Brassica* plants, especially *Brassica campestris* L., sequesters allelochemicals that lower their palatability and nutritive value (Ahuja et al. 2010). This results in slower development and reduced oviposition in many of ladybirds (Pervez and Omkar 2004; Omkar and Mishra 2005) that feed on such aphids. Hence, it would be interesting to quantify the effect of a preferred aphid-prey raised on a toxic host plant with that of a well-established toxic prey raised on a toxic host. Giorgi et al. (2009) review the evolution of food preferences in ladybirds. Previous studies on food preferences of predaceous ladybirds used single species of aphid separately (Omkar et al. 1997, 1999; Omkar and Bind 1998; Omkar and Pervez 2001) and recorded their effect on development and reproduction (Pervez and Omkar 2004; Omkar and Mishra 2005). However, providing several food choices in the same microhabitat, *i.e.* a cafeteria setup, could be more accurate in quantifying prey preference in ladybirds (Ferrer et al. 2008; Nedved and Salvucci 2008; Šenkeříková and Nedvěd 2013; Guroo et al. 2017).

Propylea dissecta is an aphidophagous ladybird occurring in agro-ecosystems in North India and have a high aphid biocontrol potential (Omkar and Pervez 2004a; Pervez and Omkar 2011). Both adults and larvae of *P. dissecta* prefer *A. craccivora* reared on *D. lablab* over *L. erysimi*

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reared on *Brassica campestris* (Pervez and Omkar 2004; Omkar and Mishra 2005). Comparative demographics of this predator attacking five species of aphids indicates that its abundance is more synchronized with *A. craccivora*, while the advent of *L. erysimi* marks its decline in the field (Omkar and Pervez 2004b). Comparison of the functional responses of *P. dissecta* reveals its better at attacking and handling *A. craccivora* than other aphids (Pervez and Omkar 2005). The question, therefore, is could a highly preferred prey in terms of ease of attack and handling, consumption and net reproduction be rejected or less preferred if cultured on a toxic plant? Considering the fact that plant allelochemicals have a major role in the preference and suitability of prey, we hypothesize that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed in different proportions with *L. erysimi*. Hence, we performed laboratory experiments to investigate the prey preference of *P. dissecta* for aphids reared on toxic plants.

Materials and Methods

Stock maintenance

We collected adults of *P. dissecta* in the suburbs of Kashipur, Uttarakhand, Northern India (30.2937°N, 79.5603°E) and brought them to the laboratory. They were sexed and paired in Petri dishes (2.0 × 9.0 cm diameter) containing an *ad libitum* supply of the aphids, *A. craccivora* and *L. erysimi* infesting pieces of leaves/twigs of *R. sceleratus* and *B. campestris*, respectively (n = 10), and then kept in an Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. We monitored mating pairs daily and any eggs they laid were transferred to a new Petri dish (size as above). On hatching, we transferred the neonates to muslin covered beakers (1 litre) and provided them with an *ad libitum* diet until they completed their larval development. We sexed the emerging F_1 adults, which were then isolated and raised on their parental diets.

Prey preference in a cafeteria setup

Prey preference was studied by offering the aphids, *A. craccivora (Ac)* and *L. erysimi (Le)* to adult male and female *P. dissecta* in three ratios, *i.e. Ac : Le* 50 : 100, 75 : 75 and 100 : 50. For this purpose, a 12-hour starved 10-day-old adult male *P. dissecta* was placed in a glass beaker containing the two aphids, *Ac* and *Le* (50 : 100 respectively) infesting plant twigs of their respective host plants (as mentioned above). The beaker was covered with muslin fastened with a rubber band and kept in the Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. Similar treatments were carried using the other two mixtures of aphids (*Ac: Le* = 75 : 75 and *Ac : Le* = 100 : 50). After 24 hours, the beaker was taken out and the number of unconsumed aphids counted to quantify the number of aphids consumed. The experiment was replicated ten times (n = 10). The experiment was repeated using adult female *P. dissecta* as the predator. We calculated Manly's preference index (Manly 1972) for each treatment using the formula, $\beta = \log (N_A / r_A) / [\log (N_A / r_A) + \log (N_B / r_B)]$, where N_A and N_B are the number of prey A and prey B offered to the ladybird and r_A and r_B are the numbers of unconsumed prey. This index overcomes the error associated with prey depletion, i.e. it is applicable in those experiments, where killed prey items are not replaced (Cook 1978; Sherratt and Harvey 1993). If β is close to 1, the predator prefers prey A and if close to 0, prey B. An index value close to 0.5 indicates no preference. We tested the β obtained for each treatment for significant difference from a value of (0.5) using a one sample t-test and statistical software, SAS Version 9.0. Prey preference was also analyzed using the *C* index ($C = (E_A \times N_B) / (E_B \times N_A)$) (Sherratt and Harvey 1993), where E_A and E_B are the number of prey A and prey B consumed. A *C* value between 0 and 1 indicates a preference for prey B and a value more than 1 indicates preference for prey A. We subjected the *C*-index for each treatment to a one sample t-test to determine whether it was significantly different from a value of 1.0 using SAS 9.0. We subjected data on the number of prey consumed to Wilcoxon's matched-pairs signed rank test and the proportion of each prey consumed in a cafeteria setup to a two sample t-test in SAS Version 9.0. We subjected the proportions of aphids, *A. craccivora* and *L. erysimi* consumed by adult male and female *P. dissecta* to a two-way ANOVA using 'aphid species' and 'sex' as independent variables and 'proportion of prey consumed' as a dependent variable using SAS 9.0.

Response to monotypic diet

Adult male and female *P. dissecta* were each provided with a monotypic diet of *Ac* or *Le* to determine their feeding propensity. For this purpose, an adult male was kept in a glass beaker containing 100 third instars of *Ac* infesting a twig of the respective host plant (as above). The beaker was covered with a muslin cloth fastened with a rubber band and kept in the Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. After 24hours, the beakers were taken out and the number of live aphids counted to quantify the number of aphids consumed (n = 10). The experiment was repeated using adult female *P. dissecta* as the predator. We tested the data on prey consumption for normality using the Kolmogorov–Smirnoff test and homogeneity of variance using Bartlett's test in statistical software, SAS Version 9.0. The data on the consumption of aphids when provided with a monotypic diet were subjected to a two sample t-test using SAS 9.0 and the means compared. We subjected the data on the number of the two species of aphids consumed by the adult male and female *P. dissecta* to a two-way ANOVA using 'aphid species' and 'sex' as independent variables and 'prey consumed' as dependent variable in SAS 9.0.

Results

Prey preference in a cafeteria setup

Adult male *P. dissecta* preferred the aphid, *L. erysimi* over *A. craccivora* at all three ratios of the mixed diet tested, *i.e.* at 50 : 100 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.701$; $P = 0.0069$), 75 : 75 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.803$; $P = 0.005$) and 100 : 50 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.701$; $P = 0.0069$; Fig. 1a). However, adult females only preferred *L. erysimi* over *A. craccivora* when an equal proportions of the aphids were provided (75*Ac* : 75*Le*) ($Z_{(1, 18)} = -2.701$; $P = 0.0069$) and at the 100 *Ac* : 50*Le* ratio ($Z_{(1, 18)} = -2.599$; $P = 0.009$). The proportion of prey consumed at the 50*Ac* : 100*Le* ratio by female *P. dissecta* was not statistically significant ($Z_{(1, 18)} = -2.599$; $P = 0.009$; Fig. 1b). Two-way ANOVA revealed a significant main effect of 'aphid' species on the proportion of prey consumed ($F = 44.20$; $P < 0.0001$; d.f. = 1). The main effect 'sex' was not found to be statistically significant ($F = 44.20$; $P < 0.0001$; d.f. = 1). The interaction between 'sex' and 'aphid' did not differ significantly ($F = 2.64$; $P = 0.107$; d.f. = 1). Both β and C indices had significantly negative t-values in all the combinations except for adult females provided with the aphid proportion 50*Ac* : 100*Le* (Table 1).

Response to a monotypic diet

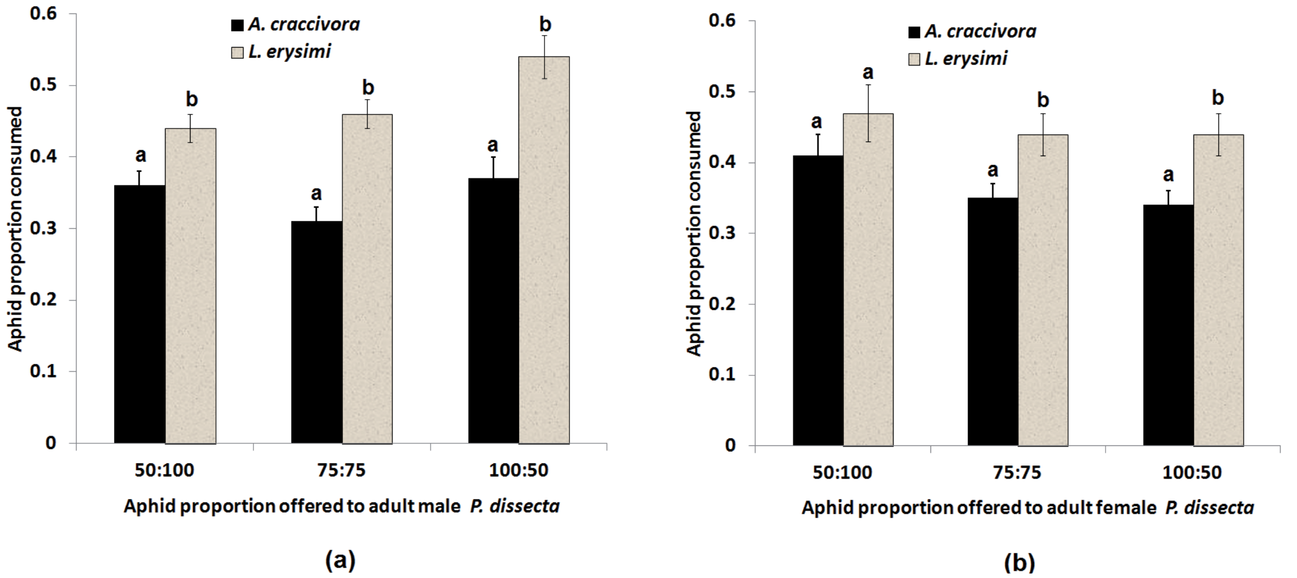
Both adult male ($t = -0.22$; $P = 0.83$; d. f. = 1, 9) and female ($t = 0.92$; $P = 0.383$; d. f. = 1, 9) *P. dissecta* consumed the aphids, *A. craccivora* and *L. erysimi* raised on toxic hosts, equally (Fig. 2). The two-way ANOVA reveals that the main effects 'aphid species' ($F = 0.11$; $P = 0.745$; d.f. = 1) and 'sex' ($F = 0.18$; $P = 0.713$; d.f. = 1), along with the interaction 'aphid species' x 'sex' ($F = 0.43$; $P = 0.516$; d.f. = 1, 39) were not statistically significant.

Table 1 Mean values of the β and *C* indices of *P. dissecta* provided with diets made of different proportions of the aphids *A. craccivora* and *L. erysimi*. The predator prefers *A. craccivora* if β is close to 1 and *L. erysimi* if β is close to 0; β close to 0.5 indicates no preference. *C* index more than 1 indicates a preference for *A. craccivora,* while *C* index between 0 and 1 indicates a preference for *L. erysimi*.

Life stage	Prey ratio	*β* index	t-value	*C* index	t-value
Adult Male	50:100	0.44 ± 0.02	t = −3.57; P = 0.006	0.84 ± 0.03	t = −3.87; P = 0.004
	75:75	0.37 ± 0.02	t = −6.98; P < 0.0001	0.67 ± 0.03	t = −8.14; P < 0.0001
	100:50	0.37 ± 0.03	t = −5.54; P < 0.0001	0.69 ± 0.04	t = −5.92; P < 0.0001
Adult Female	50:100	0.46 ± 0.03	t = −1.06; P = 0.318	0.95 ± 0.06	t = −0.40; P = 0.7
	75:75	0.42 ± 0.02	t = −4.58; P < 0.001	0.80 ± 0.04	t = −4.23; P < 0.001
	100:50	0.42 ± 0.02	t = −4.14; P = 0.003	0.78 ± 0.04	t = −4.07; P = 0.003

Discussion

Both adult male and female *P. dissecta* consumed more *L. erysimi* than *A. craccivora* in almost all mixed diet combinations. This supports our hypothesis that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed with *L. erysimi*. It is also evident from the preference (β and *C*) indices that *L. erysimi* is preferred over the toxic *A. craccivora*. Our finding agrees with that of Guroo et al. (2017) who report similar preference indices for the larvae and adults of *Coccinella septempunctata* L. for *L. erysimi* when mixed with the toxic prey, *Brevicoryne brassicae* L.. However, *C. septempunctata* prefers to consume *L. erysimi* over other species of aphid (Omkar et al. 1997;



(a) (b)

Fig. 1 Proportion of aphids (*A. craccivora; L. erysimi)* consumed by adult (a) male and (b) female *P. dissecta*. Error bars show Standard Error. Different letters indicate the data in the two columns differ significantly.

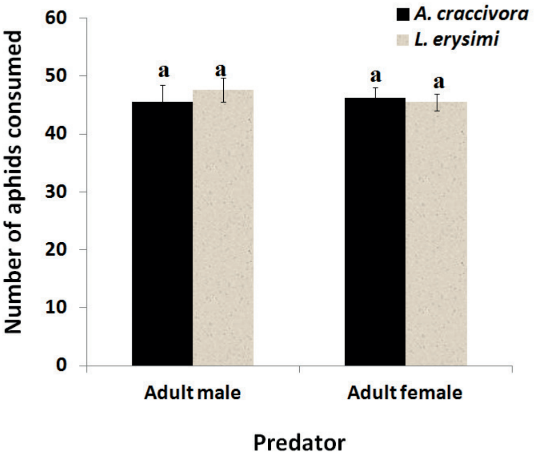


Fig. 2 Prey consumption by adult male and female *P. dissecta* when fed on a single species of aphid. Error bars denote Standard Error. Same letter indicates that data are not significantly different.

Omkar and Srivastava 2003; Ali and Rizvi 2007), whereas *P. dissecta* does not (Pervez and Omkar 2004; Omkar and Mishra 2005). It is clear that plant allelochemicals and toxicants have a direct effect on the quality of aphids in terms of their palatability for predators. *Ranunculus* sp. have secondary metabolites, like glycosides, phenolic compounds, steroids, di and tri terpenes, coumarins and flavanoids, which when sequestered by herbivores may harm predators (Hachelaf et al. 2013). This is the first laboratory study using *A. craccivora* raised on the toxic buttercup, *R. sceleratus*. Previous studies on *P. dissecta* indicate that *L. erysimi* is its least preferred and less suitable prey (Pervez and Omkar 2004; Omkar and Mishra 2005) because this aphid contains secondary metabolites and glucosinolates (Francis et al. 2002; Ahuja et al. 2010). Preference for *L. erysimi* clearly indicates that allelochemicals from *Ranunculus* sp. are more toxic and have a greater effect on the quality of *A. craccivora*.

Recently, Guroo et al. (2017) infer that ladybirds can preferentially feed on toxic or rejected prey if also provided with nutritious prey, as the latter may supply the necessary nutrients absent in the former. Hence, the possible essential nutrients lacking in *L. erysimi* might have been replaced by eating *A. craccivora* resulting in the preference for the former species by *P. dissecta*. The seven-spotted ladybird, *Coccinella septempunctata* L. eats twice as many of the toxic aphid, *Aphis sambuci* L. than of the nutritious aphids, *viz. Acyrthosiphon pisum* (Harris) and *Aphis philadelphi* (Nedved and Salvucci 2008). Šenkeříková and Nedvěd (2013) report that the Harlequin ladybird, *Harmonia axyridis* (Pallas) consumes a greater number of the toxic aphid, *A. sambuci* than of the suitable aphid, *Aphis fabae cirsiiacanthoidis (*syn. *A. philadelphi)*. Similarly, the two spotted ladybird, *Adalia bipunctata* (L.) consumes more *A. craccivora* than of the more suitable aphid, *Acyrthosiphon pisum* (Harris) (Ferrer et al. 2008). Soares et al. (2004) report that a mixed aphid diet is better than a monotypic aphid diet in certain situations (Soares et al. 2004). Continuous rearing on toxic prey for a few generations can also skew the preference of ladybirds towards toxic prey rather than the previously considered more nutritious prey (Rana et al. 2002).

We found a similar reluctant behaviour of both adult male and female *P. dissecta* when provided with monotypic diets. The main effects of 'aphid species' and 'sex' were not statistically significant, which reveals similarity in terms of biochemical contents of the two aphids, and the response of the two sexes of adults towards these aphids. This indicates that both prey were preferred equally when provided separately, and the significant preference for *L. erysimi* in the cafeteria experiment reveals that prey preference was skewed towards this prey. This furthers affirms our theory that possible vital nutrients missing in *L. erysimi* might have been supplemented by eating *A. craccivora*. We conclude that: (i) host plant allelochemicals have a direct effect on the prey preference of ladybirds, (ii) a change in host plant can result in a most preferred prey becoming less preferred, and (iii) mixing two toxic similar diets can make one diet more suitable than the other.

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FACTORS ASSOCIATED WITH THE DISTRIBUTIONS OF ORCHIDS IN THE JESENÍKY MOUNTAINS, CZECH REPUBLIC

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ABSTRACT

Species distribution models are a useful tool and are now often used in many branches of biology, especially when dealing with threatened organisms. In combination with GIS techniques, these models are especially important and valuable for predicting the occurrence of rare species, for example orchids. Orchids are an endangered plant group, protected worldwide. Questions about their conservation are therefore highly discussed, but not all factors affecting their survival and distribution are known. Here we present an example of using SDMs for analysing orchid species occurrence data from the Jeseníky Mountains in the Czech Republic. Our data were analysed using the MaxEnt program, which produces species distribution maps and thus allows the prediction of the potential occurrence of orchids at yet unknown localities. This program also determines the environmental factors affecting species distribution. This is important for the better protection of orchids, because only by knowing these factors can new localities be found or the management plans that are crucial for maintaining orchid localities be improved. We studied the most abundant orchid species in the given region. We determined the most important factors affecting their occurrence and also areas, where new sites are most likely to be discovered and depicted them in potential distribution maps. This approach can help in finding new localities of orchids and in understanding, which environmental factors influence the occurrence of endangered orchids.

Keywords: orchids, climatic factors, environmental factors, Maxent, species distribution models

Introduction

Recently, questions concerning species diversity have become more and more important for scientists worldwide because the diversity of life on Earth is in rapid decline (Dirzo and Raven 2003; Possingham and Wilson 2005). Thus, understanding the main factors determining species diversity (Possingham and Wilson 2005) and identifying important areas for conservation (Tsiftsis et al. 2011) is important because this is crucial for their survival. This especially holds for threatened groups such as orchids (Efimov 2011; Feldman and Prat 2011).

The orchid family is one of the largest and most diverse in taxa in the flowering plant kingdom, with estimates of 880 genera and about 20,000 to 35,000 species (Dressler 1993; Chase et al. 2003; Cribb et al. 2003; Tsiftsis et al. 2011). Many characteristics, such as great species richness, their specific role in ecosystems or endangered situations make it crucial to explore the distribution and conservation status of Orchidaceae (Zhang et al. 2015).

Predictive modelling of the geographic distributions of species based on the environmental conditions at sites of known occurrence is an important technique in analytical biology, with applications in conservation and reserve planning, ecology, evolution, epidemiology, invasive-species management and other fields (Yom-Tov and Kadmon 1998; Corsi et al. 1999; Peterson et al. 1999; Scott et al. 2002; Welk et al. 2002; Peterson and Shaw 2003; Phillips et al. 2006). Species distribution models (SDMs) are widely used in many branches of biology (Elith and Leathwick 2009) and are especially useful for threatened species (Guisan et al. 2013). These numerical tools combine species occurrence records with environmental data (Elith and Leathwick 2009). In combination with GIS techniques, these models are especially important and useful for predicting occurrence of rare species (Guisan and Thuiller 2005), like endangered species of Orchidaceae.

In this study, we used the maximum entropy algorithm in the MaxEnt application (Elith et al. 2006; Phillips et al. 2006; Phillips and Dudík 2008; Elith et al. 2011). This algorithm uses maximum entropy and Bayesian methods to estimate the probability distribution for each species based on their presence or absence. Since becoming available in 2004, MaxEnt has been utilized extensively for modelling species distributions. This approach is used by conservationists for predicting the distribution of a species from a set of occurrence records and environmental variables (Elith et al. 2011; Fourcade et al. 2014) as well as many other fields of biology and ecology that cover diverse aims across ecological, evolutionary, conservation and biosecurity applications (Elith et al. 2011). Presence-only modelling methods require a set of known species occurrences together with predictor variables such as topographic, climatic, edaphic, biogeographic and/or remotely sensed data (Phillips et al. 2006; Phillips

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and Dudík 2008). An output of the MaxEnt program are maps of the distribution of suitable niches and information on the input variables needed to be put into the model.

As previously stated, species distribution data and modelling are increasingly being used. Although the relationship between species distribution and environmental variables has been extensively investigated (Peppler-Lisbach and Schröder 2004; Ferrier and Guisan 2006; Ferrier et al. 2009), these relationships were rarely used in the past to explain the spatial arrangement of high conservation value areas (Gelfand et al. 2005). Despite the long history of studies on orchids, only a minute part of previous papers concerning potential and spatial distribution, niche conservatism, phytogeography, migration routes or conservation strategies of this taxonomic group included the use of species distribution models (e.g. Tsiftsis et al. 2011; Angulo et al. 2012; Kolanowska 2013; Wan et al. 2014; Vogt-Schilb et al. 2015; Hernandez-Ruiz et al. 2016; Reina-Rodríguez et al. 2016; Vollering et al. 2016).

All recently published studies on orchids use only climatic variables in the modelling of species' distributions. Here both climatic variables and the effect of vegetation cover (mainly consolidated layer of ecosystem, KVES) are seen as determining the distribution of species of orchids in the Czech Republic. These two factors have not been previously seen as acting together in determining the distribution of orchids.

Methods

The study site was located in the mountain range in the Hrubý and Nízký Jeseník Nature Conservation Area, in the Highlands of Hanušovice and surroundings in the Czech Republic (Fig. 1). This area is situated in the north-east of the Jeseníky Nature Conservation Area (NCA) at altitudes from about 300 to 1400 masl. Because of the wide range in altitude, many different types of habitats occur in this region, such as peat bogs, meadows and pastures, oak-hornbeam forests or are naturally forest-free areas. Many of the different habitats in the Jeseníky NCA are managed and suitable for many species of orchids, even critically endangered species such as *Listera cordata* or *Orchis mascula*.

As a source of data we used information in 5 databases: those of the Nature Conservation Agency of the Czech Republic (2006), the Czech National Phytosociological Database and the Floristic Documentation, all deposited in the Department of Botany and Zoology, Faculty of Science of the Masaryk University in Brno (2005), the database of the South Bohemian Branch of the Czech Botanical Society (2017) and that of the inheritance of the late František Procházka (10,000 items, digitized from original cards). All the data from these databases are deposited in one comprehensive database at the Global Change Research Institute CAS, Department of Biodiversity Research in České Budějovice, but in order to protect the orchids at the localities studied, there is no public access to either of these databases.

During 2017, we visited as many localities as possible to check whether particular species of orchids were still present there. If the species was found, the number of flowering plants was counted and all important information, such as accurate GPS coordinates, the state of the locality and whether it was mown were recorded. A total of 146 localities was checked.

Because of the special demands we used the methods in MaxEnt and only the four most numerous species

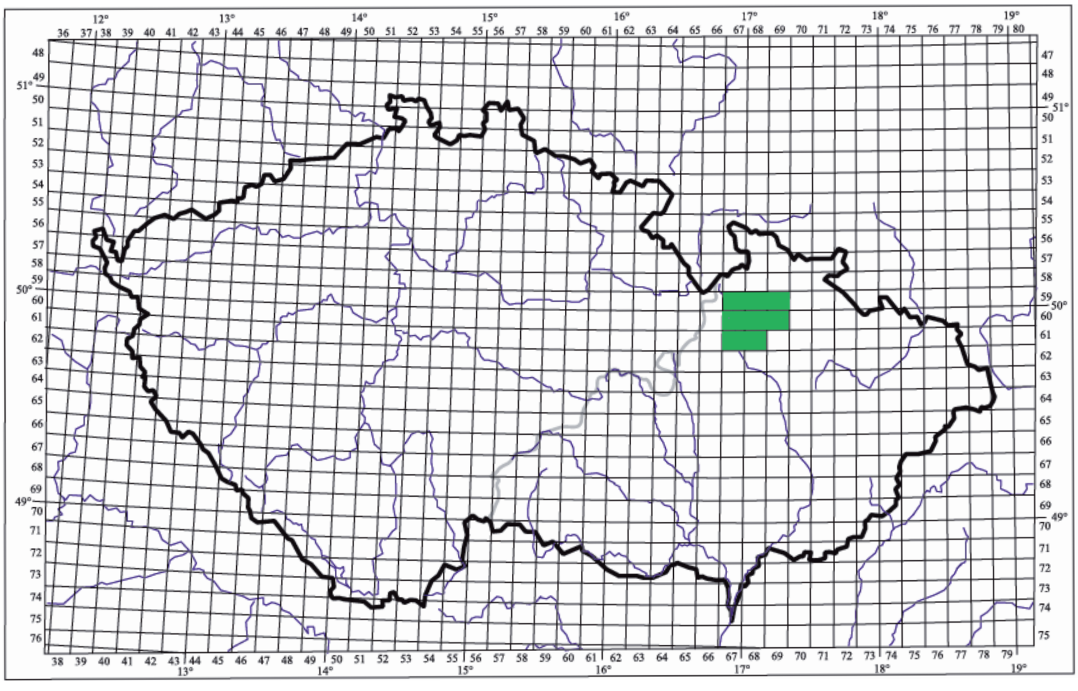

Fig. 1 Map showing the location of the study site, depicted in green, in the Czech Republic – Jeseníky Nature Conservation Area.

were incorporated in all analyses. The first species analysed was *Dactylorhiza fuchsii* (Druce) Soó, which occurs in wet as well as in dry meadows, the second was *Dactylorhiza majalis* (Rchb.) P. F. Hunt & Summerh., which lives in wet meadows, the third was *Gymnadenia conopsea* (L.) R. Brown, which usually occurs in xerothermic meadows or pastures and the fourth was *Platanthera bifolia* Rich., which flourishes in open deciduous forests.

A set of environmental and habitat variables was obtained from datasets for the Czech Republic. The list of all variables used in the analysis of the four most numerous species is described in Table 1.

All analyses were done using the MaxEnt program version 3.3.2 (Phillips et al. 2006; Phillips and Dudík 2008; Elith et al. 2011). In this program, the jack knife procedure reveals how the species react to different environmental factors. Two different blue bars are always displayed in the resulting figure. The length of the dark-blue bar indicates the importance of the effect of the selected factor. The length of the light-blue bar indicates how much information would be lost, if the corresponding factor were excluded from the analysis. Thus, deletion from the model of a factor associated with a long light-blue bar would result in a large loss of explanatory power. Then we performed the analysis for each species.

Before describing them, we have to elucidate the meaning of one factor used in the analyses that consists of 40 sub-factors: the "consolidated layer of ecosystems" (KVES) (AOPK ČR 2013). KVES is a list of 40 types of habitat, named KVES_1, KVES_2, …, KVES_40. For example, KVES_6 is mesophilic meadow, KVES_29 is deciduous forest etc. (see Table 2 for further examples). Based on our many years research on orchids and information in the literature on ecological requirements of individual orchid species (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005), we thought that these factors might be important determinants of the occurrence of these species and therefore included them in the analyses. KVES without a number is the presence of a certain class of habitat, which is therefore a categorical variable. If this is statistically significant, it means that the occurrence of the corresponding species of orchid depends on a particular habitat. Sometimes, environmental heterogeneity (here called KVES_var – see Table 2), expressed as the number of different types of KVES per unit area (sometimes also called "grain size" in the literature, especially that on landscape ecology) may be important, a large KVES_var indicates that the landscape consists of a mosaic of many small units, like fields, pastures, meadows, forests etc., which usually indicates a low intensity of agriculture and consequently a good habitat for protected species. Therefore, we always included KVES_var in our analysis.

In the analysis, the influence of climatic factors, environmental variables and other basic abiotic factors on orchid distribution was studied. There is a list of these factors in Table 1 and their description in Table 2. The climatic data were obtained from the Global Change Research Institute CAS and a climate character for the timeline of 1981–2011 was created. The objective of this analysis was to determine the extent to which these variables are associated with the occurrence of the species of orchid studied. However, at least some of the other most important environmental non-climatic factors were also included, so as not to produce a purely climatic model, which does not seem to be appropriate in our case, as our experience and information in the literature indicates that climate alone cannot account for the presence of orchids in these temperate and rather flat regions (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindl-

Table 1 The list of variables used in the analysis.

Dactylorhiza fuchsii	*Dactylorhiza majalis*	*Gymnadenia conopsea*	*Platanthera bifolia*
alkali	dem	dem	dem
dem	frost_days	KVES	KVES
frost_days	KVES	KVES_6	KVES_6
KVES	KVES_6	KVES_var	KVES_29
KVES_6	KVES_9	reactivity	KVES_var
KVES_9	KVES_20	solar_rad	reactivity
KVES_20	KVES_23	temp	solar_rad
KVES_23	KVES_var	TPI	TPI
KVES_var	reactivity	vert_het	vert_het
reactivity	solar_rad		
temp	temp		
TPI	TPI		
vert_het	vert_het		

Table 2 Description of all important factors used in all analyses.

Code	Description
alkali	alkalinity of bedrock
dem	altitude
frost_days	number of freezing days per year
KVES – 4 – 6 – 7 – 9 – 11 – 19 – 20 – 23 – 29 – 30 – var	consolidated layer of ecosystems – alluvial and wet meadows – mesophilic meadows – alpine meadows – floodplain and wetland forests – brash forests – wetlands and coastal vegetation – peat bogs and water springs – swamps and marshes – deciduous forests – mixed forests – habitat heterogeneity (number of different types of habitats)
reactivity	reactivity of rocks in a bedrock
solar_rad	solar radiation - total amount of incoming solar insolation (WH/m^2)
temp	temperature
TPI	topographic position index
vert_het	vertical heterogeneity (standard deviation in terms of altitude)

Table 3 Percentage contribution of particular factors for each of the species studied.

Factor	Percentage contribution *Dactylorhiza fuchsii*	Factor	Percentage contribution *Dactylorhiza majalis*	Factor	Percentage contribution *Gymnadenia conopsea*	Factor	Percentage contribution *Platanthera bifolia*
frost_days	**35**	**reactivity**	**20.5**	**dem**	**47.8**	**solar_rad**	**27.1**
reactivity	**17**	**KVES_9**	**19**	**KVES**	**21.5**	**KVES**	**25.7**
KVES_20	8.9	**KVES_6**	**14.4**	**KVES_var**	**11.7**	**KVES_var**	**21.6**
KVES_9	8.8	dem	13.8	KVES_6	9.7	reactivity	11.4
KVES_var	6.6	KVES	12.8	reactivity	4.3	KVES_6	5.9
KVES	6.5	KVES_var	10.4	vert_het	1.8	dem	4.6
alcali	6.2	vert_het	3.8	saolr_rad	1.7	vert_het	2.3
TPI	3.2	KVES_20	2.3	TPI	1.2	KVES_29	1.5
KVES_6	2.5	frost_days	1.3	temp	0.3	TPI	0.1
dem	2.3	salor_rad	1.2				
vert_het	1.5	TPI	0.5				
temp	1.3	temp	0.1				
KVES_23	0.1	KVES_23	0				

mann 2004; Průša 2005). Therefore, we selected factors based on our experience and information in the orchid literature, such as descriptions of the ecological requirements of the species of orchids studied (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005). We also added TPI (topographic position index) and vertical heterogeneity (vert_het; see Table 1) as they might be important for the occurrence of particular species of orchids. TPI classifies landscape in terms of slope, landform category and its position in the terrain, for example, whether at the top of a hill, in a valley or near a depression. Another important environmental variable is vertical heterogeneity (vert_het). This factor indicates the degree to which the landscape undulates near the locality studied, in terms of altitudinal range. All of these factors are described in Table 2. The influence of alkalinity and type of rock at a particular locality were also included in the analysis (Chuman et al. 2017) because according to the literature, some orchids only grow in soils on one or two types of rock (Procházka and Velísek 1983; Dykyjová 2003; Průša 2005). The final potential distribution map was then created.

In our results, only the most important factors for each species of orchid are described.

The list of factors used in the analysis is given in Table 1 and detailed descriptions of each factor in Table 2.

Results and Discussion

Dactylorhiza fuchsii (Druce) Soó

The results of the jack knife procedure illustrated in Fig. 2 indicate that many of the variables included in the analysis have an effect on the distribution of *D. fuchsii*. According to the Maxent analysis, the most important factors are the number of frost days per year (frost_days; 35%) and reactivity of the bedrocks at a particular locality (reactivity; 17%). Other variables had only a small effect (less than 10% of variability explained) and thus were not included in the following description. A detailed list of the contributions (%) of particular factors for each of the species studied is shown in Table 3.

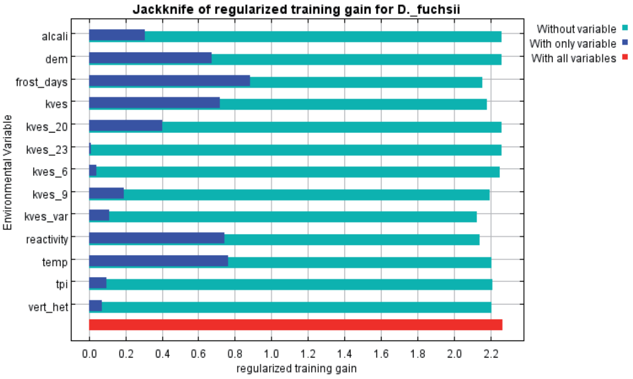

Fig. 2 The graph of the results of the jack knife procedure of selected factors for *Dactylorhiza fuchsii*.

A closer look at the associations of the most important variables with the distribution of this species (Fig. 3) reveals certain patterns.

Fig. 3a indicates that *D. fuchsii* has a high probability of being present in areas with more frost days per year. From this picture, it is clearly visible that this species is almost absent in areas where there are only a few frost days per year. This means that *D. fuchsii* should occur at high altitudes where the temperatures are much colder than in the lowlands, which is in agreement with information in the literature (see Procházka and Velísek 1983; Průša 2005).

In Fig. 3b, the association with the reactivity of the bedrock at a particular locality is shown. According to this, *D. fuchsii* should occur with high probabili-

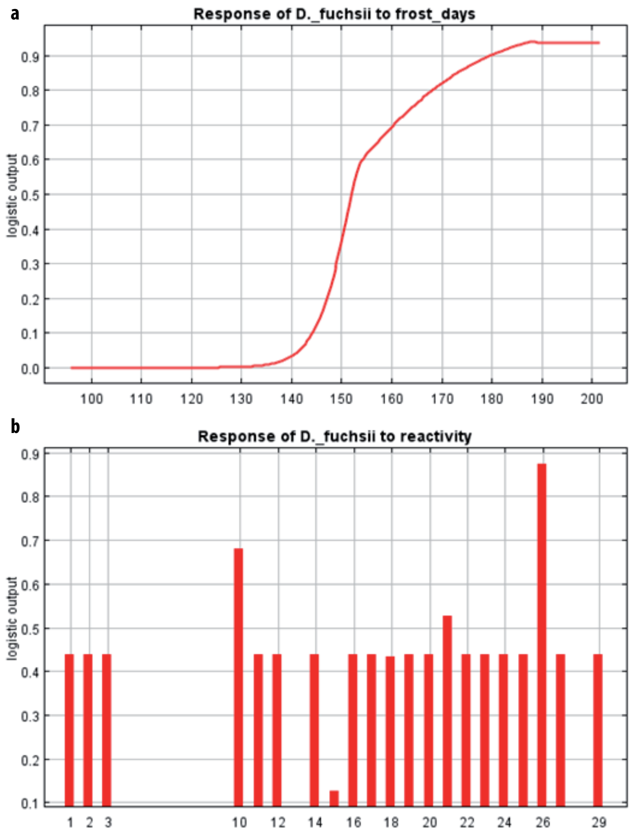

Fig. 3 Association of *Dactylorhiza fuchsii* with a) number of frost days per year (frost_days) and b) reactivity of bedrock at a particular locality (reactivity).

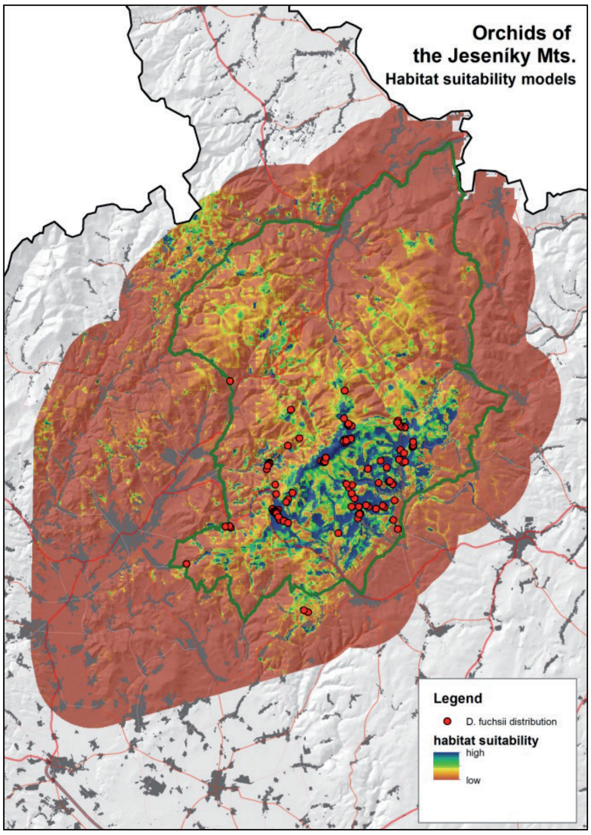

Fig. 4 Map of the potential distribution of *Dactyorhiza fuchsii* in the Jeseníky Mountains. The green line on the map is the border of the Jeseníky Nature Conservation Area.

ty on sandstone, conglomerates or arkose (number 26) and metamorphic rocks like phyllite (number 10) and low probability on consolidated sediment rocks like greywacke, siltstone or shale (number 15).

In Fig. 4, is a map showing the potential distribution of *D. fuchsii*. It is clear that there are still some places in the region studied that are also suitable for this species but all of them are in the vicinity of its current distribution. The most suitable places for finding new localities are mainly around Skřítek National Nature Reserve and west of the village Karlov pod Pradědem in the highest parts of the Jeseníky mountains.

Dactylorhiza majalis (Rchb.) P. F. Hunt & Summerh.

The results of the jack knife procedure illustrated in Fig. 5 indicates the various factors associated with the distribution of *D. majalis*. Many of the selected variables have an effect but according to the analysis, the most important factors are the reactivity of the bedrock at a particular locality (reactivity; 20.5%), presence of floodplain and wetland forests (KVES_9; 19%) and mesophilic meadows (KVES_6; 14.4%) nearby. We did not include consolidated layer of ecosystems (KVES) and altitude as important even though they appear to be important, because as stated above, KVES without a number means that the occurrence of a particular species of orchid is habitat dependent. The jack knife procedure revealed certain types of KVES (KVES_6 and 9) as more important, thus they were not included as a separate factor again. In the case of altitude (dem), the analysis indicates 700 m as the most probable altitude for the occurrence of *D. majalis*, but the distribution of this species extends from lowlands to mountains (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005). The indicated altitude (700 m) is due to the mean altitude of the area studied in the Jeseníky Mountains, thus this factor was also not included as an important variable (see Table 3 for more information).

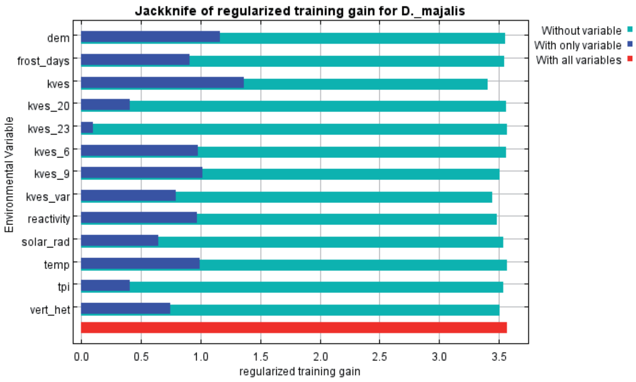

Fig. 5 The graph of the results of the jack knife procedure of selected factors for *Dactylorhiza majalis*.

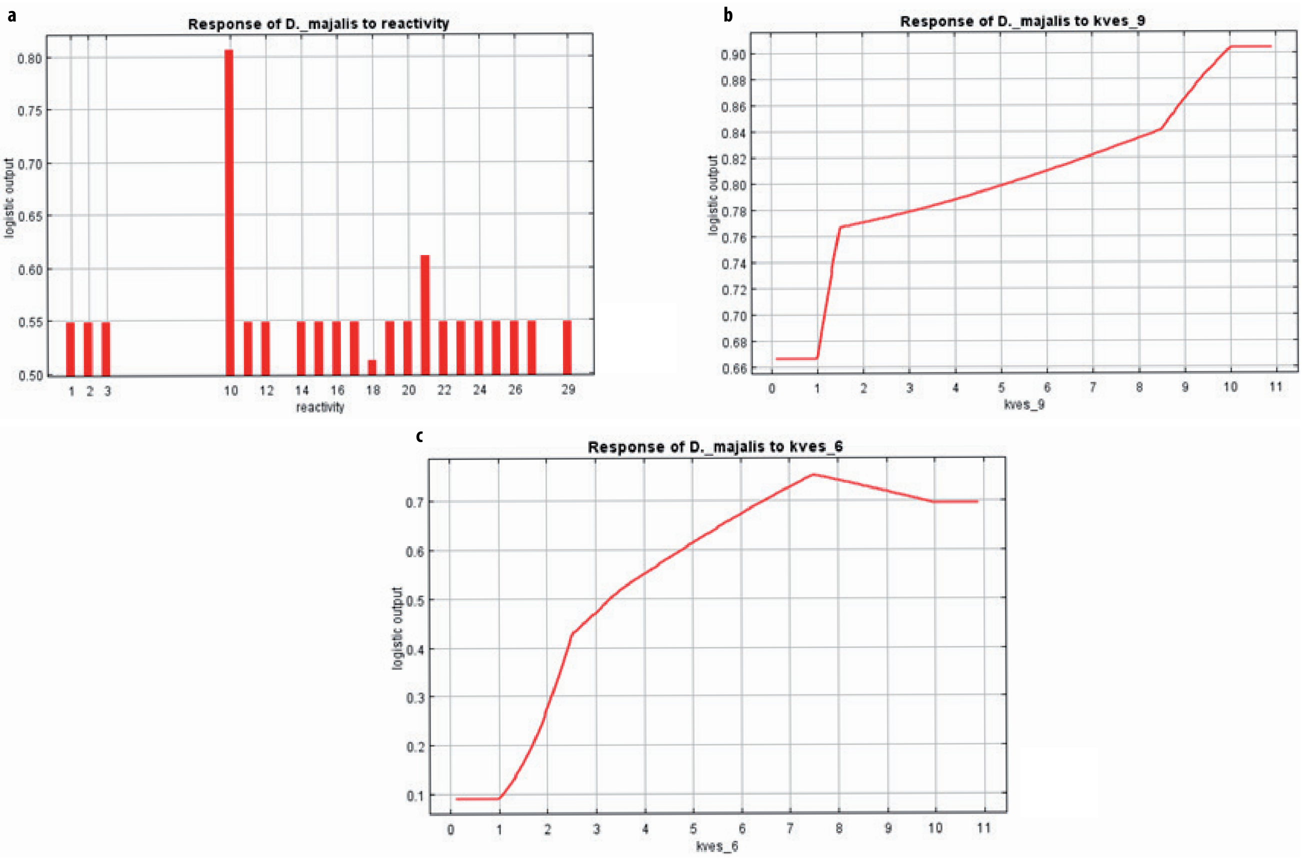

Fig. 6 Association of *Dactylorhiza majalis* with the a) reactivity of the bedrock at a particular locality (reactivity), b) presence of floodplain and wetland forests near selected localities (KVES_9) and c) presence of mesophilic meadows near selected localities (KVES_6).

A closer inspection of the factors that were significantly associated with the distribution of *D. majalis* (Fig. 6) proved interesting.

Fig. 6a indicates the association of this species with the reactivity of the bedrock (reactivity). It occurs mainly in areas with rock types 10 and 21, which are metamorphic rocks like phyllite (No. 10) or metagabbro and dolerite (No. 21). This species does not flourish on metagranite, migmatite or metagreywacke (No. 18).

Fig. 6b clearly shows that the higher probability of occurrence of *D. majalis* is in areas with high occurrence of floodplain and wetland forests (KVES_9). Although *D. majalis* is mainly a meadow species (Jersáková and Kindlmann 2004; Průša 2005), it is also possible to find it in small open patches in open forests (e.g. forest and grassy clearings or small wet meadows).

Fig. 6c indicates the association of the distribution of this species with mesophilic meadows (KVES_6). It is clear that the occurrence of this species is closely associated with mesophilic meadows. This means that *D. majalis* is more likely to occur in areas in the vicinity of mesophilic meadows. Both previous figures of KVES indicate that if there are any wet areas nearby, the probability of *D. majalis* being present is almost zero. This is in accordance with the literature, which indicates that these types of habitat are suitable for this species (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005).

Map of the potential distribution of *D. majalis* (Fig. 7) indicates there are other potentially suitable localities for this species in the Jeseníky Mountains, but most are near to existing localities. New localities are most likely to be found around Vysoká Hole hill and in the Skřítek National Nature Reserve. Other potentially suitable areas are between the villages of Karlova Studánka and Andělská Hora and in the vicinity of the village Nová Rudná.

Gymnadenia conopsea (L.) R. Brown

The results of the jack knife procedure are illustrated in Fig. 8, which indicates that distribution of this species is associated with many of the variables considered. The analysis indicates that the most important variables are altitude (dem; 47.8%), consolidated layer of ecosystems (KVES; 21.5%) and habitat heterogeneity (KVES_var; 11.7%). In this case, it is relevant to mention also the fourth most important factor because it is connected with the KVES variable, which indicates that the occurrence of *G. conopsea* is dependent on a particular type of habitat, mesophilic meadows (KVES_6), which is congruent with information in the literature (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005). Although this factor explained less than 10% of the variability and was not included in the list of the most important factors, it is important to mention it. The detailed contribution of each factor is shown in Table 3.

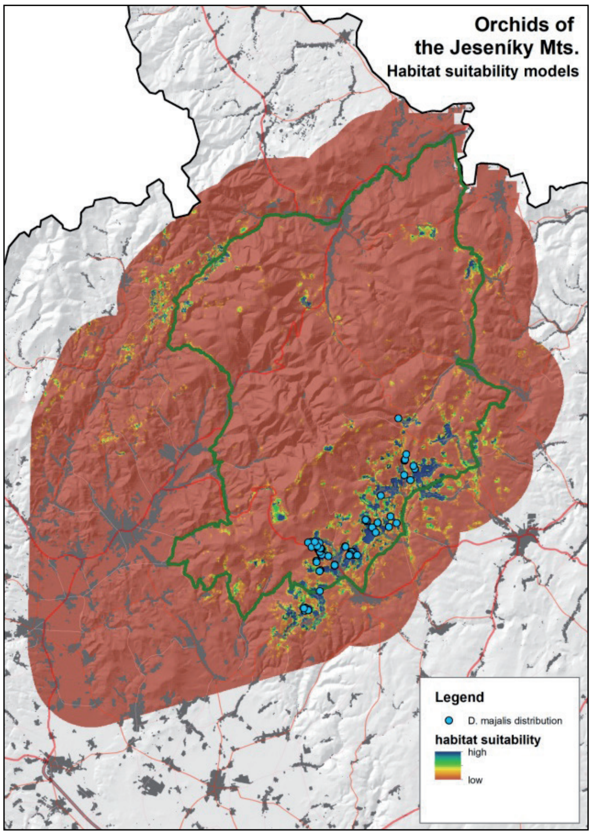

Fig. 7 Map showing the potential distribution of *Dactyorhiza majalis* in the Jeseníky Mountains. The green line indicates the borders of the Jeseníky Nature Conservation Area.

A closer inspection of the most important variables associated with the distribution of *G. conopsea* (Fig. 9) proved interesting.

Fig. 9a indicates the important association between altitude (dem) and the distribution of this species. It is clear that *G. conopsea* occurs mainly at high altitudes in highlands and mountains, and rarely in lowlands. Průša (2005) mentions that this species is absent in lowlands.

Fig. 9b indicates that the distribution of this species is associated with the consolidated layer of ecosystems (KVES) and this species occurs mainly in wetlands of various types (KVES_19, KVES_6 and KVES_4), natural shrub land (KVES_17) and alpine meadows (KVES_7). Especially its possible occurrence in alpine meadows is interesting because this habitat only occurs on the highest parts of mountains in the Czech Republic and one of the areas is in the area studied, the Jeseníky Mountains. All of these results are in accordance with information in the literature (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005), which indicate that this species mainly occurs in from hilly areas to mountains and in wet meadows as well as in xerothermic pastures and shrub land.

Fig. 9c indicates that the third most important factor is habitat heterogeneity (KVES_var). This indicates that the distribution of *G. conopsea* is mainly associated with landscapes consisting of a mosaic of many smaller biotopes. This confirms our expectation and experience gained from field studies that this species is more likely be found in these types of landscapes, probably because they are little affected by agriculture.

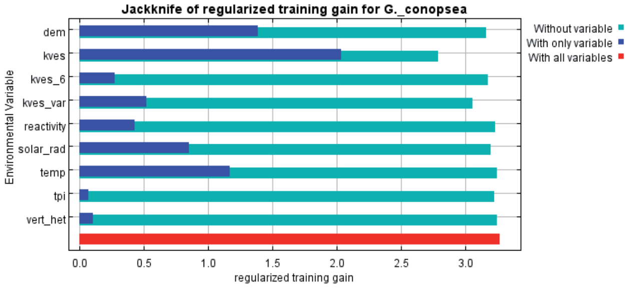

Fig. 8 The graph of the results of jack knife procedure of selected factors for *Gymnadenia conopsea*.

In Fig. 10, there is a map of the potential distribution of *G. conopsea* in the area of the Jeseníky Mountains studied. It is clear that there is still a possibility of finding new localities for this orchid. Except for the current localities, the most suitable habitats are likely to be mainly on the highest mountains (e.g. Vysoká Hole, Praděd, Mravenečník) and in small areas east of the city of Jeseníky and north of village Branná outside of the Jeseníky NCA.

Platanthera bifolia Rich.

The results of the jack knife procedure are displayed in Fig. 11. The analysis revealed that solar radiation (solar_rad; 27.1%) is the most important factor associated with the distribution of *Platanthera bifolia*. Other important factors are consolidated layer of ecosystems (KVES; 25.7%) and habitat heterogeneity (KVES_var; 21.6%). For more information about the association with various factors see Table 3.

Closer inspection of the factors most significantly associated with the distribution of *P. bifolia* (Fig. 12) proved interesting.

Fig. 12a indicates that the occurrence of this species is most closely associated with solar radiation (solar_rad). In the Czech Republic, the extent of solar radiation dose not differ across the whole country so this factor mainly tells us, that *P. bifolia* occurs in shady or sunny places. From this picture, it is clear that this species occurs mainly in places with a low value for solar radiation that is in shady places. As mentioned above, this result corresponds with the information in the literature on the habitat of *P. bifolia* (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005).

Fig. 12b indicates the biotope (KVES) most closely associated with each species. According to this analysis it is mainly brash forest (KVES_11), but also alluvial and wet meadows (KVES_4), mesophilic meadows (KVES_6), floodplain and wetland forests (KVES_9) and mixed forests (KVES_30). These results are congruent with the previous results for solar radiation that indicate this species occurs mainly in shady places. Forests are shady

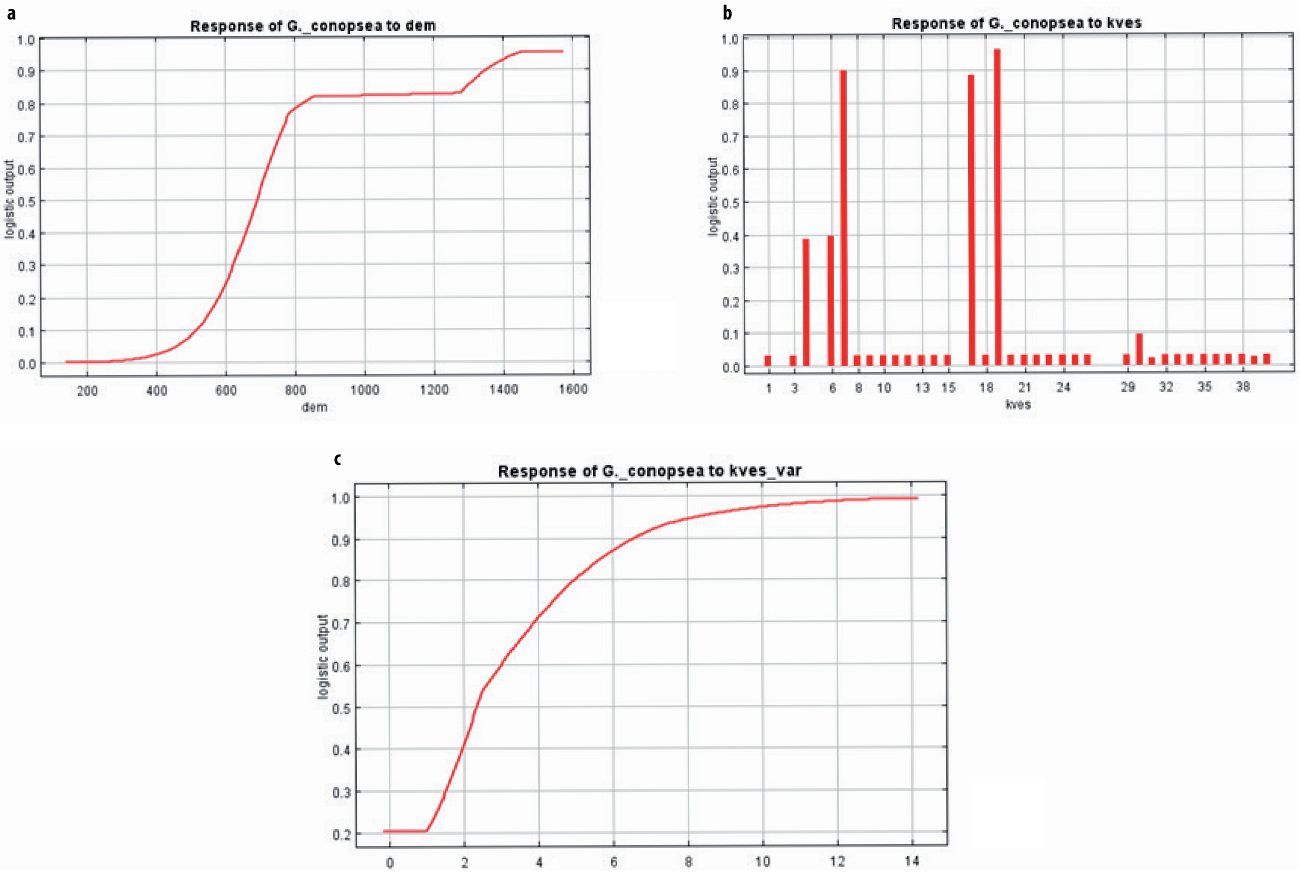

Fig. 9 Association of *Gymnadenia conopsea* with a) altitude (dem), b) consolidated layer of ecosystems (KVES) and c) habitat heterogeneity (KVES_var).

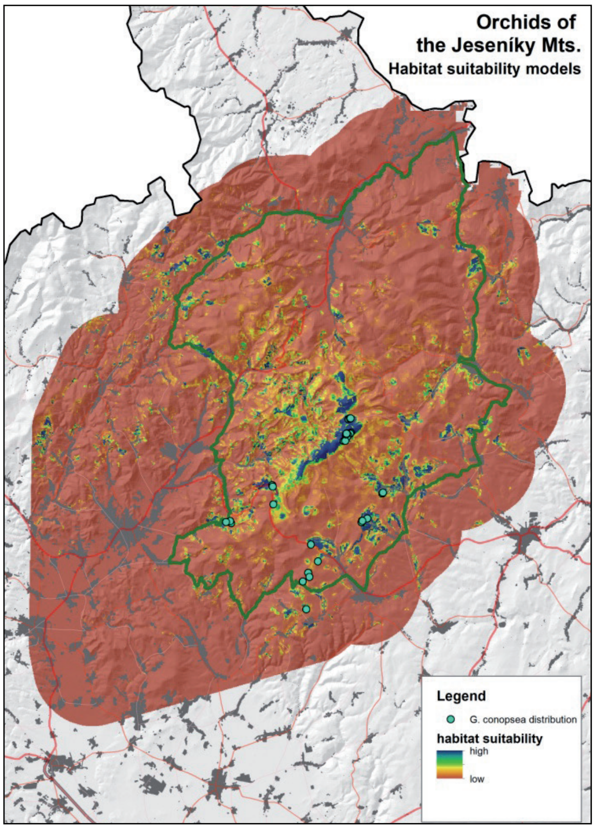

Fig. 10 Map showing the potential distribution of *Gymnadenia conopsea* in the Jeseníky Mountains. The green line indicates the borders of Jeseníky Nature Conservation Area

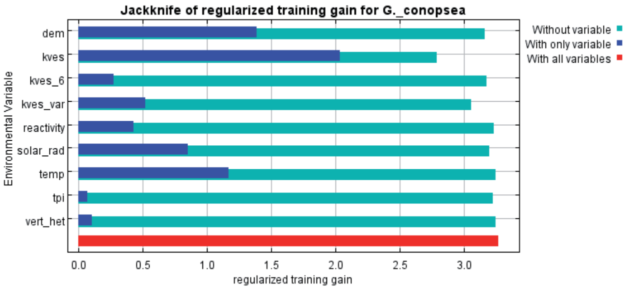

Fig. 11 The graph of the results of the jack knife procedure of selected factors for *Platanthera bifolia*.

places and tall grasses in mesophilic meadows also provide shade. Wet meadows can also include tall vegetation or border on forests. Our results are in accordance with the information in the literature (Procházka and Velísek 1983; Dykyjová 2003; Jersáková and Kindlmann 2004; Průša 2005), which indicate that this species occurs in forests as well as meadows, and also occurs in wet and dry places.

In Fig. 12c, the association between the distribution of *P. bifolia* and habitat heterogeneity (KVES_var) is displayed. Clearly, this species occurs mainly in areas of high environmental heterogeneity, that is, areas that consist of many small habitats. The probability of this species occurring in an area covered by a single habitat is almost zero.

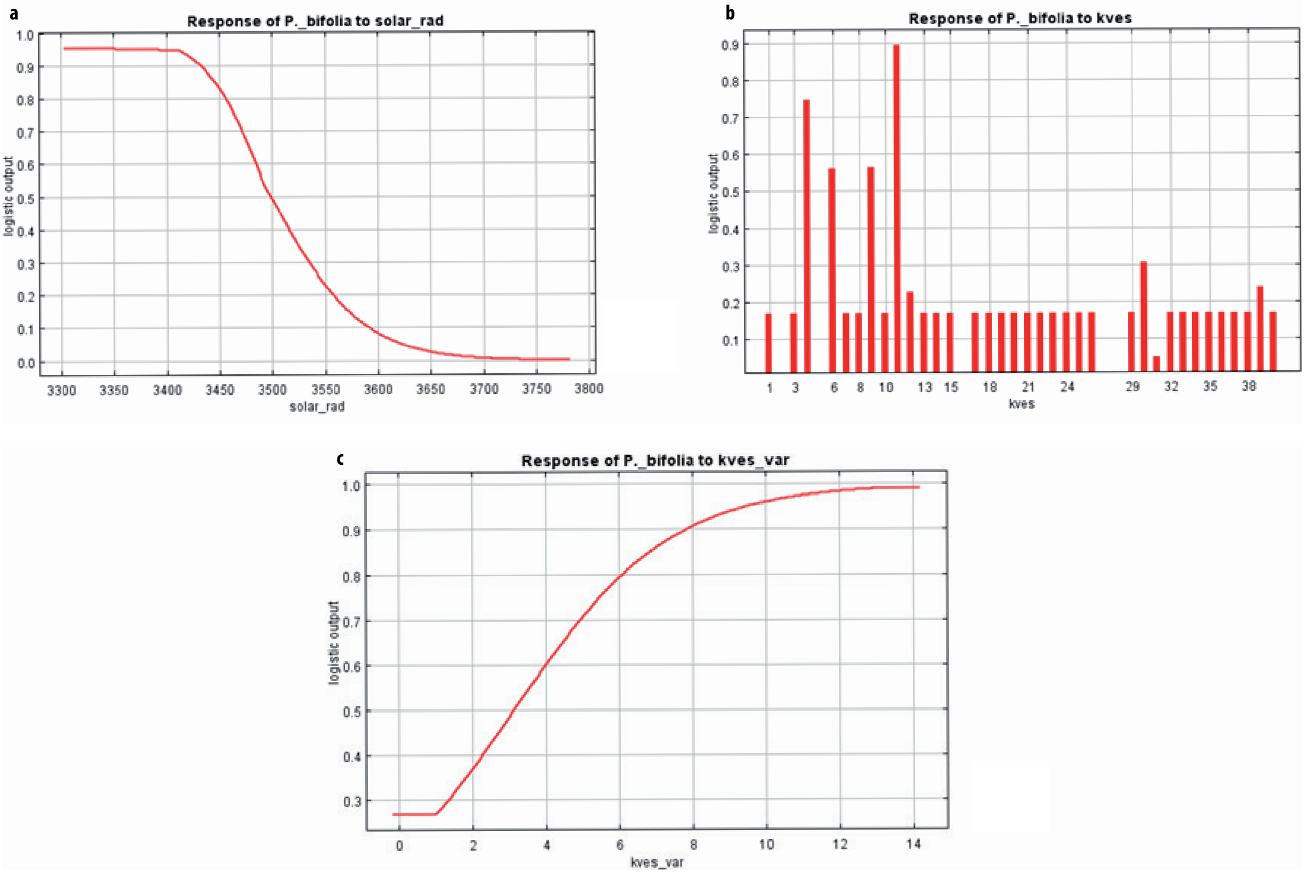

Fig. 12 Association of *Platanthera bifolia* with a) solar radiation (solar_rad), b) consolidated layer of ecosystems (KVES) and c) habitat heterogeneity (KVES_var).

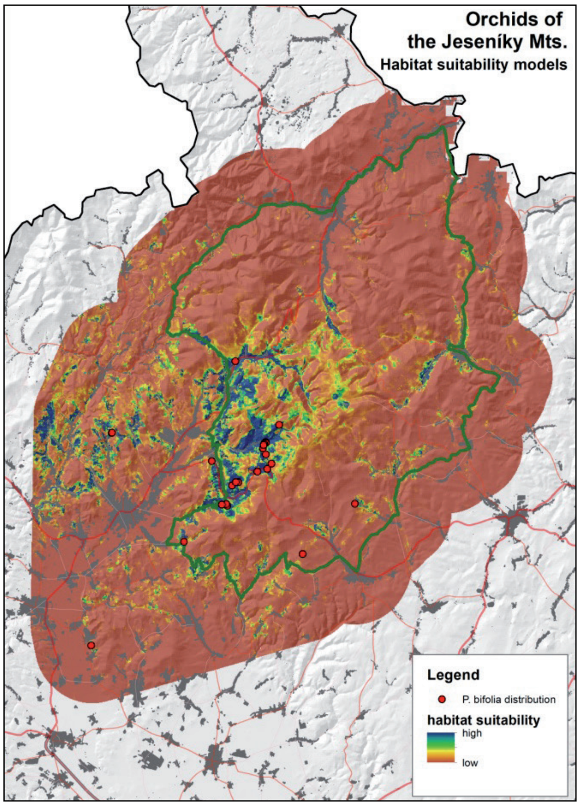

Fig. 13 Map showing the potential distribution of *Platanthera bifolia* in the Jeseníky Mountains. The green line is the border of the Jeseníky Nature Conservation Area.

Fig. 13 is a map showing the potential distribution of *P. bifolia* in the area studied. This map shows four other potential places that are suitable for the occurrence of this species: east of the village Sobotín around the stream Merta, around the village of Kouty nad Desnou, between the village of Loučná nad Desnou and the Dlouhé Stráně water reservoir, and around the village of Štědrákova in the east of the region studied.

Conclusions

The Maxent program is a useful tool for predicting the potential occurrence of endangered species in selected areas or a country, not only for orchids. Based on the results of this study, the most important factors for the species studied were different types of vegetation (consolidated layer of ecosystems; KVES) and high environmental heterogeneity and landscape structure (KVES_var). For the two species of *Dactylorhiza* studied, the common important factor was the nature of the bedrock (reactivity), or more precisely they mainly occur in areas with phyllite rocks and rarely in those with greywacke.

The results could be very helpful in identifying new, yet unknown localities of *Dactylorhiza fuchsii*, *Dactylorhiza majalis*, *Gymnadenia conopsea* and *Platanthera bifolia*, which are endangered species in the Czech Republic. Without a potential distribution map, searching for new localities would be time consuming and less effective. Such maps would also be helpful for orchid conservation as it would enable conservationists to focus on areas where it is most likely that particular species of orchids are present.

In general, this work should serve as a tool for orchid conservation and for determining the most important factors associated with the distributions of orchids in the Czech Republic.

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TRANS-PTEROSTILBENE AND ITS DERIVATIVE 2,4-DIMETHOXY-6 -HYDROXYPHENANTHRENE IN THE LEAVES OF *PARTHENOCISSUS TRICUSPIDATA*

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ABSTRACT

Trans-pterostilbene, *cis*-pterostilbene and 2,4-dimethoxy-6-hydroxyphenanthrene were detected in the leaves of *Parthenocissus tricuspidata* (Siebold et Zuccarini) Planchon. It was recorded for this plant for the first time that in autumn, when the leaves change in colour, there is an increase in content of 2,4-dimethoxy-6-hydroxyphenanthrene (DMPH), which is a derivative of *trans*-pterostilbene.

Keywords: *Parthenocissus tricuspidata*, *trans*-pterostilbene, 2,4-dimethoxy-6-hydroxyphenanthrene, *trans*-resveratrol

Introduction

Parthenocissus tricuspidata (Siebold et Zuccarini) Planchon, known as Japanese creeper, Boston ivy, Grape ivy or Japanese ivy, belongs to the family Vitaceae. It is native to eastern Asia.

The attention here is mainly on the detection and content of stilbenes, because they are known to be biologically active substances. In stem wood, in addition to *trans*-resveratrol and *trans*-piceid (Jeon et al. 2013), *trans*-ε-viniferin, pallidol, ampelopsin F, isoampelopsin F (Tanaka et al. 1998), parthenostilben A, parthenostilben B (Kim et al. 2005) and tricuspidatol A (Lins et al. 1991) are reported. In the leaves *trans*-piceid (Son et al. 2007; Park et al. 2008), longistylin A and longistylin B (Son et al. 2007) and *trans*-piceatannol (Kundaković et al. 2008) are reported.

The main goal of this study was to analyse the biologically active compounds present during the senescence of *Parthenocissus tricuspidata* leaves with the focus on *trans*-pterostilbene and its transformation products.

Materials and Methods

Plants material and preparation of extracts

The leaves of *Parthenocissus tricuspidata* (Siebold et Zuccarini) Planchon were collected at different locations in the Czech Republic in the years 2012–2014 (Table 1). The samples of leaves were frozen at −18 °C and then lyophilized. Finely ground samples were extracted with ethyl acetate for 40 min at 50 °C and the sediment was washed twice with ethyl acetate. Supernatants were pooled, ethyl acetate was evaporated in a stream of nitrogen and then the samples were diluted in methanol. The subsamples from each sample used in the analysis were prepared in triplicate.

Table 1 Localities and data sampling.

Locality		Data of collection	Leaf colour
A	Kutná Hora 49°56′54″N 15°16′6″E	20.9.2012	green; yellow-dark red
B	Hluboká nad Vltavou 49°3′8″N 14°26′3″E	9.10.2013	green; red; dark-red; yellow-green-dark-red; yellow-red
C	Lednice 48°47′60″N 16°48′12″E	10.10.2013 17.10.2014	green; green-dark red; green-yellow red
D	Starý Smolivec 49°31′39″N 13°45′7″E	18.8.2013 16.9.2013 5.10.2013	green; green-red border; green-dark red; red

Liquid chromatography

The extracts were analyzed using a HPLC (HP 1050 Ti-series, Hewlett Packard Palo Alto, CA, USA) and a Luna C18(2) column, 150 mm × 2 mm, 3 µm (Phenomenex, Torrance, CA, USA), G1315B diode array detector (DAD, Agilent) and G1321A fluorescence detector (FLD, Agilent). The compounds were identified by measurements made using a LC-MS (LCQ Accela Fleet (Thermo Fisher Scientific, San Jose, CA, USA). Separations using HPLC and LC-MS (APCI) are described in detail in Tříska et al. (2012).

As standards *trans*-resveratrol and 9-phenanthrol from Sigma-Aldrich were used and *trans*-pterostilbene was kindly provided by prof. Jan Šmidrkal, University of Chemistry and Technology, Prague. Acetonitrile and methanol were from Merck, *o*-phosphoric acid and formic acid from Sigma-Aldrich.

Data analysis

Quantification of *trans*- and *cis*-pterostilbene using HPLC was done using a calibration curve for *trans*-pterostilbene (diode array detector, at 315 nm); quantification of *trans*-resveratrol using a calibration curve for *trans*-resveratrol (diode array detector, at 315 nm); that of 2,4-dime-

Vrchotová, N., Tříska, J.: *Trans*-pterostilbene and its derivative 2,4-dimethoxy-6-hydroxyphenanthrene in the leaves of *Parthenocissus tricuspidata*
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thoxy-6-hydroxyphenanthrene using a calibration curve for 9-phenanthrol (synonym for 9-hydroxyphenanthrene) using a fluorescence detector (Ex 315 nm, Em 395 nm). LOD and LOQ for *trans*-resveratrol were 0.055 µg/ml and 0.184µg/ml, respectively, for *trans*-pterostilbene, 0.042 µg/ml and 0.142 µg/ml, for 9-phenanthrol 0.042 µg/ml and 0.141 µg/ml. Each value was based on three measurements.

Results and Discussion

The samples were collected from four locations in autumn, when the leaves had begun to change in colour from green to yellow and red. The samples from one of these locations were collected on three dates in one year, the samples from the second location were collected in two consecutive years. The locations and dates of sampling are shown in Table 1.

In the samples *trans*-resveratrol, *trans*-pterostilbene and 2,4-dimethoxy-6-hydroxyphenanthrene (DMPH) were identified. It was possible to detect also *cis*-pterostilbene in some samples. The irradiated *trans*-pterostilbene standard served as a test substance to identify compounds present in the extracts.

By irradiating the methanol solution of *trans*-pterostilbene at 254 nm for 20 hours, a mixture of *trans*-pterostilbene, DMPH and *cis*-pterostilbene was obtained. The structures of these substances are shown in Fig. 1 and their DAD spectra in Fig. 2. The samples were also measured using LC-MS (APCI in positive mode): we recorded for 2,4-dimethoxy-6-hydroxyphenanthrene molecular ion at *m/z* 255 $[M+H]^+$ and for *trans*-pterostilbene and *cis*-pterostilbene molecular ion at *m/z* 257 $[M+H]^+$.

The content of DMPH and the stilbenes studied is very variable, but the DMPH content was always much greater in autumn "coloured leaves" compared to green leaves. The highest content of DMPH was in the samples of leaves that were either completely or partially dark red (Figs 3-6). The highest content of DMPH was recorded in the leaves from site A in 2012 and from site C in 2013. The amount of *trans*-pterostilbene was only a few mg/kg; for many samples, the content of *trans*-pterostilbene was below the detection limit (e.g. in the green leaves from localities A, B and D). Only traces of *cis*-pterostilbene were detected in three samples: Locality A – yellow-dark red leaves, locality B – red leaves, locality C – green-dark red leaves 10.10.2013. In the green leaves from site C in 2014 the content of both stilbenes was below the detection limit.

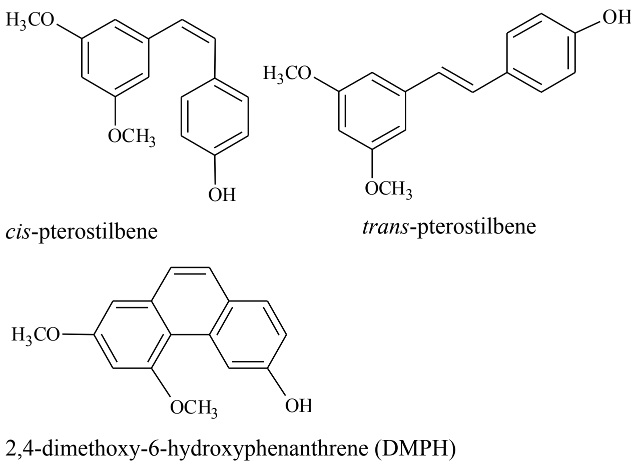

Fig. 1 The structure of analysed compounds.

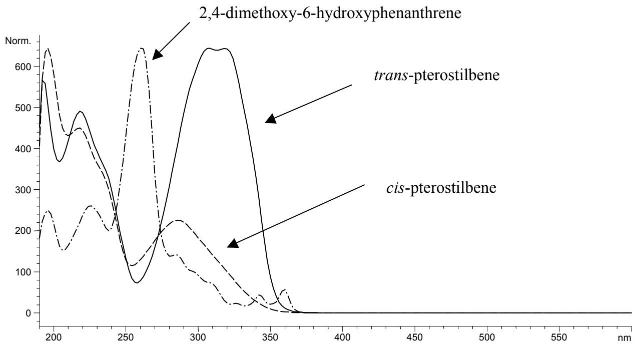

Fig. 2 DAD spectra of *trans*-pterostilbene and its derivatives.

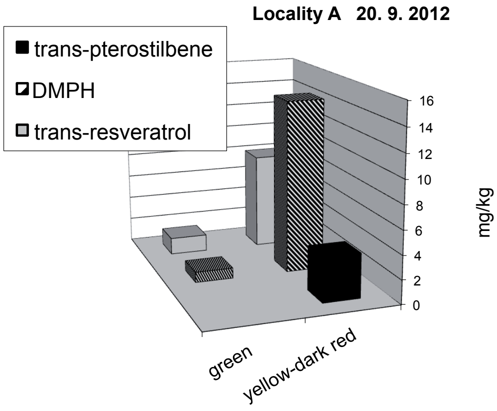

Fig. 3 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality A.

Derivatives of phenanthrene are very common biologically active compounds in the plant kingdom (Kovács et al. 2008), but to our knowledge there is no information on the presence of DMPH in plants. Only the dihydro derivative of DMPH (double bond saturated in the positions 9, 10) is mentioned in the literature under the name orchinol (Kovács et al. 2008). DMPH was patented (Hashimoto et al. 1976) as a novel growth modifier useful for controlling growth, germination of seeds and regulating the dormant stages of seeds, bulbs and buds.

Formation of phenanthrene derivatives as final products in the UV photo isomerization of *trans*-resveratrol to *cis*-resveratrol and final cyclization to the derivative phenanthrene in the leaves of *Vitis vinifera* plants following attack by *Plasmopara viticola* is described in the literature (Tříska et al. 2012). Senescence of *Parthenocissus tricuspidata* leaves, visibly manifested by the colour change in autumn, may have a similar mechanism also

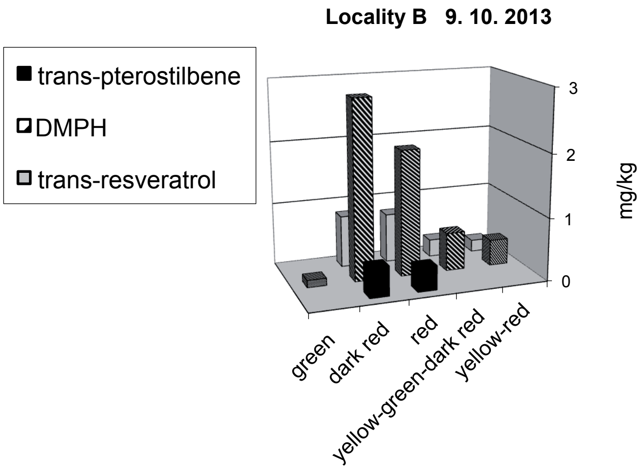

Fig. 4 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality B.

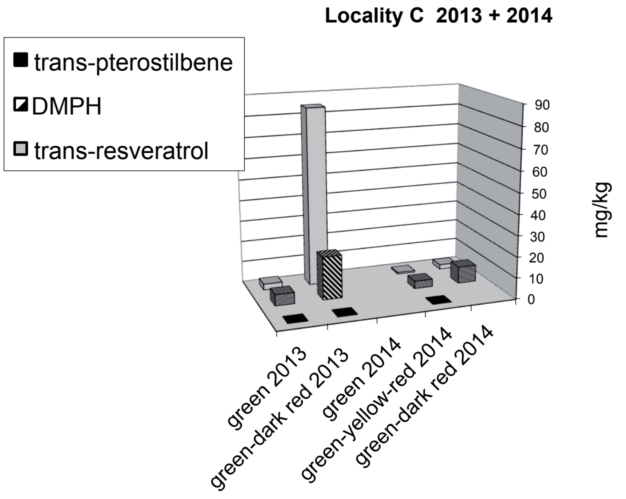

Fig. 5 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality C.

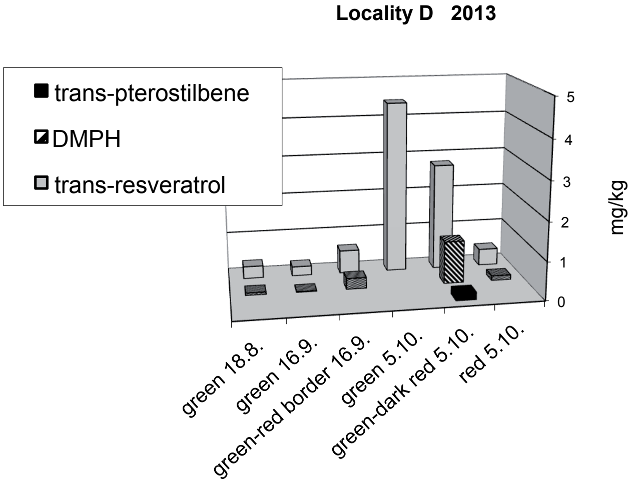

Fig. 6 The content of *trans*-resveratrol, *trans*-pterostilbene and DMPH in the leaves on the locality D.

ending with phenanthrene derivative, in this case mainly with DMPH.

Conclusions

During the senescence of the leaves of *Parthenocissus tricuspidata*, visibly manifested in autumn by the change in their colour, *trans*-pterostilbene, originally present in the leaves, is tranformed into 2,4-dimethoxy-6-hydroxyphenanthrene, which is reported here for the first time in the leaves of *Parthenocissus tricuspidata*.

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