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The Impact of Different Habitat Conditions on the Variability of Wild Populations of a Medicinal Plant Betonica officinalis L.

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Abstract. Plants are important source of beneficial bioactive compounds which may find various applications as functional ingredients, such as components of food supplements, cosmetics, and pharmaceuticals. One such medicinal plant is *Betonica officinalis*, populations of which were investigated in 2012–13. The studies were conducted in patches of *Molinietum caeruleae* dominated by: small meadow taxa (patch I); the shrub willow *Salix repens* ssp. *rosmarinifolia* (patch II); large tussock grasses *Deschampsia caespitosa* and *Molinia caerulaea* (patch III); tall-growing macroforbs *Filipendula ulmaria* and *Solidago canadensis* (patch IV). Over successive patches, the average height of plant cover increased, as did soil moisture, while light availability at ground level decreased. Much greater abundance and density of the *Betonica officinalis* population were found in patches I, III and IV, while lower values for these parameters were noted in patch II. Individuals in pre-reproductive stages were absent during whole study period in all study plots, vegetative ramet clusters were observed in plots situated in patches I and III in the first year of observations, while only generative ramet clusters occurred in plots set in patches II and IV. The number of rosettes per ramet cluster, number and dimensions of rosette leaves, height of flowering stems, number of cauline leaves, length of inflorescences, as well as number and length of flowers increased gradually over successive patches, whereas the number of generative stems per ramet cluster did not differ remarkably among populations. On the basis of the performed studies it might be concluded that the condition of populations deteriorated from patches overgrown by large-tussock grasses and characterized by considerable share of native and alien tall-growing macroforbs, *via* patch dominated by small meadow taxa, to patch prevailed by shrub willows.

Key words: *Betonica officinalis*, individual, *Molinietum caeruleae*, morphological variability, light availability, population, soil moisture.

Introduction

Plants are important sources of beneficial bioactive compounds such as phenolic and nitrogen compounds, vitamins, and terpenoids, all of which may have various applications as functional ingredients. Some of them are natural antioxidants whose intake is associated with lower risks of cancer and cardiovascular disease (CHU *et al.*, 2002; DE CARVALHO-SILVA *et al.*, 2014; GUAJARDO-FLORES *et al.*, 2013; STANKOVIC *et al.*, 2011; SUN *et al.*, 2002; YANG *et al.*, 2004, 2009). Moreover, several of these secondary metabolites possess antifungal, antimicrobial or antiviral properties (ALZOREKY & NAKAHARA, 2003; BONJAR, 2004; KUMAR *et al.*, 2006; LOPEZ *et al.*, 2001). One such medicinal plant is *Betonica officinalis*, which has been used for centuries for treatment of disorders of the respiratory and gastrointestinal tracts, nervous system, skin ulcers and infected wounds, as well as gynecological problems (VOGL *et al.*, 2013). To date, biochemical compounds of the aforementioned taxa have been studied intensively by DUŠEK *et al.*,

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(2009, 2010a), CZIGLE *et al.*, (2007), HAJDARI *et al.*, (2010), LAZAREVIĆ *et al.*, (2013) and ŠLIUMPAITĖ *et al.*, (2013). As opposed to a very large body of studies on the biochemical properties of *B. officinalis*, investigations of population and individual traits are still very scarce. Such observations were performed by DUŠEK *et al.*, (2009, 2010b), who found remarkable variability *inter alia* in in the height and width of individuals, dimensions of leaves and inflorescences, and seed production in natural localities and in field cultivation. It should be pointed out that further observations carried out *in situ* are still highly desirable. Such investigations conducted in different habitat conditions are very important for the assessment of favourable and unfavourable factors influencing the condition of populations and they might provide a basis for effective management programs.

In response to the current insufficiency of knowledge about *B. officinalis* populations, the presented investigations were performed. The main aim was to evaluate the effect of progressive increases in plant cover height and soil moisture, coincident with a gradual decrease in light availability, on population and individual traits in *Molinietum caeruleae* meadows. The specific goals concentrated on the assessment of: (1) abundance and density of populations, (2) developmental stages of individuals/ramet clusters, number and dimensions of leaf rosettes, number and height of generative stems, length of inflorescences, number of flowers per inflorescence, and flower length.

The hypotheses were following: (1) the abundance and density of populations would diminish over successive patches, (2) individuals in pre-reproductive stages would be absent in all patches, (3) dimensions of individuals and ramet clusters would increase over consecutive sites.

Materials and Methods

Study species. Betonica officinalis syn. *Stachys officinalis* (L.) Trevis is a perennial herb occurring in dry grassland, lightly grazed pastures, meadows and open woods in Europe, western Asia and North Africa. It is classified among rhizomatous species creating rosettes of leaves and upright, generative stems growing to 60 cm tall. Its cauline leaves are narrowly oval, with a heart-shaped base. The generative stems bear dense spikes of bright purple-red or occasionally white flowers, with the corolla attaining lengths of 1.0–1.5 cm. Flowering lasts from July to September. This taxon spreads notably by means of seeds (requiring a period of chilling to break dormancy) and by slow and steady vegetative growth leading to the division of senile parts of rhizomes into independent parts (FITTER & PEAT, 1994; KLIMEŠOVÁ & KLIMEŠ, 2006; KLOTZ *et al.*, 2002; SAMMUL *et al.*, 2003)

Because *B. officinalis* is a clonal species, the terms 'individual' and 'ramet cluster' were adopted as basic demographic units. An individual (genet) is a plant emerging from a single zygote. This designation might be applied in association with seedling and juvenile stages (plants with one leaf rosette), as only at these stages can it be established with certainty that it has developed from a zygote. The adult vegetative ramet cluster is understood as an integral group of leaf rosettes, the adult generative ramet clusters as an integral group of leaf rosettes and a generative stem or stems.

Field studies. The studies were carried out in the Kostrze district, on the western border of Cracow (southern Poland). The research area is located on the low flood terrace of the Vistula River, where peaty or clay- and silt-laden soils with fluctuating water tables are covered mainly by *Molinietum caeruleae* patches (DUBIEL, 2005). The abandonment of traditional land use for at least the past dozen years has favoured the encroachment of *Phragmites* swamps and willow brushwood, leading to the fragmentation of meadows (DUBIEL, 1991; 1996).

The studies were carried out in a study area consisting of three adjacent abandoned patches of *Molinietum caeruleae* sensu MATUSZKIEWICZ (2001) characterised by the presence of the following taxa: *Betonica officinalis, Dianthus superbus, Gentiana pneumonanthe, Gladiolus imbricatus, Iris* *sibirica, Molinia caerulaea, Potentilla erecta, Sanguisorba officinalis, Selinum carvifolia, Succisa pratensis*. The studied patches (study sites) differed as to dominant species: patch I was dominated by small meadow taxa, patch II by shrub willows; patch III was overgrown by large-tussock grasses and sedges; patch IV was characterised by a considerable share of native and alien tall-growing macroforbs. In each patch, the site conditions were studied. The average height of vascular plants in particular patches was evaluated on the basis of measurements of 20 randomly chosen stems of different species, performed using a folding tape measure on 10 July 2012. Light intensity at soil level and ground humidity were evaluated on the basis of 20 measurements taken randomly in each patch on 20 July 2012 between 10.00 and 12.00 a.m. Light intensity was examined with a Voltcraft MS-1300 digital light meter (accuracy ±5% + 10 digits; range 0.01–50,000 lx). Soil humidity was measured using an Omega HSM50 handheld digital soil moisture sensor (accuracy ±5% + 5 digits; range 0% to 50% moisture content of soil). Site conditions in individual patches are described in Table 1.

Table 1. The characteristics of habitat conditions in study sites.

	Patch I	Patch II	Patch III	Patch IV
Area [m²]	2500	2900	3000	1800
Dominant species (with cover exceeding 20%)	*Potentilla erecta, Lotus corniculatus*	*Salix repens* var. *rosmarinifolia*	*Molinia caerulea, Deschampsia caespitosa*	*Filipendula ulmaria, Solidago canadensis*
Subdominant species (with cover level ranging 5-20%)	*Lychnis flos-cuculi Lathyrus pratensis*	*Salix caprea, Salix cinerea*	*Carex gracilis*	*Lysimachia vulgaris*
Average height of standing vegetation [cm]*	50.3	62.7	78.7	101.9
Average light intensity at soil level**	40 600	38 100	30 400	29 000
Average soil moisture***	5.1	6.3	7.4	7.8

The values of statistical significance of differences (the Kruskal-Wallis H test, df=3) among patches in: * height of standing vegetation achieved 22.9 *(P<0.001)*, ** light intensity at soil level achieved 50.5 *(P<0.001)*, *** soil moisture achieved 38.7 *(P<0.001)*.

In July 2012, in each study patch, the abundance of individuals and ramet clusters of *B. officinalis* and their mean number per m² were evaluated. Then, in each population, one 100-m² permanent plot was established and fenced in. In this plot, the abundance of individuals and ramet clusters and their developmental stages were investigated. Subsequently, in each individual/ramet cluster, the number of leaf rosettes, number of leaves per each rosette and the maximal length and width of the longest leaf blade were studied. In each generative ramet cluster the number of generative stems per ramet cluster and the height of generative stems (from the soil surface to the top of the highest inflorescence) were also noted. Furthermore, the number of cauline leaves and length of inflorescence, as well as number of flowers per inflorescence, were studied. Moreover, the length of 10 randomly chosen flowers per each studied inflorescence was investigated. For branched generative stems, only the main branch was examined. Statistical analyses based on the non- parametrical Kruskal-Wallis H test were performed using STATISTICA 10 software (StatSoft Inc., 2010).

Results

The abundance of *B. officinalis* populations in 2012 in successive patches achieved values of 354, 287, 310 and 338, while their density amounted to 0.14, 0.10, 0.10 and 0.19, respectively. In all permanent plots, only adult ramet clusters were found; their numbers were constant during the entire observation period, reaching 18, 12, 14 and 10, respectively. Vegetative ramet clusters were observed in plots situated in patches I and III in the first year of observations, while only generative ramet clusters occurred in plots set in patches II and IV (Fig. 1).

The number of leaf rosettes increased from patch I, *via* patches II and III, to patch IV. In 2012 the mean number of rosettes per ramet cluster in patch I amounted to 1.3 (±0.6); in patch II, 4.0 (±2.6); in patch III, 4.3 (±1.8); in patch IV, 7.4 (±2.3). In 2013 this number mainly amounted to 2.3 (±1.1), 3.8 (±2.5), 5.1 (±1.9), and 6.6 (±2.8), respectively. The differences among populations were significantly different in both seasons (H_{2012}=34.1, H_{2013}=19.6; $P<0.001$). The smallest number of leaves per rosette was found in a plot located in patch I, while the largest number was observed in patch IV. The smallest length and width of rosette leaves were noted in a plot located in patch I; larger dimensions were exhibited by leaves found in patches II and IV; the largest dimensions were recorded in patch III (Fig. 2).

The number of flowering stems per generative ramet cluster did not differ remarkably among populations (Table 2). In both study seasons the height of generative stems, number of cauline leaves, inflorescence length and flower number were much lower in plots located in patches I and II than in patches III and IV (Fig. 3). The smallest flowers were found in patch I, the largest in patch IV (Table 3).

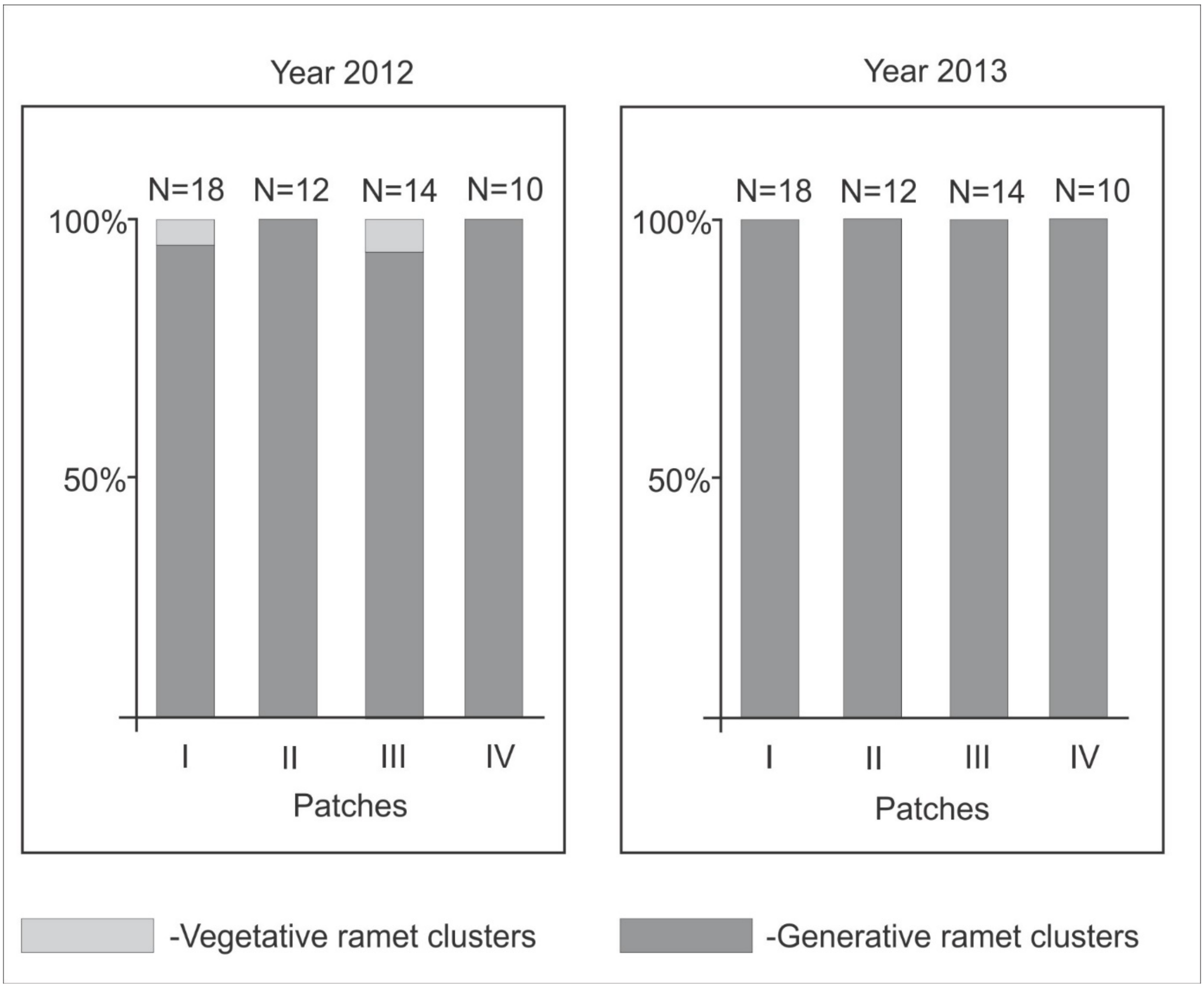

Fig. 1 The share of vegetative and generative ramet clusters of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-2013.

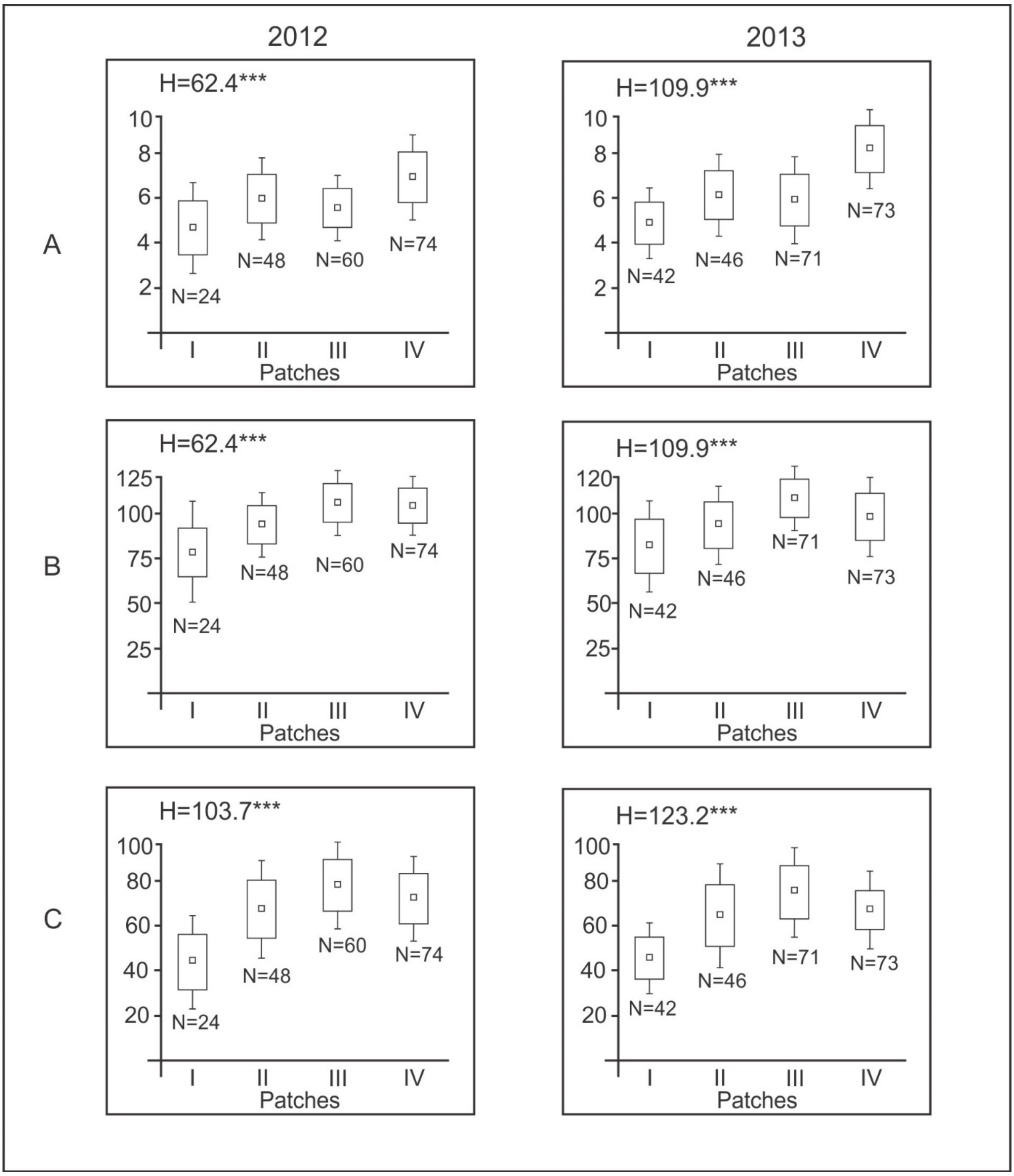

Fig. 2. The number of leaves per rosette (A), maximal length [mm] (B) and width [mm] (C) of the longest leaf blade of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-13. Box and whisker plots give the mean (square), SE (box) and SD (whiskers). Asterisks mean that there are significant differences among patches in each study year at the 0.001 level.

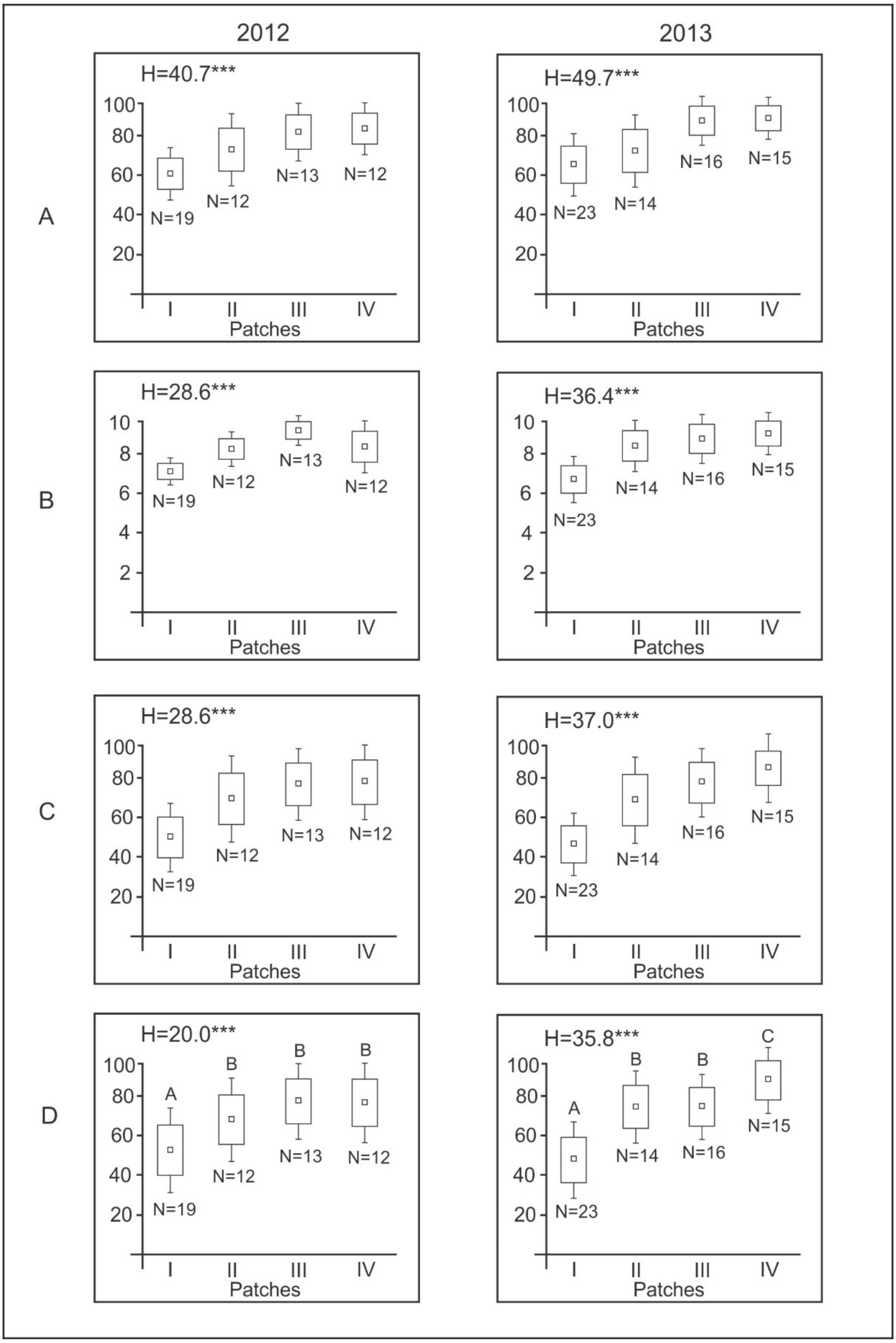

Fig. 3. The height of generative stems [cm] (A), number of cauline leaves (B), inflorescence length [mm] (C) and number of flowers per inflorescence (D) of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-13. Box and whisker plots give the mean (square), SE (box) and SD (whiskers). Asterisks mean that there are significant differences among patches in each study year at the 0.001 level.

Table 2. The number of generative ramet clusters per study plots and the mean number of generative stems per generative ramet cluster of *Betonica officinalis* within study plots set in patch dominated by small meadow taxa (patch I), prevailed by shrub willows (patch II), overgrown by large-tussock grasses (patch III) and characterized by considerable share of native and alien tall-growing macroforbs (patch IV) in the year 2012 and 2013.

	Patch	Number of generative ramet clusters	The mean number of generative stems per ramet cluster	The standard deviation	The statistical significance level (The Kruskal-Wallis H test, P value)
Year 2012	I	17	1.1	0.31	H=4.66, *P=0.12*
	II	12	1.0	0.0	
	III	13	1.0	0.0	
	IV	10	1.2	0.4	
Year 2013	I	18	1.3	0.6	H=4.48, *P=0.15*
	II	12	1.2	0.4	
	III	14	1.1	0.3	
	IV	10	1.5	0.5	

Table 3. The length of chosen 10 flowers in observed inflorescences of *Betonica officinalis* occurring within study plots set in patch dominated by small meadow taxa (patch I), prevailed by shrub willows (patch II), overgrown by large-tussock grasses (patch III) and characterized by considerable share of native and alien tall-growing macroforbs (patch IV) in the years 2012 and 2013.

	Patch	The mean length of flowers	The standard deviation	The statistical significance level (The Kruskal-Wallis H test, P value)
Year 2012	I	10.7	0.1	H=176.9, *P<0.001*
	II	11.9	0.7	
	III	11.6	1.2	
	IV	13.2	1.2	
Year 2013	I	10.6	0.9	H=30.4.7, *P<0.001*
	II	11.7	0.7	
	III	11.7	1.1	
	IV	12.3	1.1	

Discussion

The observations, showing, surprisingly, greater abundance and density of populations in patches I, III and IV than in patch II, might suggest that creeping and rooting stems of *Salix repens* ssp. *rosmarinifolia* mechanically limit the growth of *B. officinalis* individuals or even eliminate them from colonised sites. Additionally, the low abundance of *B. officinalis* populations might be a result of the activity of allochemicals produced by *Salix caprea*. The inhibition of the establishment and growth of genets of meadow taxa beneath *S. caprea* litter was observed by MUDRÁK & FROUZ (2012). The constant number of ramet clusters in both study seasons and lack of individuals in pre-reproductive stages in all permanent plots might suggest the absence of factors breaking seed dormancy and stimulating the germination process. The poor germinating ability of *B. officinalis* seeds not subjected to pre-treatment prior to sowing had already been proved in laboratory conditions (DUŠEK *et al.*, 2010a). A similar result was demonstrated in seeds of other species from the *Stachys* genera, in both laboratory (GÜLERYÜZA *et al.*, 2011) and natural (KUPFERSCHMID *et al.*, 2000) conditions. Furthermore, the absence of seedlings and juveniles observed in the present study may be due to the lack of gaps in continuous plant cover considered 'safe sites for seedling recruitment'. A similar phenomenon had already been found in populations of several taxa inhabiting overgrown *Molinietum caeruleae* meadows such as *Dianthus superbus* (KOSTRAKIEWICZ-GIERAŁT, 2013a), *Gentiana pneumonanthe* (KOSTRAKIEWICZ-GIERAŁT, 2013b) and *Gladiolus imbricatus* (KOSTRAKIEWICZ-GIERAŁT, 2014).

The lowest number of rosette leaves of *B. officinalis* noted in patch I is in disagreement with investigations documenting that low density of adjacent plants and high light availability contribute to intense multiplication of rosette leaves (CALLAGHAN & PIGLIUCCI, 2002; ERIKSSON, 1985; MEEKINS & MCCARTHY, 2000; VAN KLEUNEN *et al.*, 2007; WINKLER & STÖKLIN, 2002). The existence of the lowest number of rosette leaves in a patch dominated by small meadow taxa might be result of the activity of herbivores, notably gastropods and grasshoppers (SCHEIDEL & BRUELHEIDE, 1999; STOLL *et al.*, 2006). The production of large rosette leaves in patches with lower light availability might enhance the chances for the effective use of photoperiod in a crowded environment. The existence of the largest dimensions of rosette leaves of *B. officinalis* in patch III is in accordance with the findings of DUŠEK *et al.* (2009), who observed the occurrence of large leaves in damp sites. At the same time, it is worth mentioning that the findings of GYÖRGY (2005, 2009) showed that the thickness of leaf blades of *B. officinalis* is much greater in open land than in shaded areas.

The observations showing that the number of generative stems per ramet cluster did not differ among populations are opposed to observations carried out in populations of other rhizomatous taxa such as *Iris sibirica* (KOSTRAKIEWICZ-GIERAŁT, 2013c) and *Doronicum austriacum* (STACHURSKA-SWAKOŃ & KUZ, 2011). Combining performed observations with published data (DUŠEK *et al.*, 2010b) enables the assumption that the average length of flowering stems of *B. officinalis* did not differ between patch I and an *ex situ* collection, where the competition from neighbouring plants is strongly limited. Furthermore, presented studies support the observations of GRIME & JEFFREY (1965), who noted that genets of *B. officinalis* growing in shade are strongly etiolated. The substantial height of flowering stalks in shaded stands had already been noted in other taxa occurring in moist meadows (KOSTRAKIEWICZ-GIERAŁT, 2013a; 2013b; 2013c; 2014). The considerable height of *B. officinalis* stems in patches III and IV might be also due to substantial ground humidity, as in populations of *Fritillaria meleagris* (CSERGÖ & FRINK, 2003). At the same time, it should be added that the findings presented here partially support observations conducted in populations of *D. austriacum* (STACHURSKA-SWAKOŃ & KUZ, 2011), supplying evidence that, in populations occurring in an overshadowed place with substantial ground humidity, generative stems achieve the greatest height and create the lowest number of cauline leaves.

The obtained results showing that the length of inflorescences increases along with the growing height of adjacent plants is contrary to the observations of DUŠEK *et al.*, (2009), who found longer inflorescences of *B. officinalis* in *ex situ* cultivation compared to those in crowded natural stands. However, the obtained results are in accordance with observations carried out on populations of many taxa inhabiting *M. caeruleae* patches, which showed that inflorescence length, as well as number and size of flowers, are greater in the neighbourhood of tall-growing plants than in the vicinity of small meadow taxa (KOSTRAKIEWICZ-GIERAŁT, 2013a; 2014).

Conclusions

The obtained results did not support hypothesis that abundance of populations would diminish in subsequent patches. At the same time performed studies confirmed assumption that individuals in prereproductive stages would be absent in all places, while presumption that dimensions of genets and ramet clusters would increase in subsequent places was partially supported.

On the basis of performed studies the following conclusions were drawn:

1. The populations of *B. officinalis* occurring in patches III and IV show the most promising prospects for maintenance in colonised sites due to the large dimensions and substantial number of ramet clusters.

2. The population occurring in patch I presents an inferior state in spite of its considerable abundance.

3. The population from patch II is in the worst condition, as shown by the lowest abundance and the small size of ramet clusters.

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