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Scots pine forest in Central Europe as a habitat for *Harmonia axyridis*: temporal and spatial patterns in the population of an alien ladybird

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Abstract

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Understanding of habitat favourability has wide relevance to the invasion biology of alien species. We studied the seasonal dynamics of the alien ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) in monoculture Scots pine forest stands in south-west Slovakia, Central Europe, from April 2013 to March 2015. Adult *H. axyridis* were collected monthly across seven randomly selected pine stands of different ages and canopy closure, from the lower branches of pine trees, and larvae were recorded qualitatively. Adults were recorded all year round, most abundantly in November and least abundantly in February. The relationship between the abundance of *H. axyridis* and selected forest stand characteristics was modelled using the negative binomial Generalized Additive Model with penalized spline component in month (seasonality) effect, year, canopy closure and age effects and the random effect of forest stand (sample area effect). The abundance of *H. axyridis* was significantly influenced by the age of stand and seasonality (with month granularity) for both closed and open canopy stands, whereas the effects of canopy closure and sample area were not significant. The bimodal pattern of seasonal dynamics of *H. axyridis* on Scots pine was common for closed and open canopy stands, with two peaks reflecting the cyclic movement of the species from and to overwintering sites. *Harmonia axyridis* utilized certain pine stands preferably for foraging during the growing season and certain stands for refuge during winter. The ladybirds were found in highest numbers in the 15 year old closed canopy stand (overwintering site). The occurrence of both adults and larvae in most stands indicated a suitability of Scots pine forest for ladybird breeding. The model of year-round dynamics of *H. axyridis* has been presented for the first time within the invaded range of the ladybird in Europe.

Keywords

harlequin ladybird, invasive insect, invaded range, *Pinus sylvestris*, seasonal changes

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Introduction

The harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is the most invasive ladybird on Earth (Majerus, 2016) originating from East Asia where it has a wide range (Orlova-Bienkowskaja et al., 2015). Within this range, specifically in Japan, *H. axyridis* is mainly associated with trees and shrubs (Osawa, 2000) and mostly occurs in agricultural fields, orchards, parks, residential yards and gardens as well as in deciduous and coniferous woodlands but it is uncommon in natural forests (Osawa, 2011; Noriyuki and Osawa, 2015, 2016). This habitat generalist and predator with a broad prey range (Noriyuki et al., 2011, 2019) is also found on pine trees (Noriyuki and Osawa, 2016). In the invaded range within Europe, *H. axyridis* is also a habitat generalist (Adriaens et al., 2008; Roy et al., 2016; Honěk et al., 2018) occurring frequently in urban areas where it is often the most abundant (dominant) ladybird on lime (*Tilia* spp.) and maple trees (*Acer* spp.) (Roy et al., 2016; Viglášová et al., 2017; Brown and Roy, 2018; Honěk et al., 2015, 2019). The ladybird is also found on coniferous trees, specifically Scots pine, *Pinus sylvestris* L. (Roy et al., 2016; Brown and Roy, 2018; Holecová et al., 2018), Austrian pine, *Pinus nigra* Arn. (Adriaens et al., 2008) and Norway spruce, *Picea abies* (L.) Karsten (J. Kulfan, P. Zach, unpublished data).

Harmonia axyridis has strong dispersal capabilities (Osawa, 2000; Koch, 2003) which has been reflected by the very fast spread of this species across Europe (Brown et al., 2008; Roy et al., 2016). In newly invaded areas *H. axyridis* tended to use urban and agricultural landscapes rather than semi-natural landscapes (Brown et al., 2008; Grez et al., 2014). Soon after establishment, however, the ladybird diversified to many host plants and different habitats (Adriaens et al., 2008; Roy et al., 2016) tracking aphid populations (Honěk et al., 2019). Adults migrate from breeding to overwintering sites (Obata, 1986; Roy and Brown, 2015) and may form large aggregations (Panigaj et al., 2014). The overwintering sites of *H. axyridis* are principally man-made structures in urban areas and, less often, outside these areas in landscapes with rocks, trees and forests providing shelter from the worst of the weather (Hodek et al., 2012; Panigaj et al., 2014; Nedvěd, 2015).

Understanding of habitat preference and habitat suitability has wide relevance to invasion biology of eurytopic species such as *H. axyridis* (Roy et al., 2016). Sequential investigations of changes in the densities of *H. axyridis* as well as many other insects on trees in parks, forests or other habitats are crucial in this context but there are limitations to them in temperate regions. Leaf fall, for example, affects the timing and duration of investigations of *H. axyridis* on deciduous trees and shrubs. Within Europe, Brown and Roy (2018) studied the changes in the number of *H. axyridis* on mature lime trees *Tilia* × *europea* L. from April to October, and Honěk et al. (2019) investigated the seasonal dynamics of *H. axyridis* on lime, maple (*Acer* spp.) and birch trees (*Betula* spp.) from late April to mid November. In contrast to deciduous trees, evergreen coniferous trees enable the study of *H. axyridis* all year round. Despite this, coniferous trees within Europe have not been systematicallly studied throughout the whole year as hosts of *H. axyridis.* Brown and Roy (2018) surveyed *H. axyridis* on Scots pine from April to October, and Holecová et al. (2018) from October to March.

The Scots pine is the most widely distributed species of pine in the world, extending from Europe to northern Manchuria and the sea of Okhotsk (Critchfield and Little, 1966; Rehfeldt et al., 2002; Barna et al., 2020). Our study provides insights into the year-round dynamics of *H. axyridis* in monoculture Scots pine forest in south-west Slovakia (Central Europe). *Harmonia axyridis* was first recorded in Slovakia in 2008 (Majzlan, 2008; Panigaj et al., 2014) and in the surveyed Scots pine forest in 2012 (M. Holecová, unpublished data). Over the period April 2013–March 2015 we sampled Scots pine trees to (1) investigate changes in the densities of *H. axyridis* in Scots pine stands systematically all year round, (2) examine the effects of selected stand characteristics (variables), including tree age and canopy closure, on the densities of *H. axyridis*, and (3) assess the favourability of Scots pine forest for the reproduction and overwintering of *H. axyridis*. The study is an extension to the winter surveys of native ladybirds in the presence of *H. axyridis* from the same forest (Holecová et al., 2018).

Judging by the seasonal occurrence of *H. axyridis* on Scots pine in the invaded range (Pendleton and Pendleton, 1997–2020; Brown and Roy, 2018; Holecová et al., 2018), we expected the presence of the ladybird in Scots pine forest all year round (prediction 1). As *H. axyridis* prefers habitats within urban and agricultural landscapes over semi-natural landscapes in the early years of invasion (Brown et al., 2008; Grez et al., 2014), we expected low densities of the ladybird on pine trees (prediction 2). Considering the migratory behaviour of *H. axyridis* to and from overwintering sites (Hodek et al., 1993; Jeffries et al., 2013), we expected that this behaviour would be reflected in the seasonal dynamics of the ladybird (prediction 3).

Materials and methods

Study area

Our study was carried out in the central part of the Borská nížina lowland within the forest complex Bor in south-west Slovakia, Central Europe. Monoculture stands of Scots pine on sand dunes (Šomšák and Kubíček, 1994), replacing the former oak-pine forests (Pino-Quercetum; Kollár et al., 2011), characterize the area. The climate is sub-continental, with mean annual temperatures from 9.0 to 9.6°C and mean temperatures in January between –1.0 and –2.0 °C (Bochníček et al., 2015). The two successive summers of 2013 and 2014 were very warm and very dry (Holecová et al., 2016) and the winters of 2013/2014 and 2014/2015 were both exceptionally mild (Holecová et al., 2018).

Mononoculture Scots pine stands aged 5 to 100 years were sampled. Four stands comprised closed canopy stands

(i.e. a dense growth of pines in which the branches from neighbouring trees meet), three stands were open canopy stands (less dense growth of pines with the branches not touching). Elevations of stands ranged from 159 and 222 m asl. Characteristics of the sampled stands are provided in Table 1.

Sampling of *Harmonia axyridis*

Over a two year period (April 2013–March 2015) adult *H. axyridis* were collected in the south-eastern margins of monoculture pine stands. Collections were made monthly, in the middle of each month except for November 2013 when logistical difficulties did not allow sampling. *Harmonia axyridis* was recorded on days with favourable weather conditions (dry and not too windy) between 10:00 and 14:00 h CET by tree beating. Each sample constituted specimens from 20 low (up to 3 m above the ground) branches of several close-growing trees beaten over a circular canvas beating tray of 1.0 m diameter. A total of 10 samples were taken in each stand on every sampling date. In total 32,200 branches (20 branches × 10 samples × 7 stands × 23 months), each 1 m long, were sampled. Young pines (5 years old) were sampled as whole trees. In each stand, *H. axyridis* were always obtained from different trees which were marked to avoid repeated sampling of the same trees. Collected beetles were placed in 70% ethanol and counted later in the laboratory. The larvae of *H. axyridis* were recorded qualitatively (present/absent).

Data analysis

In order to gain insights into how frequently *H. axyridis* occurred in Scots pine forest and how likely it was to record this species in the surveyed stands throughout the year, we calculated the frequency of occurrence of *H. axyridis* as the percentage of the number of stands in which the ladybird was found during the observation period April 2013–March 2015 in relation to the total number of surveyed stands (n = 7).

We modelled seasonal changes in the abundance of *H. axyridis* using the Generalized Additive Model (GAM) (Hastie and Tibshirani, 1990; Wood, 2017) with negative binomial distribution (overdispersion taken into account) and the logarithmic link, accounting for autocorrelation in the abundance data collected repeatedly in the same stands via random stand effect. We pooled the data from 10 samples within the stand for each sampling occassion. Then, we modelled the abundance of *H. axyridis* per 200 branches as a function of the factors year, canopy closure and age of pine stand as parametric fixed terms, the factor stand (sample area effect) as a random term, and the seasonal effect (in month granularity) as the non-parametric smooth term that was allowed to change between the stands with open and closed canopy (i.e. allowing for stand*seasonal interaction).

The semiparametric negative binomial GAM model for the abundance of *H. axyridis Y* in time *t* (indexed in months from the beginning of the study) and stand *s* was specified:

$$Y_{st} \sim Negbin(\mu_{st}, \theta)$$

with

$$log(\mu_{st}) = \beta_0 + \sum_r \beta_r . I(year_t = r) + \sum_c \gamma_c . I(closure_s = c) + \sum_a \delta_a . I(age_a = a) + l_s + \sum_c s_c(month_t) . I(closure_s = c)$$

$$l_s \sim N(0, \sigma^2)$$

where:

β_0 is the intercept,

$\sum_r \beta_r . I(year_t = r)$ is the saturated effect of year (with the usual identifiability constraint – we used "contrast treatment" or baseline constraints for all factors in the model),

$\sum_c \gamma_c . I(closure_s = c)$ is the forest stand closure effect,

$\sum_a \delta_a . I(age_a = a)$ is the saturated forest stand effect,

l_s is the random (normally distributed) sample area effect,

$s_c(month_t)$ is a smooth (penalized spline modelled) seasonal effect which is allowed to differ between open and closed canopy forest stands. Because seasonal effect is necessarily periodic, we enforced periodicity (and smoothness in the whole periodic function) by penalized cyclic spline.

Table 1. Characteristics of Scots pine stands (n =7) (stand i.d., site, geographical coordinates, age of pine trees, canopy closure, habitat description). LNV, Lakšárska Nová Ves; STUD, Studienka

Stand	Site	Longitude	Latitude	Age	Canopy	Habitat
1	LNV	17.1758	48.5822	60	Open	Solitary pine trees on sand dunes, no undergrowth
2	LNV	17.1761	48.5816	10	Closed	Dense pine forest, no undergrowth
3	LNV	17.1727	48.5808	25	Closed	Dense pine forest, no undergrowth
4	LNV	17.1644	48.5819	15	Closed	Dense pine forest, no undergrowth
5	STUD	17.1644	48.5402	100	Closed	Dense pine forest, scattered undergrowth
6	STUD	17.1375	48.5377	20	Open	Patches of pine trees, rich grassy undergrowth
7	STUD	17.1369	48.5416	5	Open	Young pine trees in plantation, no undergrowth

θ is a parameter related inversely to the overdispersion, namely $Var(Y_{st}) = \mu_{st} + \frac{\mu_{st}^2}{\theta}$.

The model was fitted simultaneously via penalized likelihood optimization, with crossvalidated choice of penalization constants for the smooth terms (Wood, 2008). Statistical analyses were performed in R (R Core Team, 2020). The package mgcv in R (Wood, 2017) was used to apply the GAM modelling.

Results

From April 2013 to March 2015 adult *H. axyridis* were recorded in each month of the year and in each pine stand (n = 7). In particular months, however, the ladybird was not recorded in all stands (F < 100%). The ladybird occurred most frequently in April and May, and between October and December, and least frequently in February. The highest frequency of *H. axyridis* (F = 87%) indicated the presence of the ladybird in six of the seven stands, the lowest frequency (F = 14%) the presence in a single stand only (Fig. 1).

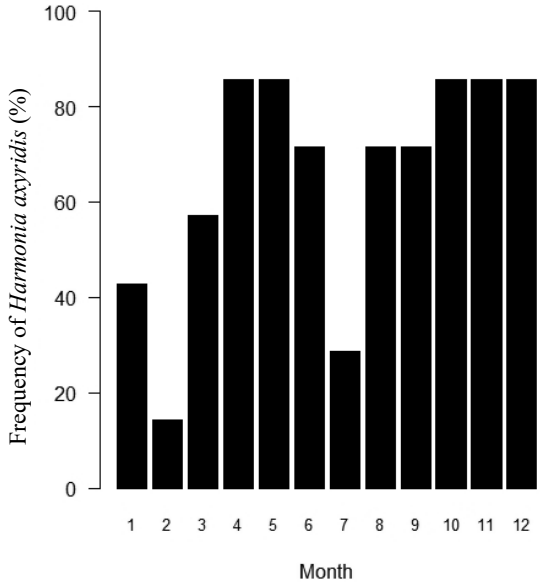

Fig. 1. Frequency of occurrence (%) of *Harmonia axyridis* in Scots pine forest throughout the year. The presence/absence data on the alien ladybird were collected monthly across pine stands (n = 7) in south-west Slovakia over April 2013–March 2015.

In total 500 adult *H. axyridis* were recorded, thus the numbers were low over the study period, within the range 0–195 specimens per 200 branches. Of the recorded beetles, 11% were found in spring (March–May), 5% in summer (June–August), 60% in autumn (September–November) and 24% in winter (December–February). The ladybirds were found most abundantly in November 2014 (195 specimens). During the winter of 2014/2015 *H. axyridis* abundance strongly decreased to 70 specimens in December, 41 specimens in January and just 7 specimens in February.

The abundance of adult *H. axyridis* greatly varied between pine stands. The adults were found in highest numbers in the 15 year old closed canopy stand (stand 4, Table 1) which yielded 67% of the recorded specimens. They were least abundant in the patches of pine trees aged 20 years (stand 6) (2% of all specimens). The pattern of decrease in the number of *H. axyridis* from spring to summer, followed by increase in the autumn and decrease during the winter, was recorded across stands 1–5 but not stands 6–7 where low numbers did not allow determination of any seasonal trend. In the 15 year old closed canopy stand *H. axyridis* was not recorded in summer (June–August) but it was recorded in high numbers in late autumn (November).

The larvae of *H. axyridis* were found in stands 1–6 in low numbers, in most cases as a single or few specimens, from June to September. No larvae were recorded in the 5 year old plantation (stand 7). In the 15 year old closed canopy stand (stand 4) the larvae of *H. axyridis* were not found in summer (June–August).

The negative binomial GAM model showed that the factor year did not significantly affect the abundance of *H. axyridis* (df = 2, χ^2 = 0.837, p = 0.658). The abundance of *H. axyridis* was significantly influenced by the age of stand (df = 5, χ^2 = 16.779, p = 0.005), and it was significantly higher in the 10, 15 and 60 year old stands than in the 5 year old stand (baseline) (p < 0.05). The effect of month was significant for both closed (edf = 5.611, χ^2 = 42.74, p < 0.0001) and open canopy stands (edf = 4.396, χ^2 = 12.97, p = 0.007). The effect of canopy closure proved not significant in influencing the number of *H. axyridis* (df = 1, χ^2 = 0.598, p = 0.439). The effect of forest stand (sample area effect) was also not significant (edf = –2.803e-16, χ^2 = 0.0, p = 0.076). The model explained 61% of the deviance in the abundance data with the overdispersion (θ = 0.686).

The seasonal changes in the abundance of *H. axyridis* were shown in the GAM model separately for the stands with closed canopy (Fig. 2) and those with open canopy (Fig. 3).

Bimodal seasonal variation was apparent in both categories of stand, with peaks of *H. axyridis* in May and November. The seasonal abundance curves differed in shape between the open and the closed canopy stands in certain respects. The closed canopy stands hosted much higher numbers of *H. axyridis* during the winter and showed a much greater decrease in the number of ladybirds in the summer, in comparison to the open canopy stands. Judging from the overlapping confidence intervals, the size of the first abundance peak of *H. axyridis* did not differ significantly between the closed and open canopy stands (Figs 2, 3).

Discussion

Analysing systematically collected data, information about the occurrence of *H. axyridis* in Scots pine forest

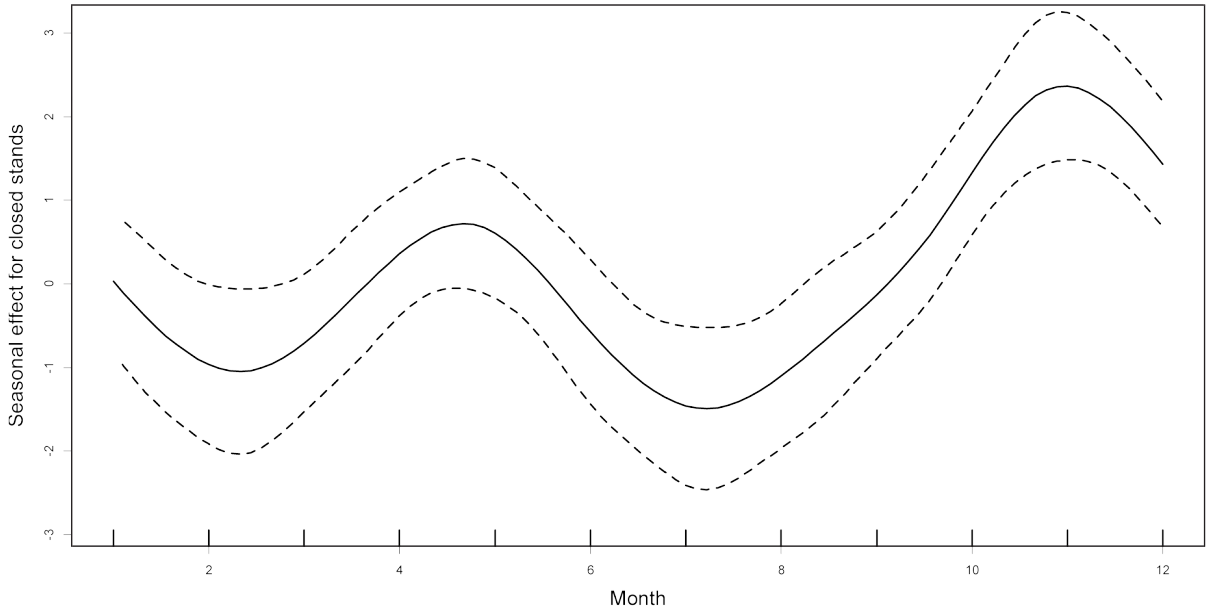

Fig. 2. Seasonal variability of *Harmonia axyridis* counts. Plot of the $s_{closed}(month_t)$ component of the negative binomial model (Scots pine stands with closed canopy). Solid line – the fitted (predicted) abundance of *Harmonia axyridis*, dotted lines – the 95% confidence intervals.

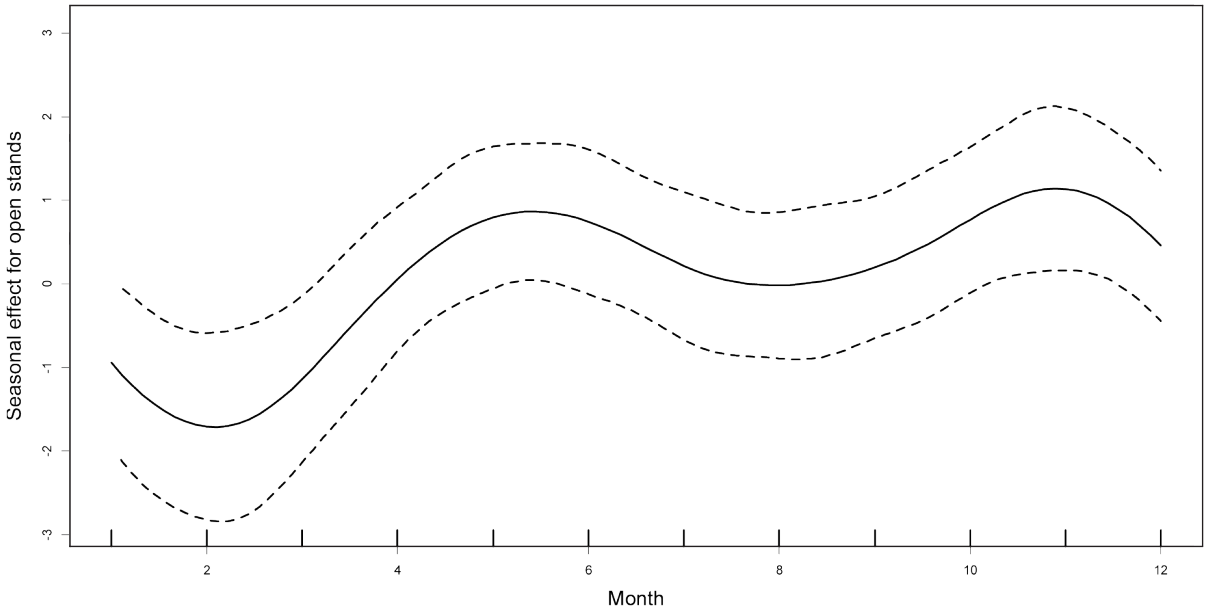

Fig. 3. Seasonal variability of *Harmonia axyridis* counts. Plot of the $s_{open}(month_t)$ component of the negative binomial model (Scots pine stands with open canopy). Solid line – the fitted (predicted) abundance of *Harmonia axyridis*, dotted lines – the 95% confidence intervals.

throughout the year has been gained, with the model of year-round changes in European populations of this ladybird presented for the first time.

Habitat preferences in generalist species of ladybirds are harder to interpret than in species with highly specified ecologies (Majerus, 2016). *Harmonia axyridis* is adapted to an extreme continental climate (Roy et al., 2016). We recorded this species on Scots pine trees growing in poor soils (sandy dunes) with extreme climatic conditions all year round (prediction 1). However, we found relatively few adults and larvae in relation to sampling effort. Low densities of *H. axyridis* may confirm that in the early years of invasion the ladybird focuses on habitats within urban and agricultural landscapes rather than semi-natural landscapes (cf. Brown et al., 2008; Grez et al., 2014) (prediction 2).

The recorded changes in the number of *H. axyridis* may reflect variation in habitats, microclimates and diet-

ary resources of the ladybird from one season of the year to another as well as its migratory behaviour. The bimodal pattern of seasonal dynamics of adult *H. axyridis* in Scots pine forest, common for closed and open canopy stands, involved two peaks reflecting the movement of *H. axyridis* from and to overwintering sites (prediction 3). In the spring, between March and April, deciduous trees lack foliage, and herbaceous plants are not yet available to the ladybird: there is a lack of aphid prey on the plants. Thus at this time evergreen Scots pine trees may intercept adult *H. axyridis* from various overwintering sites („brushing effect of conifers"), resulting in the first abundance peak of the ladybird in May, followed by decrease in abundance during summer. The second (larger) peak in November reflects the migration of *H. axyridis* to overwintering sites. In temperate regions, late summer and early autumn is a crucial time for ladybirds to build up fat reserves and prepare for the winter (Majerus, 2016). Within Europe, adult *H. axyridis* migrate to overwintering sites in October (Roy and Brown, 2015; Panigaj et al., 2014). From mid October to mid November we found them frequently and abundantly on pine branches as a result of this characteristic migratory behaviour. We showed that the number of *H. axyridis* lowered markedly between mid November and mid December and remained low during the rest of the mild winter. The recorded low abundance of *H. axyridis* on pines during the winter paralleled the trends in Britain (Roy and Brown, 2015) where the ladybird is extremely difficult to find openly on Scots pine during this most adverse time of the year for ladybirds (Pendleton and Pendleton, 1997–2020).

Many ladybirds prefer sheltered sites such as crevices in rocks and in the bark of trees, buildings and monuments for overwintering (Majerus, 1994; Koch and Galvan, 2008; Roy and Brown, 2015), and the overwintering site can be critical for their survival (Raak-van den Berg et al., 2012; Majerus, 2016). *Harmonia axyridis* within Europe usually overwinter in buildings (Roy and Brown, 2015) but they may also spend the winter in semi-natural habitats (Panigaj et al., 2014). Two of the three pine stands with the highest abundance of *H. axyridis* (stand 3 and 4) were closed canopy stands. One of them, the 15 year old stand (stand 4), yielded no *H. axyridis* during the two successive summers (June–August) but attracted relatively high numbers of adult *H. axyridis* in late autumn. Why did the beetles choose this particular stand for overwintering? It is known that prominent landscape features such as hills attract *H. axyridis* during the autumn migration (Hodek et al., 1993). Our study, however, was conducted in a lowland area without any prominent landscape features. Therefore, the local conditions providing protection of adults against wind, rain or snow, and particularly the stand characteristics such as the southern aspect of forest edge, high tree density in the forest margin (branches of closely-growing pines touching), and high degree of shelter offered by nearby old mature pine trees, could be crucial for *H. axyridis* selecting this particular stand as an overwintering site. Our results suggest that *H. axyridis* may alternate habitats in space and time; the ladybird apparently utilized certain pine stands preferably for foraging during the growing season and certain stands for refuge during the winter. Many species of ladybirds spend periods in dormancy in habitats that differ from those uti-lized for reproduction and development (Hodek, 2012; Ceryngier, 2015).

Dietary requirements obviously influence where a species lives and breeds (Majerus, 2016). Much greater decrease in the number of *H. axyridis* in the closed canopy stands than the open canopy stands in the summer could be explained by the ladybird's seasonal preference for open habitats with more light and, possibly, higher prey (aphids) availability (Honěk et al., 2018). *Harmonia axyridis* appears to have a high ability to track aphid populations in space and time (Osawa, 2000; Koch, 2003). In both the native and invaded ranges, seasonal changes in the number of *H. axyridis* are significantly correlated with those of aphids (Osawa, 2000; Honěk et al., 2019). We found patchy colonies of a widely spread Scots pine aphid *Cinara pini* (L.) but did not record the number of this or other aphid species on pines. The Scots pine aphids are so mobile that the hatchlings of *H. axyridis* have difficulty capturing them in contrast to most arboreal aphids which walk very slowly (Noriyuki and Osawa, 2016; Noriyuki, 2011). The within-crown distribution of *C. pini* changes over the season (Larsson, 1985) which may affect the distribution of predators of this aphid on pines.

High ability of prey searching and reproduction maintains a stable population of *H. axyridis* in heterogeneous and temporary habitats in the native range (Osawa, 2000). Within this range the habitats of *H. axyridis* can be categorized as (1) habitats for survival and reproduction and (2) habitats for temporary refuge (Osawa, 2000), and the same habitat categorization may apply to the invaded range of the ladybird. Our results showed that adult *H. axyridis* were found in habitats within both categories, but with a prevalence for the second category. A question arises as to whether the Scots pine forest on sand dunes in Central Europe is a favourable habitat for *H. axyridis*. A favourable habitat was defined as one that allowed a species to both survive and breed (Southwood, 1977; Majerus, 2016). Judging from the records of both adults and larvae of *H. axyridis* on the lower branches of pine trees in most stands during the growing season, the Scots pine forest may be considered a favourable habitat for the ladybird. However, as the lower branches of pines hosted few *H. axyridis* over the growing season, this microhabitat is regarded as inferior for the ladybird as a breeding habitat. The decreasing number of *H. axyridis* from spring to summer, with very low numbers of the ladybird in pine stands in summer, did not parallel the seasonal dynamics of this species (increase in number during the growing season) on lime and maple trees in urban areas in Slovakia and Czech Republic (Viglášová et al., 2017). In agreement with this, *H. axyridis* rapidly dominated at lime tree sites in England but comprised a relatively small proportion of the ladybird assemblage at the Scots pine tree site (Brown and Roy, 2018). Regarding the weak persistence of *H. axyridis* on the lower branches of pine trees during the winter, choosing this microhabitat by the ladybird for overwintering was suboptimal.

The results are in agreement with our predictions. *Harmonia axyridis* occurred in a Scots pine forest all year round in low densities and its seasonal dynamics reflected movement of adults from and to overwintering sites. A closer look is needed at what makes a Scots pine tree or Scots pine forest favourable for *H. axyridis* and what hampers the occurrence of this species in this habitat. The trophic relationships between *H. axyridis,* aphids or other insects, and persistence of this successful invader in heterogeneous pine stands with extreme microclimates (Holecová et al., 2016), require greater attention.

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References

Adriaens,T., San Martin y Gomez, G., Maes, D., 2008. Invasion history, habitat preferences and phenology of the invasive ladybird Harmonia axyridis in Belgium. *BioControl,* 53: 69–88.

Barna, M., Ferezliev, A., Tsakov, H., Mihál, I., 2020. Investigations of mature Scots pine stands in windthrow areas in Norway spruce forests in Western Rhodopes. *Folia Oecologica*, 47:1–9.

Bochníček, O., Borsányi, P., Čepčeková, E., Faško, P., Chmelík, M., Jančovičová, Ľ., Kapolková, H., Labudová, L., Mikulová, K., Mišaga, O., Nejedlík, P., Pribullová, A., Snopková, Z., Šťastný, P., Švec, M., Turňa, M., 2015. *Klimatický atlas Slovenska. Climate atlas of Slovakia*. 1st ed. Bratislava: Slovenský hydrometeorologický ústav. 132 p.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovář, I., Loomans, A.J., Majerus, M.E.N., Nedvěd, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I., Roy, D.B., 2008. Harmonia axyridis in Europe: spread and distribution of a non-native coccinellid. *BioControl*, 53: 5–21.

Brown, P.M.J., Roy, H.E., 2018. Native ladybird decline caused by the invasive harlequin ladybird Harmonia axyridis: evidence from a long-term field study. *Insect Conservation and Diversity*, 11: 230–239.

Ceryngier, P., 2015. Ecology of dormancy in ladybird beetles (Coleoptera: Coccinellidae). *Acta Societatis Zoologicae Bohemicae*,79: 29–44.

Critchfield, W.B., Little, E.I., 1966. *Geographic distribution of the pines of the world*. Miscellaneous publication, 991. Washington D.C.: U.S. Department of Agriculture, Forest Service. 97 p.

Grez, A.A., Zaviezo, T., Gardiner, M.M., 2014. Local predator composition and landscape affects biological control of aphids in alfalfa fields. *Biological Control*, 76: 1–9.

Hastie, T.J., Tibshirani, R.J., 1990. *Generalized additive models*. London: Chapman & Hall/CRC. 352 p.

Hodek, I., 2012. Diapause/dormancy. In Hodek I., van Emden H. F., Honěk, A. (eds). *Ecology and behaviour of the ladybird beetles* (*Coccinellidae*). Chichester: Wiley-Blackwell, p. 275–342.

Hodek, I., Iperti, G., Hodkova, M., 1993. Long-distance flights in Coccinellidae (Coleoptera). *European Journal of Entomology*, 90: 403–414.

Hodek., I., van Emden, H. F., Honěk, A., 2012. *Ecology and behaviour of ladybird beetles (Coccinellidae)*. Oxford, Chichester: Willey-Blackwell. 561 p.

Holecová, M., Šebestová, M., Hollá, K., 2016. *Synecological specifics in diversity and dynamics of selected beetle assemblages associated with Scots pine canopies in the Borská nížina lowland (SW Slovakia)*. Bratislava: Comenius University in Bratislava. 82 p.

Holecová, M., Zach, P., Hollá, K., Šebestová, M., Klesniaková, M., Šestáková, A., Honěk, A., Nedvěd, O., Parák, M., Martinková, Z., Holec, J., Viglášová, S., Brown, P.M.J., Roy, H.E., Kulfan, J., 2018. Overwintering of ladybirds (Coleoptera: Coccinellidae) on Scots pine in Central Europe. *European Journal of Entomology*, 115: 658–667.

Honěk, A., Brabec, M., Martinková, Z., Dixon, A.F.G, Pekár, S., Skuhrovec, J., 2019. Factors determining local and seasonal variation in abundance of Harmonia axyridis (Coleoptera: Coccinellidae) in Central Europe. *European Journal of Entomology*, 116: 93–103.

Honěk, A., Martinková, Z., Dixon, A.F.G., 2015. Detecting seasonal variation in composition of adult Coccinellidae communities. *Ecological Entomology*, 40: 543–552.

Honěk, A, Martinková, Z., Štrobach, J., 2018. Effect of aphid abundance and urbanization on the abundance of Harmonia axyridis (Coleoptera: Coccinellidae). *European Journal of Entomology*, 115: 703–707.

Jeffries D. L., Chapman J., Roy, H. E., Humphries, S., Harrington, R., Brown, P. M. J., Lawson Handley, L.J., 2013. Characteristics and drivers of high-altitude ladybird flight: insights from vertical-looking entomological radar. *Public Library of Science One,* 8: 1–12.

Koch, R.L., 2003. The multicolored Asian lady beetle, Harmonia axyridis: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3: 1–16.

Koch, R.L., Galvan, T.L., 2008. Bad side of a good beetle: the North American experience with Harmonia axyridis. Biocontrol, 53: 23–35.

Kollár, J., Balkovič, J., Mazúrová, A., Šimonovič, V., 2011. Phytocoenological and production evaluation of the natural oak and secondary pine forests of the Borská nížina lowland. *Ekológia (Bratislava),* 30: 369–380.

Larsson, S., 1985. Seasonal changes in the within-crown distribution of the aphid Cinara pini on Scots pine. *Oikos*, 45: 217–222.

Majerus, M., 1994. *Ladybirds*. New Naturalist Series, no. 81. London: Harper Collins. 367 p.

Majerus, M., 2016. Roy, H.E., Brown, P.M.J (eds). *A natural history of ladybird beetles*. Cambridge: Cambridge University Press. 397 p.

Majzlan, O., 2008. Faunistical contributions from Slovakia, Coleoptera, 4. *Naturae Tutela*, 12: 207–210.

Nedvěd, O., 2015. *Brouci čeledi slunéčkovití (Coccinellidae) střední Evropy. Ladybird beetles (Coccinellidae) of Central Europe*. Zoologické Klíče = Zoological Keys, 4. Praha: Academia. 304 p.

Noriyuki, S., Osawa, N., 2015. Geographic variation of color polymorphism in two sibling ladybird species, Harmonia yedoensis and H. axyridis (Coleoptera: Coccinellidae). *Entomological Science,* 18: 502–508.

Noriyuki, S., Osawa, N., Nishida, T., 2011. Prey capture performance in hatchlings of two sibling Harmonia ladybird species in relation to maternal investment through sibling cannibalism. *Ecological Entomology*, 36: 282–289.

Noriyuki, S., Osawa, N., 2016. Reproductive interference and niche partitioning in aphidophagous insects. *Psyche: A Journal of Entomology,* 2016: art. ID 4751280.

Noriyuki, S., Sugo, J., Ohata, M., Osawa, N., 2019. Habitat generalization of a predatory ladybird, Harmonia yedoensis (Coleoptera: Coccinellidae), in an allopatric area with respect to its sibling species Harmonia axyridis. *Entomological Science*, 22: 389–392.

Obata, S., 1986. Determination of hibernation site in the ladybird beetle, Harmonia axyridis Pallas (Coleoptera, Coccinellidae). *Kontyu*, 54: 218–223.

Orlova-Bienkowskaja, M.J., Ukrainsky, A.S., Brown, P.M.J., 2015. Harmonia axyridis (Coleoptera: Coccinellidae) in Asia: a re-examination of the native range and invasion to southeastern Kazakhstan and Kyrgyzstan. *Biological Invasions*, 17: 1941–1948.

Osawa, N., 2000. Population field studies on the aphidophagous ladybird beetle Harmonia axyridis (Coleoptera: Coccinellidae): resource tracking and population characteristics. *Population Ecology*, 42:115–127.

Osawa, N., 2011. Ecology of Harmonia axyridis in natural habitats within its native range. *BioControl*, 56: 613–621.

Panigaj, Ľ., Zach, P., Honěk, A., Nedvěd, O., Kulfan, J., Martinková, Z., Selyemová, D., Viglášová, Roy, H.E., 2014. The invasion history, distribution and colour pattern forms of the harlequin ladybird beetle Harmonia axyridis (Coleoptera, Coccinellidae) in Slovakia, Central Europe. *Zookeys*, 412: 89–102.

Pendleton, T., Pendleton, D., 1997–2020. Known ladybird over-wintering sites. [online]. In *Eakring Birds* [the web site dedicated to Nottinghamshire's invertebrate fauna]. [cit. 2020-06-24]. http://www.eakringbirds.com/eakringbirds3/ladybirdswintering.htm

R Core Team, 2020. *R: a language and environment for statistical computing*. [online]. Vienna, Austria: R Foundation for Statistical Computing. [cit. 2020-05-31]. https://www.R-project.org/.

Raak-van den Berg, C.L., Stam, J.M., de Jong, P.W., Hemerik, L., van Lenteren, J.C., 2012. Winter survival of Harmonia axyridis in The Netherlands. *Biological Control*, 60: 68–76.

Rehfeldt, G.E., Tchebakov, N.M., Parefenova, Y.I., Wykoff, W.R., Kuzmina, N.A., Milyutin, L.I., 2002. Intraspecific responses to climate in Pinus sylvestris. *Global Change Biology,* 8: 912–929.

Roy, H.E., Brown, P.M.J., 2015. Ten years of invasion: Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) in Britain. *Ecological Entomology*, 40: 336–348.

Roy, H.E., Brown, P.M.J., Adriaens, T., Berkvens, N., Borges, I., Clusella-Trullas, S., Comont, R. F, De Clercq, P., Eschen, R., Estoup, A., Evans, E.W., Facon, B., Gardiner, M.M., Gil, A., Grez, A. A., Guillemaud, T., Haelewaters, D., Herz, A., Honek, A., Howe, A.G., Hui, C., Hutchison, W.D., Kenis, M., Koch, R.L., Kulfan, J., Lawson, H., Handley, L., Lombaert, E., Loomans, A., Losey, J., Lukashuk, A.O., Maes, D., Magro, A., Murray, K.M., Martin, G.S., Martinkova, Z., Minnaar, I.A., Nedved, O., Orlova-Bienkowskaja, M.J., Osawa, N., Rabitsch, W., Ravn, H.P., Rondoni, G., Rorke, S.L., Ryndevich, S.K., Saethre, M.G., Sloggett, J., Soares, A.O., Stals, R., Tinsley, M.,C., Vandereycken, A., van Wielink, P., Viglasova, S., Zach, P., Zakharov, I.A., Zaviezo, T., Zhao, Z., 2016. The harlequin ladybird, Harmonia axyridis: global perspectives on invasion history and ecology. *Biological Invasions*, 18: 997–1044.

Southwood, T.R.E., 1977. Habitat the templet for ecological studies? *Journal of Animal Ecology*, 46: 337–365.

Šomšák, L., Kubíček, F., 1994. Phytocoenological and production evaluation of the original and secondary pine forests of the Záhorská nížina lowlands. I. Alliance Pino-Quercion. *Ekológia (Bratislava)***,** 13: 335–348.

Viglášová S., Nedvěd O., Zach P., Kulfan J., Parák M., Honěk A., Martinková Z., E. Roy H.E., 2017. Species assemblages of ladybirds including the harlequin ladybird Harmonia axyridis: a comparison at large spatial scale in urban habitats. *BioControl*, 62: 409–421.

Wood, S.N., 2008. Fast stable direct fitting and smoothness selection for generalized additive models. *Journal of the Royal Statistical Society. Series B*, 70: 495–518.

Wood, S.N., 2017. Generalized additive models: an introduction with R. 2nd ed. Boca Raton: Chapman & Hall/CRC. 496 p.

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