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Predominance of *Vibidia duodecimguttata* (Poda, 1761) in the assemblages of ladybird beetles (Coleoptera: Coccinellidae) overwintering in floodplain forests

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We investigated assemblages of ladybird beetles overwintering in the litter of willow-poplar floodplain forests (*Salici-Populetum*) that grew along the banks of the river Vistula in the Mazovian Lowland (central Poland). Altogether, in six study sites, 14 ladybird species were recorded, of which the very clear dominant was a rare mycophagous species, *Vibidia duodecimguttata*. Its contribution to all Coccinellidae sampled in early dormancy period (November-December) was 81% and to those sampled in late dormancy period (March-April) – 76%. Total numbers of ladybirds and the numbers of *V. duodecimguttata* sampled in individual sites were negatively related to the soil humidity in these sites. Degree of the forest degradation expressed as the coverage of sampling sites by a non-native tree, *Acer negundo*, was not found to affect the abundance of the beetles. The rate of winter mortality of *V. duodecimguttata* was relatively low, accounting for 12.2% for pooled data from the six sites.

Key words: Coccinellidae, floodplain forests, overwintering, *Vibidia duodecimguttata*

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INTRODUCTION

Our knowledge on the overwintering ecology of ladybird beetles largely concerns several migratory species that perform long-distance flights to hills or mountains to form winter aggregations there (Hodek et al. 1993). The species in this group which were studied in more detail are *Ceratomegilla undecimnotata* (Schneider) (Hodek 1960, Honěk et al. 2007), *Harmonia axyridis* (Pallas) (Obata 1986) and *Aiolocaria hexaspilota* (Hope) (Kuznetsov 1977) in the Palaearctic, and *Hippodamia convergens* Guérin (Hagen 1962) in the Nearctic region. Some other species, e.g. *Coccinella septempunctata* L. or *Hippodamia variegata* (Goeze), may either migrate to hilltops or overwinter at lower elevations near their breeding sites (Honěk 1989, Ceryngier 2000, Honěk et al. 2007), but even individuals staying in lowlands tend to choose at least slightly elevated, dry and sunny places (Hemptinne 1988, Turnock & Wise 2004). Dor-

mancy under such conditions has been believed to reduce ladybird winter mortality due to a lower incidence of fungal diseases (Iperti 1966, Hemptinne 1988, Honěk 1989) and/or a lower risk of being flooded by thawing snow or overflowing watercourses (Semyanov 1965). In a few ladybird species, however, a tendency for selecting moist rather than dry overwintering sites was found. In the case of the North American *Coleomegilla maculata* (DeGeer), the preference for relatively humid habitats has been postulated to be due to the susceptibility of this species to desiccation (Hodek 2012). Our recent investigations (Godeau & Ceryngier 2011) showed that in central Europe two species of the genus *Calvia*, *C. quindecimguttata* (F.) and *C. decemguttata* (L.), also seem to prefer overwintering under humid conditions. Both were found to overwinter in the litter of marshy alder forest (*Ribeso nigri-Alnetum*) more frequently than in much drier litter of the oak-pine mixed forest (*Querco roboris-Pinetum*). This finding inclined us to explore other humid habitats as potential hibernation quarters of some ladybird species.

Willow-poplar floodplain forests (*Salici-Populetum*) are rather humid habitats. They typically grow along big lowland rivers, on alluvial terraces bordering the river bed. The soil of *Salici-Populetum* is usually relatively moist and may be periodically flooded, especially in early spring. It is mainly made of silt, i.e. a non-structured soil of thin granulometry, allowing a fast percolation during dry episodes. However, humidity as well as the frequency and duration of flooding may considerably differ among sites, taking into account that the elevation of alluvial terraces may vary in a wide range – from 1.5 m to several meters above the summer water level in a river (Sienkiewicz et al. 2001).

Willow-poplar floodplain forests are nowadays infrequent in Poland, as in the whole of Europe. Moreover, their extant patches are often distorted and floristically impoverished, with native species being replaced by non-native ones (Sienkiewicz et al. 2001).

The main aim of this study was to determine the species composition and relative abundance of Coccinellidae overwintering in the litter of willow-poplar floodplain forests. We also wanted to check whether ladybird abundance and species composition were dependent on the environmental variables important for the characteristics of floodplain habitats. We assumed that the soil humidity and the degree of occupancy of the forest vegetation by non-native species belong to such variables. In the area investigated in this study, the only but very common alien woody species found in the willow-poplar forests was the box elder (*Acer negundo* L.). Hence, the coverage of the study sites by this species was considered a measure of the forest degradation.

MATERIAL AND METHODS

Study sites

Samples of overwintering ladybirds were collected in six stands of the willow-poplar floodplain forest growing along the banks of the river Vistula in Warsaw and its vicinity (Mazovian Lowland, central Poland). Geographical position of the sampling sites is shown in Fig. 1 and Table 1.

Ladybird sampling

In each site, ladybirds were sampled twice during their dormancy period: (1) in November-December 2011 (early dormancy) and (2) in March-April 2012 (late dormancy) (see Table 1 for detailed dates). Ladybirds were randomly sifted from the litter using a Winkler sieve. Individual sampling sessions usually lasted about 3 hours. Two shorter sessions instead of one 3-hour session were performed in December in the sites V and VI.

Habitat description

To compare the relative moisture of the sites, mean gravimetric water content was measured in the surface soil layer (0-5 cm). Ten soil samples

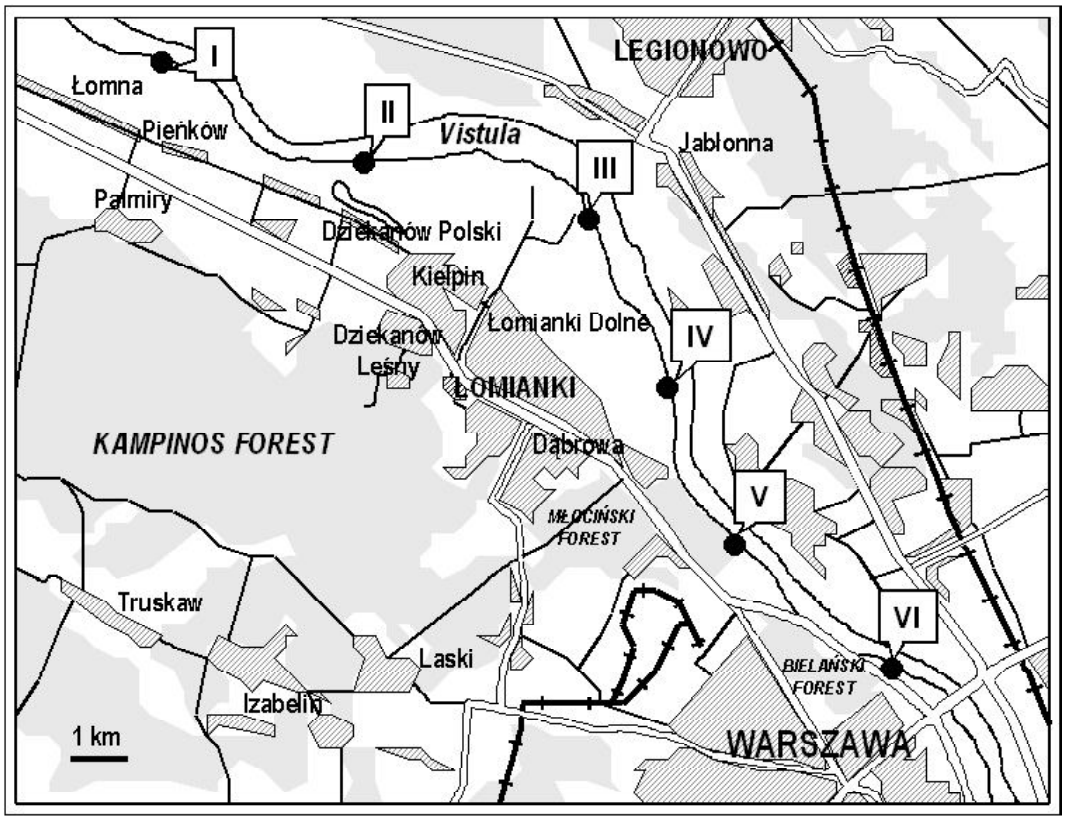

Fig. 1. Localization of the ladybird sampling sites (I-VI).

were taken randomly from each site on the same day (21 November 2012). Samples were weighted and then dried at 105°C to be weighted again after reaching a constant weight. The gravimetric percent water content was determined according to the formula:

$GWC = [(W_F - W_D) / W_F] 100$, where W_F = fresh weight, W_D = dry weight of the sample

Tree and shrub strata of the vegetation were described in each study site using Braun-Blanquet cover-abundance scale: + = cover less than 5%, few individuals, 1 = cover less than 5%, many individuals, 2 = cover range 5-25%, 3 = cover range 25-50%, 4 = cover range 50-75%, and 5 = cover range 75-100%. Two relevés per site, each on the area of 20 m x 20 m (400 m^2) were collected.

Table 1. Geographical position of the sampling sites and the dates of collecting samples in individual sites

Sampling site	Coordinates	UTM	Date of sampling	
			early dormancy	late dormancy
I	52°24'N, 20°48'E	DD80	30 Nov. 2011	21 Mar. 2012
II	52°23'N, 20°50'E	DD80	23 Nov. 2011	11 Apr. 2012
III	52°22'N, 20°54'E	DD90	16 Nov. 2011	4 Apr. 2012
IV	52°20'N, 20°55'E	DC99	7 Dec. 2011	28 Mar. 2012
V	52°19'N, 20°56'E	DC99	20 & 30 Dec. 2011	18 Apr. 2012
VI	52°18'N, 20°59'E	DC99	29-30 Dec. 2011	17 Apr. 2012

To quantify the level of forest degradation in individual sites, total cover of *A. negundo* was assessed by converting its ratings in Braun-Blanquet scale to midpoints of cover ranges. This conversion was done after Wikum & Shanholtzer (1978) as: + = 0.1%, 1 = 2.5%, 2 = 15%, 3 = 37.5%, 4 = 62.5%, and 5 = 87.5%. The mean cover of *A. negundo* on a given site was calculated as a sum of midpoint values recorded in all considered strata in both relevés divided by 2 (number of relevés per site).

Habitat characteristics of the sampling sites are summarized in Table 2. It can be seen there that the least degraded site III clearly differed from the remaining sites as to the vegetation structure and the cover of *A. negundo*.

Data analysis

The relationships between environmental variables (soil moisture and cover of *A. negundo*) and ladybird abundance were tested with the Spearman's rank correlation. Differences in the proportion of dead individuals in the samples were checked with the chi-square test. Calculations were performed using STATISTICA 6.0 software.

RESULTS

Altogether, 14 species of Coccinellidae were found in the litter of the studied floodplain forest patches with *Vibidia duodecimguttata* (Poda) being a very clear dominant in all but one sample (Table 3). This species accounted for 81% of all ladybirds collected in early dormancy and for 76 % of those collected in late dormancy. The contribution to ladybird assemblages of the next most abundant species, *Calvia quatuordecimguttata* (L.), was only 6% in early dormancy and 5% in late dormancy.

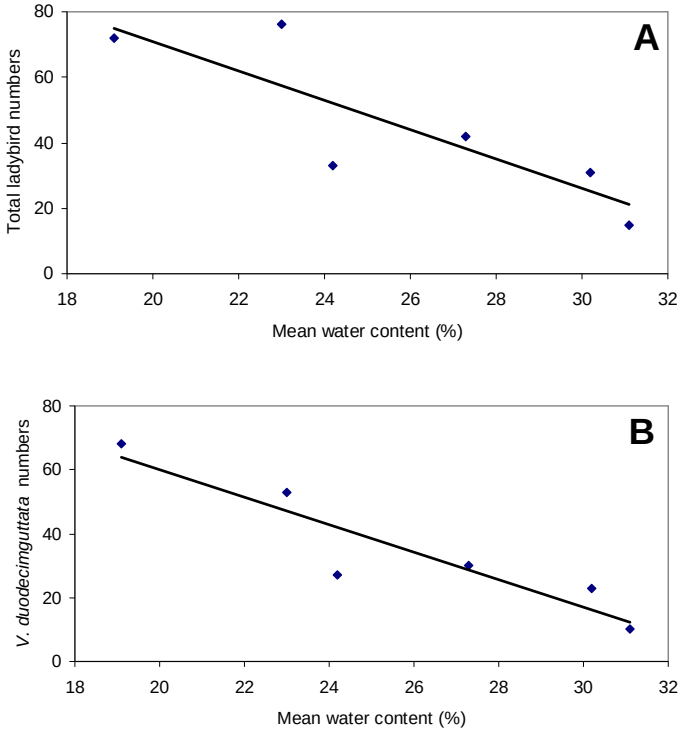

Fig. 2. Relationships between gravimetric water content in the soil of ladybird sampling sites and the total ladybird abundance (A), and the abundance of *V. duodecimguttata* (B).

Spearman's correlation revealed negative relationships between water content in the soil of the hibernation sites and the total numbers of ladybirds (R = -0.89, P = 0.02) as well as the numbers of *V. duodecimguttata* (R = -0.94, P = 0.005) collected from these sites (Fig. 2).

In the well preserved and least degraded site III the recorded species richness of overwintering Coccinellidae was the highest (9 species vs. 3-7 species in other sites). However, no relationship was found between the cover of *A. negundo* and the abundance of *V. duodecimguttata* (Spearman's correlation: R = 0.14, P = 0.78) or the total abundance of ladybirds (R = -0.09, P = 0.87).

In early dormancy samples, 2.9% (4 out of 140 individuals) of all Coccinellidae and 3.5% (4 out of 113 individuals) of *V. duodecimguttata* were collected dead. In late dormancy period, the contribution of dead individuals to all individuals in the samples was much higher (17 out of 129 individuals = 13.2% for all Coccinellidae and 12 out of 98 individuals = 12.2% for *V. duodecimguttata*). Difference between the proportion of dead individuals in early and late dormancy samples was statistically significant both for all Coccinellidae (chi-square test: $\chi^2 = 9.94$, df =1, $P < 0.01$) and for *V. duodecimguttata* ($\chi^2 = 5.68$, df = 1, $P < 0.02$).

DISCUSSION

Relatively numerous occurrence and well-marked predominance of *V. duodecimguttata* recorded in this study is an interesting finding. Although widely distributed in the Palaearctic region (Kovář 2007), in many parts of Europe this ladybird is regarded as very rare (De Gunst 1978, Adriaens & Maes 2004, San Martin et al. 2006, Lillig & Eisinger 2008, Nicolas 2010). In Poland, it has recently been reported only a few times, usually as single specimens (Gutowski et al. 2006, Ruta et al. 2009, Ceryngier 2011, Florek et al. 2011).

Very little is known about overwintering ecology of *V. duodecimguttata*. The only published information on this known to us comes from the observation made by Ruscinsky (1933, ref. in Hodek 2012) in the historical region of Bessarabia (southeastern Europe). Ruscinsky found several large aggregations of *V. duodecimguttata*, each consisting of about 2500 individuals, overwintering on the top of a hill. In our study sites aggregating of *V. duodecimguttata* for dormancy is rather unlikely. Although we did not determine ladybird densities and distribution patterns, our sampling effort and the numbers of collected individuals indicate that the densities were low, not exceeding a few individuals per square meter. Other difference between Ruscinsky's report and our finding is the type of habitat chosen by *V. duodecimguttata* for overwintering. While the Bessarabian beetles spent the period of dormancy in elevated, probably dry places, those recorded in this study overwintered in a flat terrain with rather high humidity. Nevertheless, within our sampling sites, quite diversified as to the soil humidity, a tendency in *V. duodecimguttata* could be found for selecting drier conditions. It thus seems that, although tolerant to high humidity, *V. duodecimguttata* prefers less humid places for overwintering. As pointed out by several authors (Iperti 1966, Hemptinne 1988, Honěk 1989), such a preference may imply reduced winter mortality due to lower incidence of fungal diseases. Unfortunately, the relationship between soil humidity and ladybird mortality could not be tested in our study because of too low numbers of dead and living individuals sampled from individual sites.

Mortality rate of overwintering ladybirds is often recorded to increase rapidly in late dormancy (Lipa et al. 1975, Mills 1981, Wright & Laing 1982). It is thus not surprising that we found much more dead ladybird individuals in the spring samples than in the earlier, autumn-winter samples. The recorded levels of winter mortality of *V. duodecimguttata* as well as all sampled Coccinellidae were, however, not very high comparing to those reported for other ladybird species. Barron & Wilson (1998), for example, experimentally assessed winter mortality of *C. septempunctata* in Britain as ranging between

Table 2. Habitat characteristics of the sampling sites. Braun-Blanquet cover-abundance scale: + = cover less than 5%, few individuals; 1 = cover less than 5%, many individuals; 2 = cover range 5-25%; 3 = cover range 25-50%; 4 = cover range 50-75%. Note that the mean cover of *Acer negundo*, as taking three forest strata into account, may exceed 100%

Site	I		II		III		IV		V		VI	
Relevé	1	2	1	2	1	2	1	2	1	2	1	2
Soil water content (mean ± SD) (%)	24.2 ± 2.9		19.1 ± 2.0		23.0 ± 2.3		30.2 ± 1.3		31.1 ± 3.5		27.3 ± 3.2	
Woody vegetation												
A1 (canopy stratum):												
Salix alba	+	2	1	+	2	1	4	2	3	3	3	2
Populus alba		2	2	3	2	3		+			+	1
Populus nigra			+		+	1		+			+	1
Salix fragilis			+				1	1	1	+		+
Ulmus minor						1						
Acer negundo											1	+
A2 (subcanopy stratum):												
Acer negundo	3	2	4	3	1	+	2	3	2	2	4	4
Salix alba		3	1		2	1	2	1	1	2	1	1
Populus alba				1	+							
Prunus padus					1	+			+	+		
Ulmus minor					2	3						
Acer campestre					+	1						
Acer platanoides		+										
Ulmus laevis									+			
Crataegus monogyna	+											
Salix viminalis	1											
B (shrub stratum):												
Acer negundo	3	3	3	3	2	+	3	4	3	4	3	3
Sambucus nigra	1	+	1	2	+	1	+	+		+	+	
Ulmus minor					1	2					+	
Euonymus europaea				+	3	2						
Cornus sanguinea					2	2						
Acer campestre					+	+						
Malus sp.					1							
Salix viminalis	+											
Fraxinus excelsior												+
Number of woody plant species	7		7		11		6		7		8	
Mean cover of *Acer negundo* (%)	63.75		87.50		8.85		76.25		65.00		101.30	

9% and 72% depending on which physiological group of the beetles is considered. In experiments conducted by Wright & Laing (1982) in Ontario (Canada), mortality of overwintering *Coleomegilla maculata* ranged between 24% and 100%. The rate of winter mortality of *Ceratomegilla undecimnotata* recorded by Iperti (1966) in southeastern France was different in different kinds of hibernation shelters used by this species. The beetles overwintering in rock cracks and piles of stones suffered only 1.3% and 8.6% mortality, respectively, while mortality of those hidden in plant material attained 34%. In the latter, least suitable kind of hibernaculum, *C. undecimnotata* were often killed by entomopathogenic fungi of the genus *Beauveria*. These pathogens, known as one of the most important mortality factors of overwintering ladybird beetles, require high humidity for completing their life cycle and spread in host population (Ceryngier et al. 2012). Such conditions occurred in our study sites, but among

Table 3. Numbers of individual ladybird beetle species collected from the six floodplain forest sites in early dormancy and late dormancy period. In brackets – numbers of dead individuals

Species	early dormancy						
	I	II	III	IV	V	VI	Total
Adalia decempunctata (L.)	-	-	1	-	-	-	1
Calvia decemguttata (L.)	-	-	-	-	-	-	-
Calvia quatuordecimguttata (L.)	-	1	2	2	1	3	9
Chilocorus bipustulatus (L.)	-	-	1	-	-	-	1
Coccinella septempunctata L.	-	1	-	-	-	2	3
Coccinula quatuordecimpustulata (L.)	-	-	-	1	-	-	1
Exochomus quadripustulatus (L.)	-	-	-	1	-	-	1
Harmonia axyridis (Pallas)	-	-	-	1	-	1	2
Propylea quatuordecimpunctata (L.)	-	-	1	-	1	1	3
Scymnus ferrugatus (Moll)	-	-	-	-	-	-	-
Scymnus suturalis Thunberg	-	-	1	-	-	-	1
Sospita vigintiguttata (L.)	-	-	-	-	-	-	-
Stethorus pusillus (Herbst)	4	-	-	1	-	-	5
Vibidia duodecimguttata (Poda)	10	38 (1)	22	12 (2)	10	21 (1)	113 (4)
Total	14	40 (1)	28	18 (2)	12	28 (1)	140 (4)

Species	late dormancy						
	I	II	III	IV	V	VI	Total
Adalia decempunctata (L.)	-	1 (1)	1	-	-	-	2 (1)
Calvia decemguttata (L.)	-	-	-	-	-	1	1
Calvia quatuordecimguttata (L.)	1	-	4 (1)	1	-	1	7 (1)
Chilocorus bipustulatus (L.)	-	-	-	-	-	-	-
Coccinella septempunctata L.	-	1	-	1 (1)	-	-	2 (1)
Coccinula quatuordecimpustulata (L.)	-	-	-	-	-	-	-
Exochomus quadripustulatus (L.)	-	-	-	-	-	-	-
Harmonia axyridis (Pallas)	-	-	1 (1)	-	-	-	1 (1)
Propylea quatuordecimpunctata (L.)	1 (1)	-	1	-	3	1	6 (1)
Scymnus ferrugatus (Moll)	-	-	9	-	-	-	9
Scymnus suturalis Thunberg	-	-	-	-	-	-	-
Sospita vigintiguttata (L.)	-	-	1	-	-	-	1
Stethorus pusillus (Herbst)	-	-	-	-	-	2	2
Vibidia duodecimguttata (Poda)	17 (3)	30 (2)	31 (5)	11 (2)	-	9	98 (12)
Total	19 (4)	32 (3)	48 (7)	13 (3)	3	14	129 (17)

V. duodecimguttata overwintering there neither the prevalence of mycosis (unpubl. data) nor the total mortality rate was high. It is possible that *V. duodecimguttata* might be little susceptible to the fungal pathogens present in the soil and litter of floodplain forests. In insects spending a substantial part of their lifetime under conditions favourable for entomopathogens, low susceptibility to the latter is likely to evolve. In epigeic insects (e.g. carabid or staphylinid beetles) that are in contact with soil and litter reservoirs of entomopathogens throughout their life (not only during hibernation), high resistance to fungal infections is usually recorded (Steenberg et al. 1995, Riedel & Steenberg 1998, Hicks et al. 2001, Traugott et al. 2005). On the other hand, low prevalence of mycosis and low mortality recorded in this study might result from low densities of singly (not in aggregations) overwintering ladybirds, as in low host densities transmission of the pathogens among hosts may be impeded

(Hajek & St. Leger 1994). The importance of density and distribution pattern of dormant ladybirds for their infestation by entomopathogenic fungi was shown in the studies of *Coccinella septempunctata* overwintering in submontane and montane localities in south-western Poland. In submontane site, where the beetles overwintered in low density and not aggregated, the incidence of mycosis was much lower than in two montane sites with dense populations of aggregated beetles (Ceryngier 2000).

As other members of the coccinellid tribe Halyziini, *V. duodecimguttata* is a specialized feeder of the powdery mildew fungi (Ascomycetes: Erysiphales, Erysiphaceae) (Sutherland & Parrella 2009). It is associated with the Erysiphaceae that parasitize broad-leaved trees and shrubs rather than herbaceous plants. Martelli (1914) found larvae and adults of *V. duodecimguttata* feeding on *Podosphaera pannosa* (Wallr.) de Bary infesting peach and *Phyllactinia guttata* (Wallr.) Lév. infesting hazel in Sicily, while Lichtenstein (1917) noted *Phyllactinia* sp. from ash trees as the food of larvae and adults of this ladybird in Montpellier (southern France). In autumns of 2011 and 2012, we observed high numbers of *V. duodecimguttata* on some powdery mildew infected trees and shrubs (*Acer campestre* L., *A. negundo*, *Cornus sanguinea* L.) within our sampling site III (unpubl. data). This indicates that floodplain forests may serve not only as overwintering sites, but also as suitable habitats for *V. duodecimguttata* during its active life.

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