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Temporal Variations of Chrysomelid Abundance and Herbivory in Hybrid Poplar (*Populus* × *canadensis* Moench) Plantation in Serbia

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ABSTRACT

Life cycles, feeding patterns and herbivory intensities of two chrysomelid species (*Crepidodera pluta* and *Phratora vitellinae*) were analysed in the poplar (*Populus* × *canadensis* Moench) plantation over the period of six months in 2014. Herbivory was measured as leaf area lost and a number of damaged leaves per branch. For *C. pluta* was found to have one generation per year, with peak abundance in August, while *P. vitellinae* had one or two generations and a maximum abundance in July. Both species expressed the lowest intensity of herbivory during the post hibernation period in April, and the highest levels of herbivory in July. Beetles completed their life cycles on *P.* × *canadensis* but did not attain maximum potential growth rates by feeding on this plant, which we consider as a suboptimal food source. Chrysomelid damage was found to be insignificant and did not affect tree vitality.

Key words: Leaf beetles, life cycle, herbivory, abundance, poplars.

INTRODUCTION

Beetles of the family Chrysomelidae commonly occur in willow and poplar forest ecosystems. Willows and poplars represent a primary source of food for many species (Derngburg, 1996). Certain species feed on those trees alternatively, when their primary host plants are not available for some reason. Chrysomelid herbivory can cause substantial leaf loss and can affect plant health and its susceptibility to secondary infections. This is particularly important in forest plantations and nurseries. In commercial poplar plantations, species from the genera *Crepidodera* Chevrolat, 1837 and *Phratora* Chevrolat, 1843 are often perceived as harmful (Peacock and Herrick, 2000; Sage and Tucker, 1998; Urban, 2006, 2011a, 2011b).

Two analyzed chrysomelid species *Crepidodera pluta* (Latreille, 1804) and *Phratora vitellinae* (Linnaeus, 1758) belong to subfamilies Alticinae and Chrysomelinae respectively. *C. pluta* is distributed throughout most of the Palaearctic region, while *P. vitellinae* is additionally introduced to North America and therefore has Holarctic distribution (Беньковский, 2011; Gruev and Döberl, 1997; Löbl and Smetana, 2010; Urban, 2006). Both species feed on plants from the family Salicaceae (*Populus* L. and *Salix* L.). It is interesting to note that both are more commonly found on species from the genus *Salix* L., although during this study they were exclusively caught on poplars. *C. pluta* is known to feed on *Salix alba* L., *S. daphnoides* Vill., *Salix × fragilis* L. and *Populus tremula* L. (Kapp *et al.*, 1999; Konstantinov, 1996; Parry, 1986). According to literature sources, *P. vitellinae* can feed on *Alnus hirsuta* (Spach) Rupr., *Betula* spp., *Populus × canadensis* Moench, *P. deltoides* Bartr. ex Marsh., *Populus nigra* L., *P. tremula* L., *P. trichocarpa* Torrey and Gray, *Salix acutifolia* Willd., *S. alba* L., *S. caprea* L., *S. cinerea* L., *S. dasyclados* Wimm., *Salix × fragilis* L., *S. hegetschweileri* Heer, *S. myrsinifolia* Salisb., *S. phylicifolia* L., *S. purpurea* L., *S. repens* L., *Salix × rubra* Huds., *S. triandra* L. and *S. viminalis* L. (Rowell-Rahier, 1984; Urban, 2006). *P. vitellinae* is a well known species, intensively studied for its economic importance, while the biology and life cycle of *C. pluta* is thought to be similar to other *Crepidodera* species.

In this field study we have investigated the life cycle and feeding patterns of two chrysomelid species sharing the same host plant. The herbivore pressure was expressed as a loss of leaf surface and a number of damaged leaves per branch. All parameters were monitored monthly during an active phase of feeding. An understanding of the feeding dynamics may help to develop methods to manage or control these potential forestry pests.

MATERIALS AND METHODS

Beetles were collected in 2014 over the period of six months (from April to September) in the area of Kupinski kut (N 44° 41.601' E 20° 01.012'). Kupinski kut is situated near the village of Kupinovo and Obedska bara, an abandoned meander of the Sava River, which is located in the south-eastern part of Srem in the Autonomous Province of Vojvodina (Northern Serbia). Obedska bara has the status of a Special Nature Reserve and is on the UNESCO's list of internationally important wet habitats (Ramsar area). Kupinski kut is dominated by forest vegetation, mainly poplars. A variety of herbaceous vegetation is present along the marsh and on moist meadows. Increased precipitation periods during spring and autumn cause periodic flooding in the area (Krajić *et al.*, 2012).

Kupinski kut (Public Enterprise Vojvodinašume, Forest Estate Sremska Mitrovica, Forest Administration Kupinovo) is one of the largest plantations of poplar clones in Europe. Predominant species planted here is *P. × canadensis* Moench (syn. *Populus × euramericana* (Dode) Guinier), a cross between *Populus nigra* L. and *P. deltoides* Bartr. ex Marsh. Forest management plans, working plans, and files date back to 1938 (Janjatović *et al.*, 2001). During the first rotation, the prevailing cultivars were 'Serotina', 'Marilandica' and 'Robusta'. During the second cycle of planting, the dominant cultivar was the clone I-214. High productivity of this clone surpassed initial forester's expectations and is now widely planted. It is primarily used in timber industry (Janjatović *et al.*, 2001).

The insects were collected using an aspirator. Only adults were collected and counted. Due to the fact that the highest damage is caused by adults (Warchalowski, 1973), larvae were not collected or counted but certain interesting observations were recorded at the time of sampling. Ethyl acetate was used as a killing agent. Insects were stored as dry preparations on sample cards. Stereomicroscope Carl Zeiss STEMI 2000-C with independent lighting Schott KL1500 LCD was used for the analysis of the material. The identification was performed on the basis of external morphological features and aedeagi characteristics of male specimens using the key Warchalowski (2003). Number of chrysomelid specimens on sampled branches and sex ratios were also recorded.

Herbivory was evaluated by measuring leaf area lost and a number of damaged leaves per branch. Ten trees were chosen at random, maintaining a distance of at least 10 m between trees. Ages of trees in stands ranged from 6 to 8 years. From each tree, one of the lower branches of the canopy accessible from the ground was sampled. Leaf surfaces and an area eaten by insects were measured from five leaves of each branch. Using a centimeter ruler as a reference scale, the surface area was later calculated using ImageJ v.1.48s software (Rasband, 2014). Feeding marks of the two species could easily be distinguished; *C. pluta* created minute often separated perforations (up to about 2 mm in diameter), while *P. vitellinae* damage was lace-like consisting of numerous juxtaposed irregular holes (2-3 mm in diameter). To minimize variation in leaf age, only mature, fully expanded leaves of the similar size were picked.

All parameters were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Due to violation of parametric assumptions, the non-parametric tests were used. Differences in the levels of herbivory were analyzed using Kruskal-Wallis tests (H), followed by Mann-Whitney tests (U) (reported at a .05 level of significance). Pearson's correlation coefficient (r) was used as a measure of effect sizes. Additional correlations between the variables were done using Spearman's tests (r_s). The statistical analysis was performed using statistical package IBM SPSS Statistics v.21 (IBM Corporation, Armonk, New York).

RESULTS

During the six months period of 2014 we collected a total of 629 adult specimens (278 of *C. pluta* and 351 of *P. vitellinae*). In March numerous *C. pluta* and *P. vitellinae* specimens were found in the surface litter below the poplar trees, which is probably their main overwintering site. *P. vitellinae* were occasionally found in small groups of up to three beetles. Several adults of *C. pluta* were also found under stones and logs, while several *P. vitellinae* were hiding within cracks in the bark of trees.

Both species during April started appearing on leaves of *P. × canadensis*. Adults were positioned on the upper and lower surface of leaves. *C. pluta* specimens readily flew over short distances between the branches, while *P. vitellinae* were found beneath the leaves in a somewhat lethargic state during this month. Mating pairs of *C. pluta* species were first observed in April and could be seen until July. Copulating pairs of *P. vitellinae* were noticed in May and in the same month egg depositions were detected on abaxial face of some leaves, usually located near central veins. Larvae were also observed during this month. In June last year's adults and larvae of *P. vitellinae* were often seen on leaves and branches of trees. We believed that the adults of *C. pluta* observed in July mostly belonged to the last year's generation and partially to the new generation. The new generation of *P. vitellinae* developed in July. Mating pairs and egg clusters were seen occasionally. August samples contained significantly more specimens of *C. pluta* than those taken in the previous months. This is probably because of the appearance of a new generation adults. On the other hand, number of *P. vitellinae* adults declined, although their larvae were visible on certain leaves indicating the possible development of the second generation. Beetle numbers started to decline at the beginning of autumn. During the last sampling done near the end of September the number of adults caught was drastically reduced. Larvae were not found and adults were scarce. In October sampling could not be done because only a few specimens of *C. pluta* and *P. vitellinae* were present. An overview of the species life cycles is given in the Table 1.

Table 1. Observational description of life stages succession for *C. pluta* and *P. vitellinae*. LA last year's adults (overwintering adults); A1 first generation adults; A2 second generation adults; E eggs; L1 first generation larvae; L2 second generation larvae; ? probable occurrence.

	APR	MAY	JUN	JUL	AUG	SEP
C. pluta	LA	LA	LA	LA, A1?	LA, A1	LA, A1
P. vitellinae	LA	LA, E, L1	LA, L1	LA, A1, E	LA, A1, L2	LA, A1, A2?

The number of *C. pluta* on poplars increased during May and reached the maximum (72 specimens collected) in August, when a majority of new generation beetles appeared. Their numbers decreased until October (Fig. 1). *P. vitellinae* numbers increased progressively from April to July. It is possible that the beetles of the overwintering and new generations combined in such a way that a maximum number was recorded in July (131 specimens). The number of collected *P. vitellinae* declined until October. Sex ratios in samples of both species fluctuated as a consequence of beetles maturity and behavior related to the mating and oviposition periods (Fig. 1). More females showed up in samples from April, when beetles started to leave their overwintering shelters. More males were present during the intensive periods of mating in May and during July.

During the sampling period almost all leaves had at least some amount of herbivore damage. Variation in the levels of herbivory over the period of six months was significantly different - $H(5) = 256.03$, $p < .001$ for *C. pluta* and $H(5) = 261.17$, $p < .001$ for *P. vitellinae* (Fig. 2). Both species expressed the lowest intensity of herbivory during the post hibernation period in April with mean values of leaf area damaged 11.77 ± 0.34 and 7.86 ± 0.28, for *C. pluta* and *P. vitellinae* respectively. Comparisons of leaf damages between April and July using the Mann-Whitney tests revealed that both species had the highest levels of herbivory in July - *C. pluta* 102.24 ± 3.10 ($U = 6.00$, $p < .05$, $r = -.86$), and *P. vitellinae* 177.77 ± 5.93 ($U = 42.00$, $p < .05$, $r = -.83$).

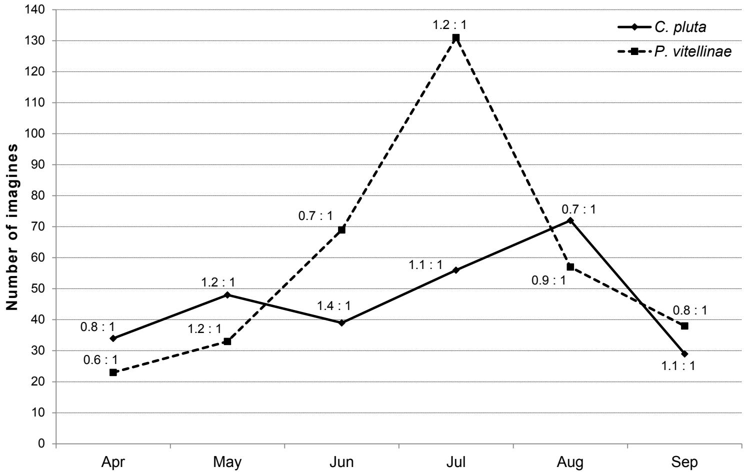

Fig. 1. Number of adults of *C. pluta* and *P. vitellinae* throughout the seasons with a sex ratio of males and females (♂ : ♀ indicated above data points).

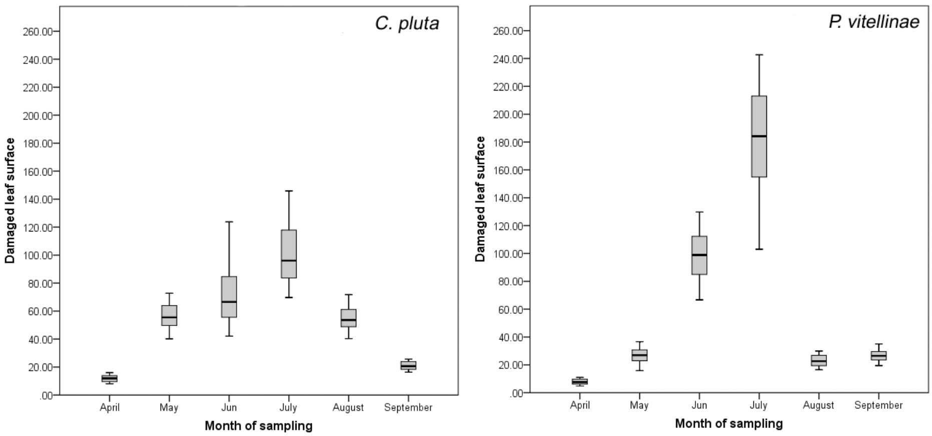

Fig. 2. Seasonal variation of herbivory expressed as leaf area lost (mm^2) for *C. pluta* and *P. vitellinae*.

Mean number of damaged leaves per branch was the lowest in April (14.40 ± 3.81) and the highest in July (27.80 ± 5.16), which complies with the levels of herbivory in those months. The total number of damaged leaves was also minimal in April and maximal in July (Fig. 3). For the sampling period of six months per cent damage of the leaf surface was significantly correlated with the number of damaged leaves for *C. pluta* (r_s = .607, p < .001) and *P. vitellinae* (r_s = .591, p < .001). It was also found that a per cent leaf damage of the two species was statistically positively correlated when an entire season was considered, r_s = .862, p < .001. However, no significant correlations were found between the herbivory of the species on the monthly basis or between the abundance of one species and the herbivory of the other.

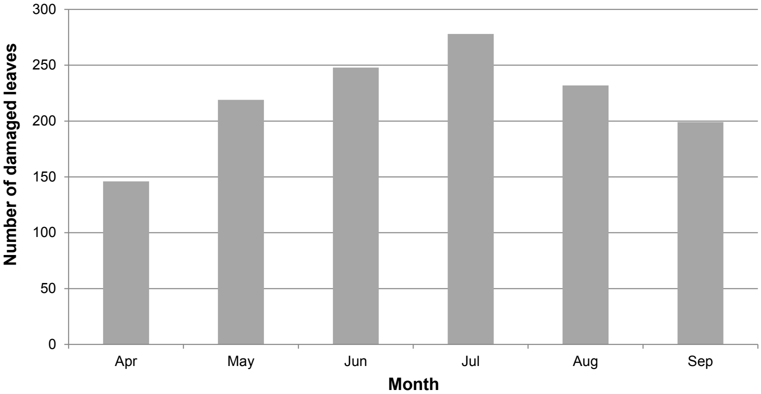

Fig. 3. Monthly variation in the number of damaged leaves.

DISCUSSION

Relatively little is known about the biology of *C. pluta* (Kapp *et al.*, 1999; Konstantinov, 1996). A part of its life cycle could not be observed because egg laying and larval development was occurring within the soil. Oviposition in the field has not been observed but it is known that in other *Crepidodera* species it can last until August (Parry, 1986). Larvae are rhizophagous (root miners), they develop and pupate during summer (Parry, 1986; Steinhausen, 2005). Due to the increase in numbers we believe that the new generation of adults developed in August, or probably even earlier, in the second half of July. Last year's beetles persisted until October and during this month a new generation of adults sought shelter in preparation for diapause. Urban (2011a) found that the new generation of adults of the similar species *Crepidodera aurata* (Marsham, 1802) occurred in the open from the end of August to the beginning of October.

Life cycle of *P. vitellinae* is generally well known and mostly takes place on the above-ground parts of the trees, on branches and leaves (Urban, 2006). The larvae we observed in May were located on the abaxial surface of leaves. They formed small aggregations, with 3-5 larvae positioned with their lateral sides in contact with each other. Larvae undergo three instars and their development takes 2-3 weeks. Pupation takes place in the surface layer of soil (Urban, 2006). Sudden increase in numbers during July indicated the emergence of the first generation adults. Based on field observations alone it was less clear if there was a second generation, and whether those new adults appeared in August or September. Beetle numbers were significantly lower during those months but larvae could be seen on some leaves in August (in small groups of up to three larvae), which could indicate the development of the second generation. Urban (2006) found two generations developing in natural conditions (Czech Republic, Moravia) and three generations per year under laboratory conditions. If the second generation did appear, then its development was only partial and probably halted before it was able to mate.

Species numbers fluctuated during the seasons mostly due to meteorological conditions and phases of the life cycles. The effect of predators and parasitoids was not ascertained. Food source was available throughout the insects' activity period and its quality rather than quantity was a controlling factor. After winter hibernation, species' numbers gradually increased toward summer. Two numerical maximums were recorded for *C. pluta* - the first maximum in May was caused by the last year's beetles, and the second in August was the result of eclosion of the new generation of adults (Fig. 1). For other species of the genus *Crepidodera* the first maximum was observed in June, while the second one was the same as in our case, in August (Steinhausen, 2005; Urban 2011a). We have recorded a slight drop in abundance in June which could be attributed to departure of females to lay eggs into the soil. During this month males were also more abundant than females (1.4 : 1). Urban (2011a) documented similar behavior for *C. aurata*. Abundance of *P. vitellinae* increased till the end of July and then gradually decreased (Fig. 1). Numerical maximum for this species coincided with the development of the new generation of adults. The second peak of abundance that could have been observed if the new generation of adults developed in August or September was not recorded. Due to already mentioned presence of larvae in those months, we can speculate that the beetle numbers did not increase, even when the second generation formed, because adults dispersed from the studied poplar plantation.

Herbivory intensity, observed as leaf area lost and a number of damaged leaves per branch, for both species increased toward July reaching the maximum values (Fig. 2, 3). For *C. pluta* this increase could be attributed to the formation of a new generation adults, since larvae do not feed on leaves. Herbivory of the last year's generation before July was greater than the combined feeding of two overlapping generations in August and September and quality of the food was at least partially responsible for the results. It is known that the tree's leaf quality decreases throughout the growing season and it can affect associated herbivores (Zehnder *et al.*, 2009). Significant leaf damage by *P. vitellinae* in June could be attributed to larvae complemented by the feeding of the last year's adults. The third larval instar that developed in this month is usually the most damaging (Urban, 2006). Eclosion of the new generation of adults in July coincided with maximal herbivory. Low herbivory in August and September was probably related to unfavorable weather conditions, high temperatures and high precipitation rate. Excessive moisture can negatively affect both larvae and pupae (Urban, 2006). Herbivory of the second generation that might have developed in August or September was negligible.

Crepidodera species are trophically primarily associated with *Salix* L. and *Populus* L. plants, although only *C. aurata* is regarded as important in forestry (Czerniakowski, 2000; Urban, 2011a; Walerys and Sadej, 2008). Economic impact of *C. pluta* is unknown and is not considered as a pest. On the other hand, *P. vitellinae* is a major chrysomelid pest in willow and poplar forests (Urban, 2006). Although the beetles were abundant during the studied period of six months, maximum damage of the leaf area was 2.84% for *C. pluta* and 5.60% for *P. vitellinae*. During spring, when leaves and buds are forming, trees are generally more susceptible to herbivore damage. However, average leaf damage expressed as median for this period (April and May) was 0.56% (n = 100) for *C. pluta* and 0.32% (n = 100) for *P. vitellinae* - an insignificant damage which did not affect tree vitality.

Frequent natural flooding of this region and natural enemies (Heteroptera - Anthocoridae, Pentatomidae; Coccinellidae; Diptera - Syrphidae, Tachinidae; various parasitic Hymenoptera; entomophagous Namatoda - Mermithidae; entomopathogenic fungus *Beauveria bassiana*, etc.) were just some of the potential regulating factors that kept species populations at relatively low levels. Concerning the quality of the food, we consider *P.* × *canadensis* Moench to be suboptimal food source for both species. During the period when observations were made their control was not necessary.

CONCLUSION

Based on the data collected in this study, *C. pluta* and *P. vitellinae* were found living in the state of competitive coexistence on hybrid poplars grown in the plantation. Populations of the two species did not seem much interdependent, at least not to an extent that they excluded or limited each other. Their growth was probably regulated by some other factors, such as intraspecific mechanisms, natural enemies and environmental conditions. We regard *P.* × *canadensis* Moench as a suboptimal food source for the developmental needs of the two analysed species, in terms that they could not attain maximum potential growth rates by feeding on this plant. Therefore, when living in *P.* × *canadensis* plantations *C. pluta* and *P. vitellinae* probably cannot reach an abundance high enough to be given a pests status. Further research and rearing in laboratory conditions is needed to better understand feeding preferences of the two species in relation to *P.* × *canadensis*.

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