Project number: NKFI-1, 112743

Project title: Pest control potential of spiders (Araneae) in apple orchard systems and possibilities for their use in conservation biological control

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Final report 2019

Start Date: 1 January 2015, End Date: 31 December 2019

Due to the integration of the Faculty of Horticultural Science Corvinus University of Budapest into Szent István University, the project was suspended for a year in 2016.

The overall aim of the project was to characterise the individual behaviour, the diet, and the pest control potential of the most abundant hunting spider species in orchards under different agricultural management. We also aimed to test whether supplementary food sources can enhance spiders and their potential to control aphid pests in apple orchards.

Provision of alternative food sources for spiders to enhance their pest control ability and to disturb ant-aphid mutualism in apple orchards

Apple is grown as a long-term perennial crop and orchards provide relatively stable ecological habitats. Only a small proportion of the diverse fauna of arthropods that can inhabit the orchard ecosystem are important pests, the majority of species being minor pests, beneficial or benign. As a part of this objective we reviewed the interacting ecosystem services provided by five contrasting naturally occurring arthropod groups in cool temperate apple orchards, and broadly quantified their economic benefits (Cross et al. 2015). Special emphasis was taken on the mutualism between the ant species, *Lasius niger* (Linnaeus) and *Lasius flavus* (Fabricius) and pest aphids, interactions with grass root aphids and competing ant species and manipulations to foster biocontrol of aphids by generalist predators such as spiders. We also reviewed beneficial epigeic arthropods including spiders, carabids and staphylinids and their role in predating the soil dwelling life stages of insect pests in apple orchards.

We concluded that if *L. niger* (or *L. flavus*) was absent from apple orchards, myrmecophilic aphid pests would be less abundant, and in many instances insecticide control of these aphids probably would not be necessary at all. Most commercial apple orchards receive 1–2 insecticide treatments for aphids per annum caused indirectly by the presence of *L. niger*. *Lasius niger* causes increased costs (i.e. has a negative economic benefit) within the orchard of €80–160 ha per season. If insecticides could not be resorted to, *L. niger* would probably indirectly cause crop losses of >10% per annum (i.e. losses of >€928–5778 ha^{-1}) owing to aphid damage. Thus, *L. niger* is a good example provider of an ecosystem disservice.

Reviewing the literature, we found that apple orchards harbour diverse and abundant epigeic predator communities. The species richness of spiders on the ground surface of apple orchards in Europe vary between 40 and 90 species per orchard. The species richness of an apple orchard carabid and staphylinid community, at high sampling effort, is usually 30–60 species and 25–100 species respectively. Epigeic predators might be important limiting factors to codling moth in intensive orchards where the young apple trees have smooth bark, and in the absence of suitable cocooning sites a high proportion of codling moth larvae pupate on the ground. They might also contribute significantly to the control of other apple pest populations although the level of suppression is not consistent and depends on several ecological factors (see in detail in Cross et al. 2015).

The rosy apple aphid, *Dysaphis plantaginea* Passerini, is a serious pest in apple orchards. It establishes close facultative mutualistic relationships with ants. We conducted an experiment manipulating the presence of the common black ant, *Lasius niger* (L.), in colonies of *D. plantaginea* in the canopy of apple trees by provision of ant-feeders (artificial nectaries) containing sucrose solution as an alternative sugar source in apple orchards in the United Kingdom and Hungary to test if this method can regulate densities of *D. plantaginea* below the threshold for economic damage through enhancing the activity of its natural enemies. Apple trees with physical ant-exclusion (sticky barriers) and without any treatment were used as controls (Nagy et al. 2015).

Ant-feeding with sucrose solution, in a similar way to physical ant exclusion, effectively reduced the numbers of ants tending *D. plantaginea* colonies and it consequently resulted in a reduction in *D. plantaginea* populations in both studies. In general, the presence of ants usually affected the predator groups Coccinellidae, Heteroptera, and hoverfly eggs and larvae negatively, but the treatments had less effect on the spider species, *Entelecara acuminate*, *Philodromus cespitum*, *Anelosimus vittatus*, *Enoplognatha ovata* and *Theridion varians* or on the total spider community. Despite marked differences in natural enemy abundance and composition found in UK and Hungary, both treatments caused significant increases in natural enemy (e.g. Dermaptera, Heteroptera, Coccinellidae, Syrphidae) pressure on *D. plantaginea*. Our results show that supplementary sugar feeding of ants is a successful method for supporting biocontrol of rosy apple aphid through enhancing the effectiveness of its natural enemies. This new biocontrol approach to control aphids in apple orchards is likely to substantially reduce the need for aphicide sprays in orchards and is compatible with organic pest control (Nagy et al. 2015).

An additional study was conducted in Újfehértó from April to August in 2015 and 2016. The treatments were as follow: sugar solution applications on the canopy, sugar solution applications on the trunk, ant feeders on the trunk (with sugar solution), ant feeders on the ground surface (agar with sugar solution), untreated control with completely randomised design. The *Dysaphis plantagiena* colonies were initiated in April and counted during the season. The other studied aphid*, Aphis pomi* colonised the experimental plots spontaneously. The aphids and their predators, among them spiders were counted and identified. Generally, the abundance of aphids, similarly to the previous study, was significantly lower on the sucrose treated trees compared to control. Ant feeders in the canopy gave the best results, followed by sugar solution applications on the canopy and feeders on the ground surface. Sugar treatments enhanced the abundance of predatory arthropods (mainly coccinellids) in the aphid colonies but had little effect on spiders (mainly *Philodromus cespitum*). Although the total prey abundance increased significantly, spiders did not aggregate on sugar-sprayed trees (Nagy et al., in prep.).

Changes in behavioural syndromes in different spider species along a pest management gradient

Circadian rhythms play an essential role in the adaptation of organisms to the environment and may show species-specific or sex-specific differences even within a closely related taxonomic group. Although spiders (Araneae) are sexually dimorphic in several morphological and behavioural features, there are very few studies on the sex-specific differences in their biological rhythms (reviewed in Mezőfi, 2020). The first step of this objective was to evaluate the circadian rhythm in the locomotor activity of the two most abundant hunting species of spider in fruit orchards in Hungary, *Carrhotus xanthogramma* (Latreille, 1819) (Salticidae) (very common in orchards in the warm regions of Europe and the Eastern Palearctic) and *Philodromus cespitum* (Walckenaer, 1802) (Philodromidae) (highly abundant in apple orchards of Europe and North America) under natural photoperiod conditions. Particular attention was paid to possible differences between the sexes in both species.

We found that *C. xanthogramma* is a strictly diurnal species with a mean activity peak in the morning in both sexes and the adult females are more active than adult males. The locomotor activity rhythm of males was richer in ultradian (shorter than a day but longer than an hour) components, although the relative power of these components was negligible compared to the main, 24-h period component. In accordance with these results, the diel pattern of locomotor activity of *C. xanthogramma* can be described by a unimodal cosine curve. Although in most jumping spiders the locomotor activity of males is greater than that of females, our results indicate the opposite pattern also occurs. In contrast to *C. xanthogramma*, both sexes of *Ph. cespitum* showed cathemeral activity (i.e., activity occurs within both the light and dark portions of the daily cycle). Prior to this study, *Ph. cespitum* was believed to be a diurnal species. Females and males of *Ph. cespitum* follow quite different activity schedules: females were most active at night, shortly before nautical dawn, whereas males were most active early in the morning. Unlike *C. xanthogramma*, *Ph. cespitum* has more ultradian components, with higher relative power especially in females, where besides the 24-h circadian component there is a particularly strong 12-h ultradian period. Based on these factors, females of *Ph. cespitum* show a bimodal and males a unimodal pattern. For detailed results, see Mezőfi et al. (2019).

The second step of this objective was to compare the individual morphological and behavioural variations (i.e. phenotypic plasticity) in *C. xanthogramma* along pesticide toxicity and prey availability gradients in apple orchards. In laboratory conditions, we assayed three functionally different behaviours, activity level (percentage of time spent moving), freezing after a looming stimulus (risk-taking) and voraciousness (willingness to attack prey) in subadult males and females (altogether 286 individuals) collected in apple orchards with different pesticide regimes and prey availability (three abandoned, three organic and three IPM orchards) in September 2017 at least 6 weeks after the last pesticide applications of the season.

Firstly, we tested the effects of the environmental conditions (i.e. pesticide regimes and prey availability) on individual body traits (sex, body-mass, prosoma width and body condition at the start of the study). We found that with increasing pesticide regimes, body-mass and prosoma width decreased significantly in males and increased significantly (mass) or numerically (prosoma width) in females. In both sexes, the body condition increased with pesticide toxicity, but all the three body parameters were independent of prey availability. Altogether, our results show that pesticide pressure affects the body traits of males and females in opposite directions. One explanation for this pattern can be that pesticide applications cause sex-specific and size-dependent mortality in *C. xanthogramma* and filter out large males and small females.

Secondly, focussing only on the individuals collected in the abandoned i.e. pesticide-free apple orchards, we measured the behavioural traits. We found that subadult females had lower activity level and took lower risk than males, and there was no difference between the sexes in the willingness to attack prey. No behavioural syndromes (as a correlation of functionally different behavioural traits) were detected neither for males nor for females. As our results indicated distinct sex-specific differences in body and behavioural traits, in the further assays, we analysed the data for the two sexes separately.

Thirdly, we examined the influence of body traits (prosoma width and body condition) and environmental conditions (pesticide regime and prey availability) on behavioural traits (activity level, freezing and voraciousness). We found that body condition affected the activity of males and freezing of females and males (marginally significantly) while prosoma width influenced the freezing for both sexes (marginally significantly) and voraciousness in males (Figure 1). Both environmental conditions (increasing pesticide toxicity and prey availability) directly and negatively affected the voraciousness in females. Furthermore, both sexes became less risk-takers with time.

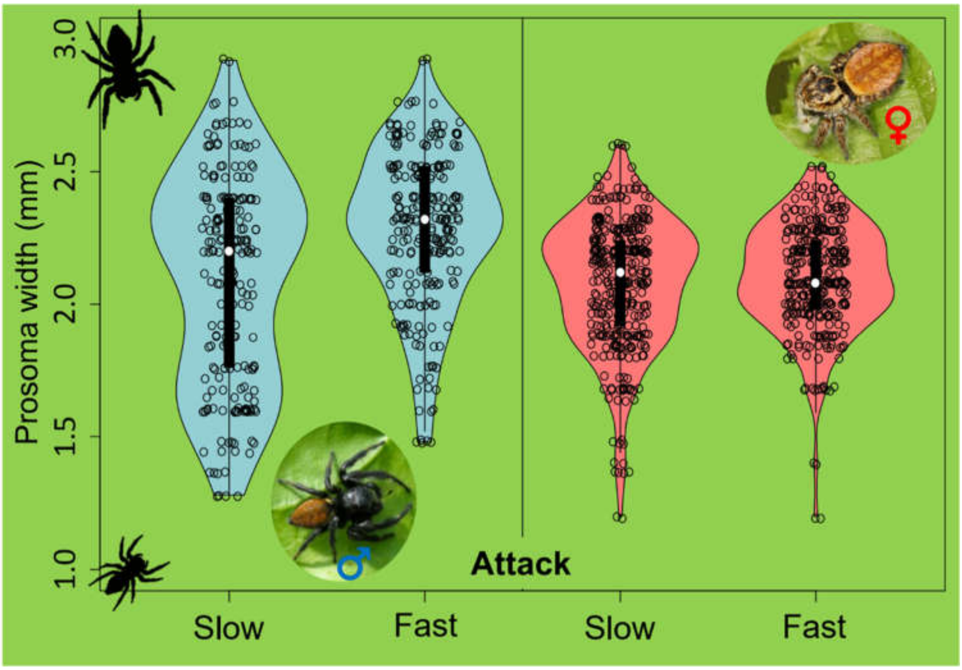

Figure 1: *Carrhotus xanthogramma* males (blue) have wider prosoma than females (red), and larger males are more voracious than smaller ones. Time to prey capture was handled as a binary variable ('slow' and 'fast' attacks). White circle – mean; black vertical rectangle – interquartile range.

Altogether, we concluded that (i) pesticide applications affect the body traits of *C. xanthogramma*, and influence the body-size and mass in males and females in opposite directions; (ii) pesticide applications have sex-specific effects on behavioural traits of *C. xanthogramma*, and (iii) these effects are mediated mainly via morphological (body size and mass) plasticity. Preliminary data were presented at conferences (Gyóni et al. 2017, Taranyi et al. 2019a, 2019b). A detailed publication of all results is in preparation.

Predation rates of common arboreal spider species on some key pests of apple

Spiders (Araneae) form abundant and diverse assemblages in agroecosystems such as fruit orchards, and thus might have an important role as natural enemies of orchard pests. Although spiders are polyphagous and opportunistic predators in general, limited information exists on their natural prey at both species and community levels. Thus, our aim in this study was to assess the natural prey (realized trophic niche) of arboreal hunting spiders, their role in trophic webs and their biological control potential with direct observation of predation events in apple orchards. Hunting spiders with prey in their chelicerae were collected in the canopy of apple trees in organic apple orchards in Hungary during the growing seasons between 2013 and 2019 and both spiders and their prey were identified and measured. Among others, the composition of the actual (captured by spiders) and the potential (available in the canopy) prey was compared, trophic niche and food web metrics were calculated, and some morphological, dimensional data of the spider-prey pairs were analysed. Species-specific differences in prey composition or pest control ability were also discussed.

By analysing a total of 878 hunting spider prey items collected from the canopy of apple trees in apple orchards in Hungary we concluded that (1) although highly polyphagous, arboreal hunting spiders forage selectively and therefore cannot be considered as entirely opportunistic predators. Two-thirds of the hunting spiders' prey were Sternorrhyncha, Brachycera or Nematocera. However, we found that more Brachycera, Nematocera [and possibly Other (non-formicid) Hymenoptera, Lepidoptera and Sternorrhyncha] and less Coleoptera (and possibly Auchenorrhyncha) were consumed by the hunting spider assemblage than would be expected from their abundance in the canopy of apple trees. (2) Hunting spider assemblages consume a large number of pests (31% of the diet) e.g., aphids, *Phyllobius* spp. (Coleoptera: Curculionidae), *Metcalfa pruinosa* (Say) (Auchenorrhyncha: Flatidae), and psyllids (Figure 2). However, this beneficial effect is strongly constrained by the high predation levels on natural enemies (intraguild predation) and on neutral insects (propensity to switch from pests to alternative prey). Hunting spider community preyed mostly (54% of the diet) on arthropods irrelevant to pest management such as Brachycera (excluding hoverflies), Nematocera, and Formicidae. The rest of the prey (15% of the diet) was made up of natural enemies such as spiders, zoophagous bugs, parasitic wasps, hoverflies, and lacewings (Figure 2). In this study, the hunting spider assemblage showed positive selection for neutral prey, neutral selection for natural enemies and negative selection for pests. Our results suggest that, similar to several multi-enemy systems, hunting spider assemblages in general may often be unable to augment the pest suppression ability of local natural enemies, but instead reduce the overall predation pressure on pests via intraguild predation.

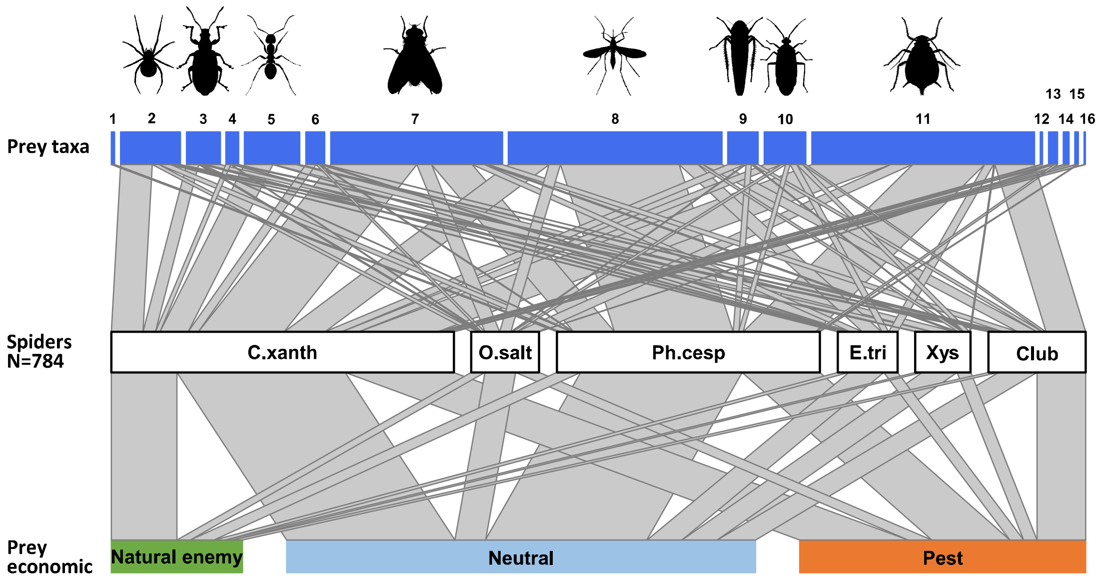

Figure 2: Trophic interactions between the most abundant hunting spider groups and the arthropod community in the canopy of apple trees. Whole growing season, N=784. The middle bars represent spider groups and upper and bottom bars represent the spiders' prey divided taxonomically and according their economic status. The width of the links between the trophic levels depict the frequency of interactions and bar widths indicate the relative abundance of each category. Numbers refer to following prey taxa: **1** Acari, **2** Araneae, **3** Coleoptera, **4** Lepidoptera, **5** Formicidae, **6** Other Hymenoptera, **7** Brachycera, **8** Nematocera, **9** Auchenorrhyncha, **10** Heteroptera, **11** Sternorrhyncha, **12** Ephemeroptera, **13** Neuroptera, **14** Psocoptera, **15** Thysanoptera, **16** Trichoptera; Spiders: **C.xanth** = *C. xanthogramma*, **O.salt** = Other salticids, **Ph.cesp** = *Ph. cespitum*, **E.tri** = *E. tricuspidata*, **Xys** = *Xysticus* spp., **Club** = *Clubiona* spp.

(3) In the trophic web, the most abundant hunting spider species/groups were *Carrhotus xanthogramma*, *Philodromus cespitum*, *Clubiona* spp., *Ebrechtella tricuspidata* (Fabricius), *Xysticus* spp. and 'Other salticids' which accounted for 89% of all hunting spiders collected with prey in their chelicerae. We found that the species/groups mediate different strengths of trophic effects on different prey taxa, and the web structure changes considerably with the season. (4) The natural prey of hunting spider species is highly overlapped, showing functional redundancy in their predation. (5) Nevertheless, hunting spider species show different trophic niche occupancy, also exhibit a certain level of stenophagy (species-specific prey preference) and select prey by its taxonomic identity and size. The prey composition of *Xysticus* spp. was markedly different from that of the other spider groups. Brachycera, Nematocera, and Sternorrhyncha were all consumed by *Xysticus* spp. in much lower proportions (16%) than by the other spiders (60–88%) (Figure 3).

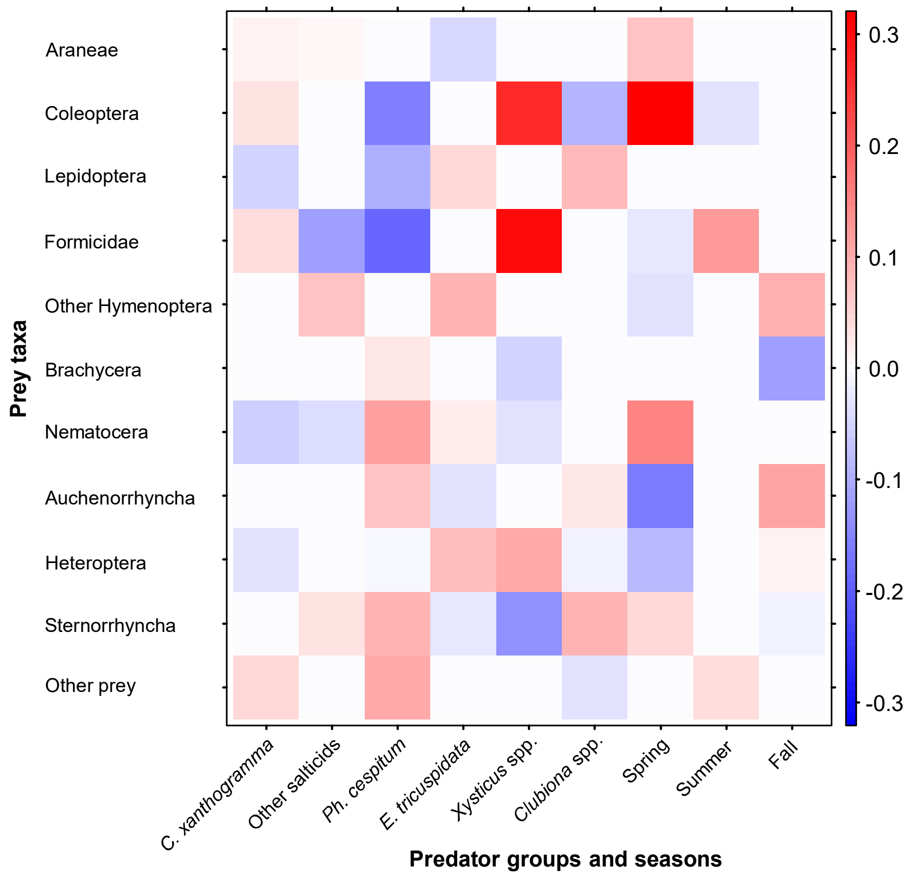

Figure 3: Fourth-corner analysis, including standardised coefficients of prey taxa vs. spider groups and seasonal predictors (GLM model-based approach with LASSO penalty). Darker colours indicate stronger associations than paler ones; positive associations are indicated by red, negative associations are indicated by blue colour.

(6) The guilds (such as stalkers, ambushers, and foliage runners) do not determine the preferred or rejected prey types consistently, thus the diet of hunting spiders classified into the same guild can be considerably different. (7) From an economic point of view, *Ph. cespitum* and *Clubiona* spp. were found to be the most effective natural enemies because of their high level of aphid (*Ph. cespitum* and *Clubiona* spp.) and Lepidoptera (*Clubiona* spp.) consumption and low level of intraguild predation. (8) The size of the prey was strongly related to the size of the hunting spiders, and on average, prey size was 67% that of the spider. Analysed separately, there was a significant exponential relationship between the six most abundant spider taxa and their prey, with prey size being 62–77% of predator size. We found that size and taxonomic identity of the prey are not independent factors. In this study, spiders of the same size caught larger Brachycera than they did Nematocera prey. (8) The trophic niche width of *C. xanthogramma* and *Ph. cespitum* increased during ontogeny where adults prey upon a wider taxonomic and size range of arthropods than juveniles. *Ph. cespitum* exhibited

an ontogenetic shift in prey type, whereas no such pattern was observed for *C. xanthogramma*. Taking into account two niche dimensions, prey type (taxonomic identity) and prey size, adults and juveniles of both species differed from each other in niche occupancy. To our knowledge, our study was the first that studied the food web of the entire hunting spider community in the canopy of fruit trees (Mezőfi et al. 2020).

Further results

We reported the first records of four spider species for Hungary: *Cyclosa sierrae* Simon, 1870 (Araneidae) *Porrhomma oblitum* (O. P.-Cambridge, 1871) (Linyphiidae), *Philodromus buchari* Kubcová, 2004 (Philodromidae) and *Icius subinermis* Simon, 1937 (Salticidae) (Korányi et al. 2017; Mezőfi & Markó 2018a, 2018b). *Cyclosa sierrae* also represented the first record of this species from Central Europe. In the past *Ph. buchari* was misidentified as *Ph. aureolus* (Clerck, 1757), *Ph. cespitum* (Walckenaer, 1802) or *Ph. longipalpis* Simon, 1870, and possibly it is a native species in Hungary. Furthermore, we provided evidence about the occurrence of *Dysdera lata* Reuss, 1834 and *Philodromus marmoratus* Kulczyński, 1891 in Hungary and for six further species we reported new data: *Brigittea vicina* (Simon, 1873) (Dictynidae), *Iberina microphthalma* (Snazell & Duffey, 1980) (Hahniidae), *Mermessus trilobatus* (Emerton, 1882) (Linyphiidae), *Pulchellodromus ruficapillus* (Simon, 1885) (Philodromidae), *Lasaeola prona* (Menge, 1868) (Theridiidae) and *Diaea livens* Simon, 1876 (Thomisidae). Most of the twelve mentioned spider species were collected in apple orchards. We provided comments on their distribution, biology, and taxonomy (Korányi et al. 2017; Mezőfi & Markó 2018a, 2018b).

We also reported the presence of *Psallus assimilis* Stichel, 1956 (Hemiptera: Heteroptera: Miridae) for the first time from Hungary. Specimens were collected from the canopy of field maple (*Acer campestre* L.) trees in Budapest, Diósd and Törökbálint in spring of 2015, 2016 and 2017. Our study indicates that *P. assimilis* is one of the most abundant heteropteran species in the canopy of field maple trees not only in suburban and urban forests but also on individual street trees in highly urbanized locations in Budapest (Korányi et al. 2018).

List of publications produced by the project

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