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Effects of landscapes, pesticide toxicity and host plant-herbivore phenological synchrony on the abundance of true bugs (Heteroptera) in apple orchards

Táji környezet, peszticid terhelés és tápnövény-fitofág fenológiai szinkronitás hatása almaültetvények poloska (Heteroptera) együtteseire

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1. BACKGROUND AND OBJECTIVES

The suborder Heterotpera (true bugs) is a species-rich and functionally diverse insect group. Approximately 37–40000 described Heteroptera species exist on Earth. Majority of the species are phytophagous and some of them are pests of crops, while others are predacious. Therefore, the role of heteropterans could be very different in different crop systems.

Fauvel (1999) in his review listed 60 predatory Heteroptera species from apple orchards in Europe, which contribute to the suppression of aphids, mites, and other small insects.

Apple is by far the most widely grown fruit crop in the temperate zone. It is one of the oldest cultivated fruit and apple production is also a rapidly growing industry. In the 2000s, approximately 600 thousand tons of apples were produced annualy in Hungary, which represented about 60% of all fruit grown. Szabolcs-Szatmár-Bereg County is the major fruit growing region in Hungary (Papp, 2004).

Landscape composition may influence the abundance of insect assemblages in agricultural fields. Most of the studies dealing with landscape diversity have focused on annual crops; however, only sparse information is available on the impact of landscape structure on pest and natural enemy populations in orchards, and true bug assemblages have only rarely been studied. Similarly, considerable attention has been devoted to the effects of global warming on ecosystem functions, however, our knowledge on the possible consequences of phenological mismatches on ecological interactions is still very limited.

The aims of our study were as follows:

- to give a comprehensive list of true bug (Heteroptera) species occuring in apple orhards in Hungary;
- to determine the composition of true bug assemblages in apple orchards in realtions to different pest amangement systems (organic orchards, orchards with integrated or conventional pest management and abandoned apple orchards);
- to determine, how increasing pesticide pressure influence the abundance of true bug (Heteroptera) species in apple orchards;
- to obesrve, how different landscape elements (proportion of arable fields, orchards, grasslands, human settlements, forest plantations, and semi-natural forests) can influence the abundance of heteropteran species in apple orchards throughout the growing season

- to define the changes in abundance of heteropterans and related arthropods (prey and predator species) and fitness parameters of manipulated apple trees (host plants for heteropterants and their prey) compared to control ones as a consequence of phenological mismatches;

2. MATERIALS AND METHODS

2.1. Faunistical investigations in the apple orchards

The faunal surveys were conducted in 25 settlements and 33 apple orchards with different plant protection (IPM – 15 orchards, convewntional – 6 orchards, organic – 6 orchards, and abandoned – 6 orchards) between 1992 and 2013.

The samples were taken by beating from the canopy (Winkler type beating net d=70 cm and 50 cm deep). The sample sizes were different.

The indentification was based on the keys of Aglyamzyanov (2006), Benedek (1969), Halászfy (1959), Kis (1984), Kis és Kondorosy (2000), Péricart (1972) and Vásárhelyi (1978, 1983).

2.2. Effects of orchard management and landscape composition on the true bugs

2.2.1. Studied orchards and sampling methods

Twelve apple orchards with different landscape context were selected as sampling sites in Szabolcs-Szatmár-Bereg County, Hungary Landscape composition around each orchard was estimated in a 1 km-radius circle based on CORINE land cover maps and aerial photographs using ArcGIS 9.2 (ESRI, 2006).

We used the following habitat types, which covered 95–100% of the total study area: arable fields (mostly corn, wheat and sunflower fields), deciduous forest plantations [mostly, black locust (*Robinia pseudoacacia*) and poplar (*Populus x euramericana*)], semi-natural grasslands (meadows and pastures), human settlements (houses, gardens and streets), orchards (almost exclusively apple orchards but also some sour cherry and walnut orchards) and semi-natural forests (mostly native riverine willow-poplar forests dominated by *Salix alba*, *S. fragilis*, *Populus alba* and *P. nigra*, hedgerows and tree lanes) (1. ábra).

Fig. 1. – Examples for three charasterictic landscape composition (Nyírmada, Győrtelek, Csaroda). Pink: forest plantation, mid-brown: arable fields, light-green: grasslands, dark-green: semi natural forest plantation, dark-brown and gray: human settlements, mid-green: tree lanes, blue: water surface, purple: conifer plantation.

Insecticides were applied 2–5 and fungicides 0–8 times in the orchards during the growing season.

The pesticide load of orchards was estimated using data from the International Organization for Biological Control (IOBC) Pesticide Side Effect Database where the acute toxicity of pesticides for non-target organisms is divided into four risk categories from harmless (score 1) to harmful (score 4). The calculation of pesticide (insecticide and fungicide) load was based on the toxicity of the applied pesticide compounds to Anthocoridae, but the toxicity scores were rescaled to a 0–3 scale (range from harmless to harmful). Cumulative IOBC toxicity scores were calculated for all orchards separately by summing the toxicity scores of each insecticide and fungicide application for either a month, or several months, or the whole growing season, regarding the activity pattern of the studied Heteroptera species. The pesticides were applied between April and August in 2012.

True bugs were collected from the whole canopy of 20 apple trees per orchards by beating method (Muther and Vogt, 2003) (with a beating funnel 35 cm radius, 50 cm depth, and a 70-cm-long beating stick) in eight occasions between 21st May and 11th October, 2012.

All Heteroptera individuals were identified to species level. Each species was classified into one of three major feeding guilds: phytophagous, zoophagous and zoo-phytophagous.

The number of aphids was counted on 20 annual shoots of apple trees (two shoots per tree for ten trees) in four occasions (in May, June, July and September) in every orchard. The most abundant aphids (Hemiptera: Aphididae) in May and June were *Aphis pomi* (De Geer) and *Aphis spiraecola* (Patch), while *Dysaphis plantaginea* (Passerini) and *Dysaphis devecta*

(Walker) were less common. Canopy dwelling mites (Acari) were counted on 20 leaves per orchard (2 leaves from 10 trees in each apple orchard) in eight occasions.

2.2.2. Data analysis

General linear mixed-effect models (GLMM, Bolker et al., 2009) were used to study the relationship between the assumed explanatory variables and the monthly, bi-monthly or seasonal (between April and October) abundance data for individual true bug species. We added the orchard as a random effect. Response variables were log(x+1) transformed to fulfil the normality requirement for model residuals. We built sets of singe-argument models, where only one explanatory variable was considered in each model to avoid collinearity (Burnham and Anderson, 2002).

For each species, we used a model selection based on information criterion corrected for small samples sizes (Akaike Information Criterion - AICc) to rank the above models per model family in terms of their ability to explain species abundances (Burnham and Anderson, 2002). In this way, the 'best approximating' model was selected as the most parsimonious explanation of the data, when Δ AICc > 2. In other cases, when the models differed in their AICc values less than 2, we applied a model averaging approach to account for model selection uncertainty and obtain robust parameter estimates (Grueber et al., 2011). During model averaging, we built all the possible models with the given set of explanatory variables and parameter estimates of the best models (Δ AICc > 2) were averaged with the models' AICc weights.

Model estimates were obtained using maximum likelihood method and diagnostics included the AICc and checking model residuals. We estimated the model parameters using the 'nlme' package (Pinheiro et al., 2011), performed model averaging with the package 'MuMIn' (Barton 2013) and applied the 'graphics' package for graphical outputs in R 3.1.2 (R Core Team, 2014). Some preliminary statistics and the interdependency of explanatory variables were tested by the software *ROPstat* (Vargha et al., 2015).

2.3. Effect of climate-induced phenological shift of apple trees

2.3.1. Studied orchards and sampling methods

We manipulated the phenology of potted, three years old apple trees (n=182, cv. Resi) by keeping them either in a greenhouse, a cool store, or outdoors from March 2013. Before flowering, trees were randomly assigned to one of eight blocks in three organic apple orchards in Eastern Hungary (Újfehértó in three blocks 62 trees, Nagykálló in two blocks 60 trees and Nagykálló-Ludastó in three blocks 60 trees), and burrowed outdoors with their pot in five treatments during April and May (Advanced1, Advanced2, Control, Delayed1, and Delayed2). We used similar numbers of trees in each of the five treatment groups and each block.

The arthropods were sampled by beating the whole canopy of each tree for 15 seconds with a 70-cm-long stick, collecting the fallen arthropods in a 35-cm-radius beating funnel and preserving them in 50% ethanol for later identification. Sampling was repeated once a week from 24 April until 18 July. The collected true bug species were classified into three guilds: apple feeders, phytophagous, and predatory (zoophagous, zoo-phytophagous) species.

Five randomly selected leaves were collected from each experimental tree on 18 July; leaf size was calculated from scanned digital images using ImageJ and Adobe Photoshop 8.0 (Adobe Systems) softwares (O'neal et al. 2002).

Green apple aphids (*Aphis pomi* de Geer) (Hemiptera: Aphididae) were sampled three times during summer (14 & 27 June, 12 July). Each time the number of shoots was counted and the proportion of young, still growing shoots was estimated on each tree. Then three growing shoots and three non-growing (old) shoots were randomly selected and aphids were counted on them.

2.3.2.Data analysis

We used generalized linear mixed effects models (GLMM) with treatment as the fixed effect and block ID as a random factor. When more than one fixed term were involved, we then performed an AICc-based model selection (Burnham & Anderson 2002) and results of the best model are reported. Continuous covariates were centred.

For occurrence data, we used a binomial error distribution, otherwise we applied the most appropriate error structure based on AICc values and diagnostic plots (Appendix S2.1). Abundance of *S. pyri* was zero on ~36% of apple trees and showed a skewed distribution on the rest of them so we fitted a model to the log-transformed non-zero abundances with a quasi-Poisson error structure (Zuur *et al.* 2009).

For the community-level analysis, we calculated and plotted Rényi's diversity profile for each treatment. Common diversity indices are special cases of Rényi diversity (Hill 1973), and one community can be regarded as more diverse than another only if its Rényi diversities are all higher (Tóthmérész 1995).

We also conducted two separate redundancy analyses (RDA) with constraint variables *orchard* and *treatment*. Species matrix was transformed with the Hellinger method (Legendre & Gallagher 2001) and significance of the constraint term was tested by a permutation test (10^4 permutations). A non-metric multidimensional scaling (NMDS) with Bray-Curtis distances was applied to visualize similarity among treatments in each orchard. All analyses were made using packages lme4 (Bates *et al.* 2014), MuMIn (Barton 2014) and vegan (Oksanen *et al.* 2013) of R statistical software (version 3.1.2, R Core Team 2015).

3. RESULTS

3.1. Faunistical investigations

The survey of true bug assemblages in apple orchards under different pest management strategies was conducted in Hungary. A total of 21 914 individuals belonging to 177 species were collected in the studied 33 apple orchards representing approximately 20% of the Heteroptera fauna of Hungary.

From the collected 21 914 individuals 19 513 belonged to 137 phytophagous species (15 203 specimes to *Stephanitis pyri* and 4310 specimens to other, non apple feeder phytophagous heteropterans). The collected 2401 predatory heteropterans comprised 40 species. Heteropteran assemblages were dominated by three families (118 species): Lygaeidae (46 species), Miridae (39) and Pentatomidae (26). The other 68 species comprised 16 families.

Mészáros et al. (1984) reported 13 additional species not found in this survey, therefore, a total of 190 Heteroptera species are now known to occur in the canopy of apple trees in Hungary, which represent 22% of the Hungarian Heteroptera fauna.

Stephanitis pyri was found to be the most widespread and abundant heteropteran pest in abandoned and organic apple orchards. Its abundance was much lower in orchards with integrated (IPM, based on selective insecticides) and especially in the orchards with conventional (based on broad spectrum insecticides) pest management.

The most frequently found phytophagous (non-apple feeder) species in the canopy of apple trees (in decreasing order of their abundance) were: *Lygus rugulipennis, Aelia acuminata, Palomena prasina, Metopoplax origani, Dolycoris baccarum, Oxycarenus pallens, Peribalus strictus, Piesma salsolae, Coreus marginatus, Piesma maculatum, Rhaphigaster nebulosa, Nysius thymi, Lygaeus equestris* and *Stictopleurus punctatonervosus*. *Aelia acuminata* and *P. prasina* were the dominant species in the abandoned orchards, while in the organic, IPM and conventional orchards *N. senecionis* and *L. rugulipennis* had high relative abundances. The most frequently found zoophagous-zoophytophagous species were: *Orius minutus, Campylomma verbasci, Himacerus apterus, Deraeocoris ruber, Nabis* spp.*, Nabis punctatus, Nabis pseudoferus, Arma custos,* and *Orius* spp. However, these species were variously abundant in the orchards where different pest management systems were applied. *Himacerus apterus* and *C. verbasci* were abundant in the abandoned and organic orchards, *O. minutus* and *C. verbasci* in the IPM orchards, while *O. minutus* and *N. punctatus* were the most common predatory species in the conventional orchards.

3.2. First record of *Deraeocoris flavilinea* (A. Costa, 1862) in Hungary

The true bug community in the canopy of three *Acer* species (*A. campestre, A. platanoides* and *A. pseudoplatanus*) was surveyed in different locations in Budapest, Hungary: Alkotás street, Botanical Garden of the Corvinus University of Budapest, and Gellért Hill. Regular samples were taken with a beating tray in 2014, and heteropterans were identified using keys by WAGNER & WEBER (1964). Among the collected 2624 heteropterans, 60 individuals were identified as *D. flavilinea*. This is the first record of this species in Hungary. Most of the individuals were collected from *A. pseudoplatanus* (44 specimens), followed by *A. campestre* (8 specimens) and *A. platanoides* (8 specimens).

The peak of activity of *D. flavilinea* was experienced in June and the sex ratio was female biased (38 ♀ *vs*. 22 ♂) (Fig. 2.).

Next year (2015), we collected *D. flavilinea* specimens in the canopy of apple trees in the next year. *Deraeocoris flavilinea* probably will contribute to the suppression of pests in apple orchards in Hungary.

Fig. 2. - *Deraeocoris flavilinea* A. Costa, 1862 male (left) and female (right) (Photo: Ákos Varga)

3.3. Effects of orchard management and landscape composition on true bugs

3.3.1. Effects of orchard management

A total of 2465 individuals of 71 true bug species were collected from the studied apple orchards. The number of species varied between 11 and 26 per orchard, and the number of individuals varied between 19 and 113.

Increasing pesticide toxicity caused a decline in the abundance of *S. pyri.* However, other phytophagous and predatory true bug species appeared to be unaffected by the pesticide load.

Weed height and the generally low abundance of aphids and mites did not influence the abundance of true bug species.

3.3.2. Effects of landscape composition

Abundance of *Stephanitis* pyri was not influenced by the landscape composition. while the abundance of other phytophagous species was negatively correlated with the proportion of grassland in May and July. In the six orchards with lower proportion of grasslands harboured 1,6 times more phytophagous species than the six orchards with higher proportion.

The abundance of *Metopoplax* origani correlated positively with the proportion forest plantation. We observed similar but not significat relationship for *Nysius senecionis* well.

The abundance of zoophagous and zoo-phytophagous speces correlated positively, in summer with the proportion of semi-natural forests; in autumn with the proportion of arable fields in the landscape. The number of predator specimens was only 1.1 times greater in spring, 5-7 times greater in autumn in the six orchards with higher proportion of semi-natural forests and arable fields.

The abundance of *Orius minutus*, *Campylomma verbasci* correlated positively with the proportion of arable fields, while in case of *Nabis* spp. we observed close to significance context. The number of *Nabis* spp. was 3.5-times greater, the *Orius minutus* 5-times greater and the *Campylomma verbasci* 7-times greater in the six orchards with higher proportion of arable fields.

3.4. Effects of climate-induced phenological shifts on true bugs

3.4.1. Effects of the treatments on tree fitness

Trees kept in the greenhouse had their flowering advanced by 6–9 days, while flowering of cool stored trees was delayed by 16–38 days compared to the control ones.

Flowering period lasted for 5–7 days in all treatments. Time elapsed between bud burst and onset of flowering was almost twice (22-25 days) as long in the most shifted treatments (11-15 days).

Leaf size was significantly larger on advanced trees and significantly smaller on delayed trees than on control ones (Fig. 1B), while shoot growth was not affected by treatment.

3.4.2. Activity of the most abundant species

A total of 3681 individuals of 52 true bug species were collected from the studied apple orchards. From the 3681 specimes 2929 adults were *Stephanitis pyri*, the other 752 true bugs belonged to 38 phytophagous and 14 zoophagous or zoo-phytophagous species. The most frequently found phytophagous (non-apple feeder) species were: *Oxycarenus pallens, Metopoplax origani*, *Nysius thymi,* while the most abundant zoophagous, zoo-phytophagous species were: *Campylomma verbasci*, *Orius minutus* and *Nabis* spp. (mainly *Nabis pseudoferus*).

The seasonal activity pattern of the true bugs suggests that the highest activity of phytophagous species was in June, July and October. For predatory species the highest activity was found in end of May, June and July and in autumn.

3.4.3 Effect of climate-induced phenological shift ont he true bugs

The abundance of *S. pyri* was significantly lower on delayed trees than on control.

Spider abundance was significantly lower on Delayed2 trees than on control trees if only treatment was included as a predictor. However, in the best model the abundance of *S. pyri* was also included as a covariate, and its interaction with treatment was significant: spider abundance showed a significant positive relationship with the abundance of *S. pyri* in Advanced2 and Control treatments.

Diversity of the phytophagous heteropterans was lower on delayed than on control and advanced trees. RDA revealed no significant effect of *treatment* on species composition

We analyzed the most abnudant aphid species, *Aphis pomi*. The green aphid abundance showed no difference between the Advanced (1, 2) and the control trees. But on the Delayed trees the abundance of *A.* pomi was significantly higher.

Abundance of zoophagous and zoo-phytophagous true bugs was similar on Delayed (1, 2) and control trees, but was significantly higher on Advanced (1, 2) trees than on control.

This might be due to the low number of zoophagous bugs in the samples, and especially to that the most dominant mullein bug *Campylomma verbasci* (Meyer-Dür) (~60% of all zoophagous true bugs) occurred much before the aphid peak and occupied mainly the delayed trees.

4. NEW SCIENTIFIC RESULTS

1. Species composition of true bug (Heteroptera) assemblages of Hungarian apple orchards were determined. 177 species were collected and determined from the canopy of apple trees.
2. A survey of true bug assemblages in apple orchards under different pest management strategies (abandoned orchards, orchards under organic, IPM and conventional pest management) was conducted in Hungary. Depending on the orchard management systems, I determined the heteropteran species that may be of importance in the canopy of apple trees.
3. *Deraeocoris flavilinea* (A. Costa, 1847) was reported first from Hungary.
4. It has been proved that the abundance of the apple feeder species, *Stephanitis pyri* decreased with the increasing cumulative toxicity of pesticides, but the abundance of indifferent phytophagous species and predatory heteropterans was unaffected by the pesticide use.
5. Unlike pesticides, the abundance of *S. pyri* was not affected by the landscape elements. The abundance of non-apple feeder and predatory species correlated positively with the proportion of semi-natural forests in the landscape in spring and summer and with the proportion of arable lands in autumn. In autumn, we found a 5–7-fold increase in the abundance of predatory true bugs in the orchards with a high amount (45–67%) of arable fields in the surrounding landscape compared to those with a low amount (14–30%) of arable fields.
6. I proved that the abundance of *S. pyri* was significantly lower on the delayed trees than on control ones, while the abundance of zoophagous-zoophytophagous species was significantly higher on delayed trees. At the same time the treatments were not affected the abundance of indifferent phytophagous species. To sum up, the significantly delayed trees can be restructuring the Heteroptera assemblages.
7. As a further result of phenological studies I proved first, that the abundance of spider assemblages in the canopy of apple trees, followed by the abundance of *S. pyri*.

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