Doctoral Theses

CHEMICAL ECOLOGY OF PESTS OF ORNAMENTAL TREES AND SHRUBS: EXAMPLE OF THE SMALL CYPRESS BARK BEETLE

Gábor Bozsik

Eötvös Loránd University, Faculty of Science
Zootaxonomy, animal ecology, hydrobiology Doctoral Program
Doctoral School of Biology

Supervisor: Gábor Szőcs, Dsc., scientific advisor
Internal consultant: Árpád Szentesi, DSc., professor emeritus

Host institute:
Department of Zoology, Plant Protection Institute, Centre for Agricultural Researches, Hungarian Academy of Sciences

Budapest, 2018

1. Introductions:

Many invasive insect species are associated to scale-leaved trees (Cupressaceae). In Hungary, the main pest of these ornamental trees is the small cypress bark beetle (CBB), [*Phloeosinus aubei* (Perris, 1855)] (Coleoptera: Curculionidae, Scolytinae). It is considered in Hungary as an invasive species (Rakk et Bürgés 1994). Its native range is in the Mediterranean area, common from the Crimea till China (Balachowsky 1949, Mendel 1984, Knížek 2011). In the last few decades it has appeared in northern European countries, too (Winter 1998, Sobczyk et Lehmann 2007, Moraal 2010). The small cypress bark beetle is a known species of the Hungarian fauna for more than 50 years (Endrődi 1959). Based on the spatial distribution of damage, it has spread massively from southwest to northeast (Both és Farkas 2005, Reiderné-Saly és Podlussány 1994). It is a typical oligophagous species, specialized on the scale-leaved conifers (Bel Habib et al 2007). It constructs two types of tunnels: (1.) hibernation tunnels, in the shoot-tips of healthy trees and (2.) galleries (nuptial chambers, maternal tunnels, larval tunnels) under the bark of trunks of decaying host trees.

The chemical ecology of bark beetles living in scale-leaved conifers, and especially that of the small cypress bark beetle, is largely unknown. In the case of *P. aubei*, we have no knowledge about the life cycle in the invasive area too.

2. Aims of my studies

My studies proceeded in two major lines:

(1) To determine the key elements of life cycle of the invasive populations of CBB in temperate climate, in particular view to the inter- and intraspecific communication.

(2) To reveal the olfactory cues of the small cypress bark beetle (chemical ecological approach). In this section I attempted to reveal the sensory mechanisms in the *Thuja occidentalis – Phloeosinus aubei* interaction.

3. Materials and methods

(1) Examinations to reveal the life cycle and host preference

Spread: Observing the occurrence and damage of the small cypress bark beetle in the eastern part of the country.

Host preference: I chose two *Juniperus* and two *Thuja* cultivars to the studies: *Thuja occidentalis* 'Smaragd', *Thuja plicata* 'Atrovirens', *Juniperus chinensis* 'Spartan' és *Juniperus scopulorum* 'Blue Arrow'. I assessed the number of adults in the overwintering tunnels by examining the shoots. I analysed the result by one-way ANOVA and if the F value proved to be significant, I used Tukey's HSD test.

Annual generations: To reveal the life cycle of the small cypress bark beetle, I monitored the seasonal pattern of the appearance of the overwintering tunnels and the galleries on

Juniperus chinensis ‘Spartan’ and *Juniperus scopulorum* ‘Blue Arrow’ cultivars, in the Tahi Tree Nursery, in 2015 and 2016.

Pioneer gender: To determine the gender which constructs the nuptial chambers, I made non-choice preference test under outdoor conditions. I presented a piece of trunk to caged males, and females, kept separately, and observed which sex would construct nuptial chambers.

To reveal whether females, bored into the trunks, would attract males, I carried out a multi repetitions choice test in Petri dishes (control: tunnels with no female inside).

To reveal how the health conditions of the host trees correlates to the frequency of nuptial chambers, I made a field survey to compare the number of nuptial chambers in healthy and decaying *Juniperus* trees.

Daily rhythm of foraging adults on the bark of the trunk: I recorded the number of foraging, crawling beetles on the bark in the mating season under field conditions by hourly observations.

Statistical analyses: I used chi-square test to compare the zero and non-zero independent cases, while in non-independent cases I used sign and binominal test.

(2) Studies for revealing the perception of host plant volatiles and for extracting pheromone from female beetles

Identification of potential kairomone compounds from *Thuja occidentalis* 'Smaragd' volatiles: I made volatile collections from cut Emerald Green Arborvitae twigs using a special pump in closed loop system with charcoal filter (Brechbühler AG, Switzerland). The bound volatile compounds were washed with 20 μl analytical *n*-hexane from the filter.

I analysed the extracts as by coupled gas chromatography – electroantennography (GC-EAD) (6890N GC Agilent Inc., USA – SYNTECH GmbH, Germany), using either male or female CBB antennae.

The compounds which evoked active antennal responses were identified in international cooperation (Univ. Hamburg, Inst. Org. Chem., Germany). I submitted the synthetic samples (Sigma-Aldrich, or Univ. Hamburg) for electroantennographic (EAG) tests in the following solutions: 10 μg/μl, 1 μg/μl, 100 ng/μl and 10 ng/μl. Similar tests were made for comparisons with some commercially available, natural plant extracts, as cedar oil, juniper oil, Korean *Thuja* oil and turpentine oil. In every case *n*-hexane was used as a control stimulus. I repeated these tests five times with male and female antennae. The relative antennal responses were evaluated.

Attempt to extract pheromone compounds: I treated unmated females taken from nuptial chambers with juvenile hormone (JH III) (Santa Cruz Biotechnology, USA). For control, I made extracts from untreated females, too. 24 hours after the treatment, I isolated the mid- and hindguts of beetles and extracted these in *n*-hexane. I analysed extracts by GC-EAD, using male *P. aubei* antennae.

Statistical analyses: To compare the antennal responses evoked by different synthetic samples to that of *n*-hexane control, or to compare responses of enantiomer pairs, I used two tailed, paired t-test. I analysed the responses to essential oils by ANOVA and if the F value proved to be significant, I used Tukey's HSD test.

4. Results

(1) Examinations to reveal the life cycle and host preference

Spread: I evidenced first the massive damage and appearance of *P. aubei* in the eastern part of Hungary (Szentistván, BAZ county).

Host preference: The *Thuja plicata* 'Atrovirens' proved to be the most preferred cultivar in the case of overwintering tunnels among the chosen cultivars, followed by *Thuja occidentalis* 'Smaragd', *Juniperus chinensis* 'Sparatn and *Juniperus scopulorum* 'Blue Arrow'.

Annual generations: Both sex overwintering in similar proportion, one by one in hibernation tunnels, from August to the middle of April. The small cypress bark beetle constructs galleries once in a year, from the middle of April to the end of June. This means that only one generation develops in the vegetation period. However, there are two flying periods in a year. The first one occurs in spring till the beginning of summer, when the beetles leave their overwintering tunnels and start to build the nuptial chambers. The second one starts at the end of summer, when the freshly emerged adults fly out from the galleries and start to bore into the overwintering tunnels.

<u>Pioneer gender:</u> The nuptial chambers and the maternal tunnels (initial and core parts of the galleries) are exclusively constructed by the females. I proved that the male locates and enters the nuptial chambers only when occupied by the female. I found that the female choose only declining trees for preparing the nuptial chambers.

<u>Daily rhythm of foraging adults on the bark of the trunk:</u> According to my studies the foraging behaviour on the bark of trunks and the flying activity of *P. aubei* adults occurs in the sunny hours, intensified in the early afternoon hours.

(2) Studies for revealing the perception of host plant volatiles and for extracting pheromone from female beetles

Identification of potential kairomone compounds among *Thuja occidentalis* 'Smaragd' volatiles: Among the several *Thuja occidentalis* 'Smaragd' volatile compounds, I found 22 active compounds, which evoked antennal responses on female *P. aubei* antennae by GC-EAD analyses. As a result of electroantennographic assays (EAG) with the identified synthetic compounds in 100 µg doses, in the case of females the mixture of *α*- and *β*-thujone evoked the significantly highest antennal responses, followed by (–)-terpinene-4-ol, (+)-camphor and *cis*-4-thujanol. In the case of male *P. aubei* antennae the highest antennal responses evoked by (–)-terpinene-4-ol, followed by *cis*-4-thujanol, the mixture of *α*- és a *β*-thujone, then (+)-camphor. The enantiomers of sulcatol evoked significantly different antennal responses from each other. In the case of terpinene-4-ol, however, I did not find significant differences between the enantiomers. As a result of EAG assay of the commercially available essential oils, I found that the juniper oil and Korean *Thuja* oil evoked significantly larger antennal responses than either turpentine or cedar oil, in 100 µg doses, in case both females and males.

Attempt to extract pheromone compounds: After the coupled gas chromatography analyses (GC-EAD) of JH III hormone treated and untreated mid- and hindgut extracts, screened on male antennae, I found active antennal responses. The identification of these compounds in under way.

5. Summary

My studies revealed that the *P. aubei* has only one generation per year in Hungary, however, associated to two flight periods. The pioneer gender is the female. She chooses declining host trees for colonization. Females construct the nuptial chambers, which is the initial part of breeding galleries. Monitoring the number of overwintering tunnels in four selected cultivars, I found sharp differences in host preference. This result could be useful for tree nurseries to select their assortments. I identified 22 antennal active components from the host volatiles (potential kairomone components), by GC-EAD analyses using female CBB antennae. Following the chemical identification of these compounds, I evaluated the electrophysiological responses of synthetic samples on *P. aubei* antennae. These results could help in developing an effective kairomone trap in the future, which would be used for monitoring the flight of CBB.

6. Publications in the subject of the theses:

Bozsik, G., Zsolnai, B., Both, Gy., Szőcs, G., Francke, W. 2014: Megfigyelések a borókaszú áttelelésérői, hazai terjedéséről és a tuja illatanyagainak szerepéről. *Növényvédelem* 50: 209-213.

Bozsik, G., Tröger, A., Francke, W., Szőcs, G. 2016. *Thuja occidentalis*: identification of volatiles and electroantennographic response by the invasive cedar bark beetle, *Phloeosinus aubei*. *Journal of Applied Entomology* 140: 434-443. (IF: 1,641)

Bozsik, G., Szőcs, G. 2017. Phenology, behavior and infestation levels of the invasive small cypress bark beetle, *Phloeosinus aubei*, on some cultivars of *Thuja* and *Juniper* spp., in Hungary. *Phytoparasitica* 45: 201-210. (IF: 0,882)

7. Citated publications

Balachowsky, A. S. 1949. Faune de France. Vol. 50. Coléoptères Scolytides. *Librairie de la Faculté des Sciences, Paris*. 320 p.

Baruch, O., Harari, A., Sapir, N., Mendel, Z. 2007. The colonization process and the gallery system of the cypress bark beetles *(Phloeosinus* spp.). *Israel Journal of Entomology* 37: 369.

Bel Habib, R., Ben Jamâa, M. L., Nouira, S. 2007. Biological characteristics of the cypress bark beetle *Phloeosinus aubei* in the Kessra forest, Center of Tunisia. *Tunisian Journal of Plant Protection* 2: 99-108.

Both, Gy., Farkas, I. 2005. Nagy veszély a borókaszú. Növényvédelem 41: 305-306.

Endrődi, S. 1959. Szúbogarak – Scolytidae. (Bark beetles – Scolytidae) *Fauna Hungariae* No. 9., Akadémiai Kiadó Budapest.

Knížek, M. 2011. Scolytinae. *In*: Löbl, I., Smetana, A. (Eds.). *Catalogue of Palaearctic Coleoptera. Vol 7*. Apollo Books, Stenstrup, Denmark. pp. 86-87, 204-251.

Mendel, Z. 1984. Life history of *Phloeosinus armatus* Reiter and *P. aubei* (Perris) (Coleoptera: Scolytidae) in Israel. *Phytoparasitica* 12: 89-97.

Moraal, L. G. 2010. Infestation of the cypress bark beetles *Phloeosinus rudis*, *P. bicolor* and *P. thujae* in the Netherlands (Coleoptera: Curculionidae, Scolytinae). *Entomologische Berichten* 70: 140-145.

Rakk, Zs., Bürgés, Gy. 1994. Pusztít a borókaszú! *Növényvédelem* 30: 7-10.

Reiderné-Saly, K., Podlussány, A. 1994. A borókaszú (*Phloeosinus aubei* Perris, 1855) terjedése, gazdanövényköre és életmódja. *Növényvédelem* 30: 23-24.

Sobczyk, T., Lehmann, M. 2007. Zur Ausbreitung des zweifarbigen Thujaborkenkäfers *Phloeosinus aubei* (Perris, 1855) in Ostdeutschland mit Anmerkungen zu *Phloeosinus thujae* (Perris, 1855) und *Phloeosinus rudis* Blandford, 1894 (Colepotera, Curculionidae, Scolytinae). *Märkische Entomologische Nachrichten* 9: 55-60.

Winter, T. 1998. *Phloeosinus aubei* (Perris) (Scolytidae) in Surrey, the first record of this bark beetle in Britain. *The Coleopterist* 1: 1-2.