Studies on the chemical communication of beetle pests by electrophysiological and behavioural methods

Doctoral thesis

VUTS JÓZSEF

Eötvös Lóránd University of Sciences Biological Doctoral School (leader: Anna Erdei DSc.)

Zootaxonomy, Animal Ecology, Hydrobiology Doctoral Program (leader: Klára Dózsa-Farkas DSc.)

Supervisors: Miklós Tóth DSc.
Árpád Szentesi DSc.

Budapest, 2010

INTRODUCTION AND PURPOSES

In recent decades Integrated Pest Management has become more and more important because of its tendency to reduce the amount of chemicals used in agriculture by combining different agrotechnical, biological and that kind of chemical methods where the applied chemical agents are non-toxic. The chemical part of Integrated Pest Management includes, besides pesticides, the use of pheromones and attractants of economically harmful insects. There are two ways for this: 1) mating disruption with pheromones and 2) using traps baited with the pheromone or attractant of the target insect species. Traps are suitable for detection, monitoring or, in some cases, mass trapping insect pests.

In the development of pest management strategies partially based on lured traps there are two ways in case of click beetles (Coleoptera: Elateridae) and scarabs (Coleoptera: Scarabaeoidea, Cetoniidae): 1) application of pheromones in traps which assumes the identification of compounds of the pheromone blend and optimalization of ratio of the identified components in field tests, 2) use of host plant-derived compounds attractive to the given species (floral volatiles and volatiles emitted by ripening fruit). To the latter case, screening of synthetic plant volatiles or identification of active components of extracts collected from fruit of the host plant and testing the active compounds also in laboratory and field tests is required. In my studies, in case of the click beetles *Agriotes lineatus* L. (1767) and *Agriotes proximus* Schwarz (1891) my aim was to increase the efficacy of the known pheromone bait of the two species. Larvae of these two species (wireworms) are dangerous pests of many crops. In the flower- and fruit-devastating scarabs *Epicometis hirta (=Tropinota hirta)* Poda (1761), *Cetonia a. aurata* L. (1758), *Potosia cuprea (=Protaetia cuprea)* Fabr. (1775) and *Oxythyrea funesta* Poda (1761), on the one hand I planned to enhance the efficiency of their known attractants, on the other hand my aim was to develop novel attractants for some of them.

Specific aims for *Agriotes lineatus* and *A. proximus*

I planned to collect volatiles from females of *A. lineatus* and *A. proximus* by ’closed-loop stripping apparatus’ (CLSA) in order to identify geranyl butanoate and geranyl octanoate previously found to be attractive to *A. lineatus* and *A. proximus* in field tests.

In order to investigate if there is difference between the two species in pheromone perception, I planned to conduct electrophysiological (EAG) and behavioural experiments. In EAG I used a serie of synthetic click beetle pheromone compounds to be tested on the antennae of *A. lineatus* and *A. proximus* and also planned to test geranyl butanoate and geranyl octanoate in dose-range experiments (0,0001-10 μg). Compounds evoking high EAG-responses were planned to be tested in field experiments.

Since *A. lineatus* and *A. proximus* are very similar in morphology and males are attracted by the same lure, I wanted to know how strong similarity DNA studies could show between them. It was clear for me that the genomic approach did not really fit into my thesis but I thought DNA tests could usefully contribute to chemical ecological studies.

Specific aims for scarab beetles

My purpose about *E. hirta, C. aurata aurata* and *P. cuprea* was to enhance trap efficiency by increasing the attractive effect of the floral bait of these species. For this, I planned to screen synthetic floral volatiles on the antennae of both sexes of *E. hirta, C. aurata aurata* and *P. cuprea* assuming that compounds evoking high antennal responses prove to be attractive in the field.

For *E. hirta* a binary lure consisting of *(E)*-anethol and *(E)*-cinnamyl alcohol in a ratio of 1 : 1, for *C. aurata aurata* and *P. cuprea* a ternary mixture of *(E)*-anethol, 1-phenylethanol and 3-methyl eugenol in a ratio of 1 : 1 : 1 have been discovered by our research group.

To date there was no trap for the detection and monitoring of *O. funesta*, an orchard pest dominantly distributed in Southern and Central Europe. In Hungary it can cause local damage. My aim was to develop a trap combining visual and chemical cues suitable for, besides detection and monitoring, mass trapping as well. To this, I based my studies mostly on earlier field tests aimed at other beetle pests. EAG-screenings of synthetic floral compounds on the antennae of *O. funesta* were also planned, similarly to *E. hirta*, *C. aurata aurata* and *P. cuprea.*

My further aims included conducting whole season-long tests with the improved or newly developped baits of the above mentioned cetoniins applied in blue (*E. hirta, C. aurata aurata* and *P. cuprea)* or fluorescent yellow *(O. funesta*) traps in Central and Southern Europe to study the performance and selectivity of these traps. I wondered if there is a difference in the range of scarabs caught by the different traps and also wanted to investigate the specificity of the traps to their target species.

In earlier field experiments aiming at the lure development of *C. aurata aurata* and *P. cuprea*, traps baited with the ternary attractant of these species plus apple pieces caught significantly more *C. aurata aurata* and *P. cuprea* than the ternary blend alone. High catches were observed only in case of the apple started to rot but not when it became dry. I planned to collect volatiles from rotting apple pieces and test the extracts in gas chromatography-coupled electroantennographic detector (GC-EAD) studies on the antennae of *C. aurata aurata* and *P. cuprea* for the purpose of identifying higly active compounds in the extracts. I supposed that any of the electrophysiologically active volatiles could be potentially used in further bait-developping experiments.

MATERIALS AND METHODS

Electrophysiology (EAG, GC-EAD)

Measurements were carried out at the Plant Protection Institute HAS. In both click beetles and scarabs, I inserted the antenna and mounted it between two glass-capillary electrodes filled with electrolitic solution (0.1 M KCl). One of the electrodes was grounded (indifferent electrode), the other one (the different or recording electrode) was

connected to a high-impedance amplifier. If the antenna holds receptors specific to a given compound receptor cells start firing at the same time. This summated response of the specific receptors is called the electroantennogram (EAG).

Besides EAG, I used gas chromatography-coupled electroantennographic detector (GC-EAD) in my studies as well. If some μl amount of an extract made either from a conspecific female or host plant is injected on a GC column and then one half of it is carried onto the antenna of an insect, the other half into the flame ionization detector (FID) of the GC, two parallel runs are obtained: one showes the responses of the antenna, and the other one the FID-trace. These two traces are identical in retention time.

Closed-loop stripping apparatus (CLSA)

Measurements were carried out at the Plant Protection Institute HAS. In a closed system air was circulated by a pump. The air stream passed through a glass container containing the beetles or the apple pieces. Volatiles emitted by the target species or objects were further carried through a carbon filter which absorbed them. Absorbed volatiles were dissolved by dichloromethane.

DNA studies

These studies were conducted at the Department of Microbiology of Eötvös Lóránd University of Sciences. I used a section of the Cytochrome oxidase I gene sequence for comparing *A. lineatus* and *A. proximus.*

Field trapping experiments

In tests on *A. lineatus* and *A. proximus* Yatlor f trap type was used. Neat solutions of geranyl butanoate, geranyl octanoate and geranyl propionate were administered in different combinations in Kartell dispensers. Experiments were run in Portugal, Bulgaria and Hungary in 2007; number of blocks: 5.

In all field trapping tests on scarab beetles Csalomon® VARb3 funnel trap type was used. Neat solutions of the test floral compounds were administered onto poliethylene bag dispensers.

In case of *E. hirta*, the known binary attractant, 4-methoxyphenethyl alcohol and methyl salicylate were tested in different combinations in Bulgaria and Hungary during two seasons in 2007 and 2008. Number of blocks: 5.

In *C. aurata aurata* and *P. cuprea*, the known ternary lure, *ß*-ionone, geraniol and (±)-lavandulol were tested in different combinations in three experiments during one season in Hungary in 2006. Number of blocks: 5-10.

For catching *O. funesta*, VARb3z fluorescent yellow traps were used. Treatments consisted of the different combinations of 2-phenylethanol, *(E)*-anethol and (±)-lavandulol. Tests were carried out in Hungary in 2004 and 2007. Number of blocks: 5.

Sites of the whole-season long trapping experiments in 2008 were: Croatia (3 sites), Bulgaria (2 sites), Italy (2 sites), Hungary (1 site). Number of blocks: 4.

SUMMARY OF RESULTS AND DISCUSSION

1) Geranyl butanoate and geranyl octanoate were successfully demonstrated in volatile collections from females of both *A. lineatus* and *A. proximus.* The 1 : 1 mixture of these two compounds were attractive to both species in field tests.
2) In EAG-screenings, pattern of antennal responses of male *A. lineatus* and *A. proximus* evoked by synthetic click beetle pheromone compounds was very similar. Sequence-analyses of the Cytochrome C I genes of field-collected specimens of *A. lineatus* and *A. proximus* showed a high-degree (>99%) similarity between the two species.

   The high degree of similarity between *A. lineatus* and *A. proximus* may indicate species disjunction. From the practical point of view a trap suitable for catching both *A. lineatus* and *A. proximus* is available.
3) Based on electrophysiological studies, in field trapping experiments 4-methoxyphenethyl alcohol proved to increase the attractive effect of the known binary lure of *E. hirta.*
4) Based on electrophysiological studies, in field trapping experiments (±)-lavandulol proved to increase the attractive effect of the known ternary bait of *C. aurata aurata* and *P. cuprea.*
5) Based on our previous findings in field tests aiming at other beetle pests + EAG-screening of synthetic floral compounds on the antennae of *O. funesta*, a binary attractant consisting of 2-phenylethanol and (±)-lavandulol was found to be most effective in attracting *O. funesta.* The funnel trap VARb3z baited with this lure is suitable for detection and monitoring of this scarab.
6) In the whole-season long experiments it was proved that the improved *Cetonia/Potosia* lure is suitable for catching, besides *C. aurata aurata* and *P. cuprea*, *E. hirta* and the closely related *T. squalida* in good numbers as well.
7) From rotting apple pieces, highly active compounds (1-hexanol, acetic acid, butyric acid, isovaleric acid, hexanoic acid and 3-methyl phenol) were isolated in GC-EAD tests on the antennae of *C. aurata aurata* and *P. cuprea* and identified by GC-MS. This can be a starting point for further bait optimalization studies.

PUBLICATIONS FOR THE THESIS

Scientific papers

TÓTH M, FURLAN L, XAVIER A, VUTS J, SUBCHEV M, TOSHOVA T, SZARUKÁN I, YATSYNIN V (2007): New sex attractant for *Agriotes proximus*: similarities in pheromonal communication with *A. lineatus* (Coleoptera: Elateridae). *IOBC wprs Bulletin* 30: 59-64.

TÓTH M, FURLAN L, XAVIER A, VUTS J, TOSHOVA T, SUBCHEV M, SZARUKÁN I, YATSYNIN V (2008): New sex attractant composition for the click beetle Agriotes proximus: similarity to the pheromone of A. lineatus. *J Chem Ecol* 34: 107-111.

VUTS J, IMREI Z, TÓTH M (2008): Development of an attractant-baited trap for *Oxythyrea funesta* Poda (Coleoptera: Scarabaeoidea, Cetoniidae). *Z Naturforsch C* 63: 761-768.

VUTS J, SZARUKÁN I, SUBCHEV M, TOSHOVA T, TÓTH M (2009): Improving the floral attractant to lure *Epicometis hirta* Poda (Coleoptera: Scarabaeoidea, Cetoniidae). *J Pest Sci* (in press).

VUTS J, IMREI Z, TÓTH M (2009): New co-attractants synergising attraction of *Cetonia a. aurata* and *Potosia cuprea* to the known floral attractant. *J Appl Entomol* (in press).

Conference abstracts:

TÓTH M, FURLAN L, XAVIER A, VUTS J, SUBCHEV M, TOSHOVA T, SZARUKÁN I, YATSYNIN V: New sex attractant for *Agriotes proximus*: similarities in pheromonal communication with *A. lineatus* (Coleoptera: Elateridae)., abstract in (HORVÁTH J; HALTRICH A; MOLNÁR J) *53rd Plant Protection Days* p. 14, Budapest, feb 20-21. 2007.

VUTS J, IMREI Z, TÓTH M: Improving the field activity of the synthetic floral bait in Cetonia a. aurata and Potosia cuprea (Coleoptera: Scarabaeoidea: Cetoniidae)., abstract in (anonym) *23rd Annual Meeting, International Society of Chemical Ecology* p. 101, Jena, július 22-26., 2007.

VUTS J, IMREI Z, TÓTH M: Improving the field activity of the synthetic floral bait in *Cetonia a. aurata* and *Potosia cuprea* (Coleoptera: Scarabaeidae: Cetoniinae)., pp. 176-183 in Proc. (KÖVICS GYJ; DÁVID I) *12nd Plant Protection Symposium* Debrecen, okt 17-18. 2007.

VUTS J: Studies on the chemical communication of some elateriid and cetoniin pests., abstract in (anonym) *3rd Sensory Ecology International Course for Postgraduates* p. 28, Lund, október 6-18., 2008.

VUTS J, TÓTH M: Application of EAG response spectra in developing attractants for cetoniin scarabs., abstract in (anonym) *18th Plant Protection Forum* p. 153, Keszthely, jan 30-feb 1. 2008.

FURTHER PUBLICATIONS IN THE TOPIC OF THE THESIS

Scientific papers

TÓTH M; IMREI Z; SZARUKÁN I; VOIGT E; SCHMERA D; VUTS J; HARMINCZ K; SUBCHEV M (2005): Chemical communication of fruit- and flower-damaging scarabs: results of one decade's research efforts. *Növényvédelem* 41: 581-588.

VOIGT E; TÓTH M; IMREI Z; VUTS J; SZÖLLÖS L; SZARUKÁN I (2005): Increasing damage of *Anomala vitis* and *Cetonia aurata* and the possibilities of environmental-friendly plant protection., *Agrofórum* 16: 63-64.

VUTS J; TÓTH M (2008): Electroantennogram response spectra - which questions can be studied and which not., *Növényvédelem* 44: 377-384.

Conference abstracts:

IMREI Z; TÓTH M; VUTS J: Ready for application: chemical communication of flower-feeding scarabs (Coleoptera: Scarabaeidae, Cetoniinae)., abstract in (KUROLI G; BALÁZS K; SZEMESSY Á) *50th Plant Protection Days*, Budapest p. 42, Budapest, feb 24-25. 2004.

TÓTH M, VOIGT E, IMREI Z, SZARUKÁN I, SCHMERA D, VUTS J, HARMINCZ K, SUBCHEV M, SIVCEV I: Semiochemical-baited traps for scarab pests damaging fruits and blossoms., abstract in (anonym) *58th International Symposium on Crop Protection* p. 196, Gent, május 23., 2006.

VUTS J, FURLAN L, SZARUKÁN I, TÓTH M: Is the pheromone of Agriotes click beetles a "classical" sex pheromone?, abstract in (anonymus) *23rd IWGO Conference & 2nd International Conference of Diabrotica Genetics*, München, április 5-8., 2009.