Studies on the chemical ecology of some mirid and chrysopid species of plant protection importance

Doctoral theses

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INTRODUCTION AND PURPOSES

Chemical ecology is an interdisciplinary science involving both chemistry and biology, and it includes studies on the chemical and ecological interactions between organisms. The compounds functioning in these relations are called semiochemicals or infochemicals. Semiochemicals conveying information and evoking behavioural or physiological responses between individuals of the same species are called pheromones, and those involved in interactions between individuals of different species are called allelochemicals. Allelochemicals can further be classified according to whether the chemical substance or substances released are beneficial for the emitter (allomones), for the receiver (kairomones) or both (synomones). Pheromones can also be classified according to their function, like sex-, aggregation-, alarm and tracking etc. pheromones.

Beside their scientific importance semichemicals may be used in agricultural practice as well. Mostly sex- and aggregation pheromones of pest species are applied for monitoring and also for pest management, including methods such as mass trapping and mating disruption techniques. Recently more and more researches are aiming at attracting beneficial predatory or parasitoid organisms with semiochemicals into the crop in question.

Electroantennographic studies are used to ascertain whether a certain compound or a combination of compounds can be perceived by the antenna of the given species. The antennal response is amplified and measurable. Nevertheless it is also important to test whether the compound or combination of compounds evoke behavioural response (for instance attraction) as well. Laboratory behavioural assays may also provide information on it, however the most convenient tests are field experiments, where the behaviour of the species is not studied in simplified laboratory environment, but in natural conditions.

In the course of my studies I performed laboratory and field experiments in respect of two mirid pests, the European tarnished plant bug (*Lygus rugulipennis* Poppius, 1911) and the alfalfa plant bug (*Adelphocoris lineolatus* (Goeze, 1778)). In the laboratory I tested whether synthetic plant volatile compounds evoke response from the antennae, and I also conducted bioacoustic tests (laser vibrometer) for the better understanding of the mating behaviour of the European tarnished plant bug. In field experiments I tested synthetic floral bait and aphid sex pheromone baits to study their attractive effect and possible interactions on green lacewings.

Chemical ecological and bioacoustic studies on the European tarnished plant bug (*Lygus rugulipennis* Poppius, 1911)

The goal of my studies was to broaden the knowledge on the chemical ecology of the European tarnished plant bug. This was partly done by eletroantennographic and field testing of different plant volatile compounds. A preliminary indication suggested that a general floral odour compound, phenylacetaldehyde might be attractive for adults of the European tarnished plant bug. My aim was to test this effect in field experiments. An other goal was to test synthetic plant volatile compounds by electroantennography (EAG), and the compounds giving high responses were tested in field experiments as well, to ascertain whether they are attractive.

The sex pheromone compounds of the European tarnished plant bug have been already published (Innocenzi et al. 2004, 2005), and the research group identifying the compounds (Natural Resources Institute, Chatham, UK) kindly provided me with sex pheromone baits, which I tested in field experiments. This was particularly important since the number of individuals attracted to the pheromones can serve as a reference to estimate the attractive effect of other stimuli (e. g. plant volatiles). In a further perspective the aim of the studies was to provide additional knowledge for future monitoring purposes.

For the better understanding of the sexual behaviour of the European tarnished plant bug, I performed bioacoustic studies in a Slovenian research group having expertise in bioacoustics (National Institute of Biology, Ljubljana, Slovenia). The aim of the studies was to ascertain whether acoustic or substrate borne vibrational signals are involved in sexual communication of this species.

Studies on the chemical ecology of the alfalfa plant bug (*Adelphocoris lineolatus* (Goeze, 1778))

Only scarce data is available on the chemical ecology of the alfalfa plant bug. Since there were no previous reports on the effect of volatiles on the behaviour of this species, new results could serve as a basis for further research. In the field experiments on the European tarnished plant bug, phenylacetaldehyde proved to be attractive to the alfalfa plant bug as well. Furthermore I also tested other plant volatile compounds in electroantennographic studies. The compounds eliciting high responses from antennae were further tested in field experiments to ascertain whether they are attractive. These compounds were tested alone and in combination with phenylacetaldehyde, to check whether the two different stimuli evoke more pronounced behavioural response when presented together.

Studies on the chemical ecology of green lacewings (Chrysopidae)

The aim of my studies on green lacewings was to provide new knowledge on the chemical ecology of this group including important predators of homopteran pests. More specifically my goal was to test potential attractants with which the local abundance and with this the population dynamic effect of green lacewings could be increased. These studies could serve with important knowledge when future application in agricultural practice is considered.

In field experiments I tested the attractive effect of aphid sex pheromones (Hooper et al. 2002) on green lacewings. Since previously the effect of these baits on green lacewings was only tested in Eastern Asia and Western Europe, the experiments in Hungary could provide new interesting data. In field experiments I also tested the ternary floral bait attracting common green lacewings developed in the Plant Protection Institute of the Centre for Agricultural Research, HAS (Tóth et al. 2009). I also tested the floral and aphid sex pheromone baits in combination to check whether application of the different stimuli influences the number of lacewings attracted.

MATERIALS AND METHODS

Electroantennographic studies

The electrophysiological studies were performed with electroantennographic (EAG) tests in the Plant Protection Institute of the Centre for Agricultural Research, HAS in Julianna-major. The electric signal elicited by synthetic volatile compounds in the antennae is detected and amplified by the electroantennographic instrument. The signal is measurable in millivolts. The signal was amplified and by an IDAC-232 high impedance DC amplifier (Syntech, Hilversum, The Netherlands).

The signal detected by the instrument is the summary of individual signals of sensillae sensitive to the given stimulus. Therefore if a compound is perceived by more sensillae, higher response will be elicited from the antennae. In the studies it is important to use the solvent and blank air as a control and a standard stimulus, which is preferably a compound which elicits a response higher than the solvent, but the response is medium high, not outstanding. The stimuli chosen for tests were general plant volatile compounds, mainly floral volatile compounds, since both the European tarnished plant bug and the alfalfa plant bug prefer to feed on flowering plants, but besides these I also tested green-leaf volatiles as well (e.g. (*Z*)-3-hexenyl-acetate), which are emitted by damaged plant tissue and were found attractive for herbivorous insects. On one individual only one test was conducted. During the measurements the stimuli were administered one by one with a special syringe, with one ml puff each.

The electroantennographic signals were recorded and analyzed by the EAG 2000 software (Syntech, Hilversum, The Netherlands).

Field experiments

In field experiments I used different bait formulations. Plant volatile compounds were formulated in polyethylene bag (PEbag) formulation. The sex pheromone baits of the European tarnished plant bug were kindly provided by Prof. David Hall (Natural Resources Institute, Chatham, Egyesült Királyság), who identified the pheromone compounds. The aphid sex pheromone baits for studies on green lacewings were provided by Prof. John Pickett and Dr. Michael Birkett (Rothamsted Research, UK).

In the first field experiment with plant bugs, the effect of phenylacetaldehyde and different trap designs (KLP+, RAG, VARL+) were tested in 3 replicates. According to the experiences of this test, the VARL+ trap design was used in further experiments.

In Pusztazámor (Southwest from Budapest), phenylacetaldehyde and other compounds eliciting high responses from antennae of the alfalfa plant bug (eugenol, methyl-anthranilate, (*E*)-cinnamaldehyde) were tested alone and in combination in 5 replicates. I also tested the combination of phenylacetaldehyde and (*E*)-cinnamaldehyde in different ratios in 5 replicates,

and different doses of the two compounds alone in 4 replicates to see whether the attractive effect shows dose-dependence.

The sex pheromone compounds were tested in different formulations in Halásztelek (South from Budapest) and in Julianna-major (Northwest from Budapest). I also tested the effect of the sex pheromone and phenylacetaldehyde baits in combination, to ascertain whether the two different stimuli influence the effect of one another. These tests were run in 5, 8 or 10 replicates.

The field experiments on green lacewings were conducted in Halásztelek and in Pusztazámor, in all cases with VARL+ trap designs and in 5 replicates. The effect of the floral bait and aphid sex pheromone baits ((4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol) were both tested in alone and in combination. A test was also run with empty rubber tubes similar in size to aphid sex pheromone baits, to ascertain whether the decrease in the number of common green lacewings attracted was due to a physical effect or not.

Bioacoustic studies

To study the possible importance of bioacoustic signals in the sexual communication of the European tarnished plant bugs, I conducted bioacoustic studies on adult bugs in Slovenia, in the National Institute of Biology, with the guidance of Prof. Andrej Cokl. The recordings were performed with a PDV-100, laser vibrometer. The device detects the laserbeam reflected from the surface, and converts the movements and vibrations of the surface into sound signals, which can be analyzed later on. During the tests laboratory-reared virgin and field collected male and female individuals were tested to ascertain whether sound or vibrational signals are involved in the sexual behaviour of the species.

The recordings were performed on plant (a resonant substrate) and on artificial, non-resonant substrate (loudspeaker membrane). The analysis of the recordings was done with the software Sound Forge 6.0 (Sonic Foundry Inc., Madison, WI, USA). In the analysis, the signals were described by the following basic characteristics: dominant frequency component, pulse duration, number of signals per pulse train and repetition time, as the time between the onset of a given and the next signal. I considered the pulses to be part of the same pulse train if these were monotonously repeated (had similar repetition times) and there were no pauses between them. When analyzing the dominant frequency of the signals, the dominant frequency component of the basic, background noise was excluded.

SUMMARY OF RESULTS AND DISCUSSION

1. The field experiments have confirmed that two general floral odour compounds, the phenylacetaldehyde and (*E*)-cinnamaldehyde are attractive to males and females of the European tarnished plant bug and the alfalfa plant bug. This effect was not known in any of these species, furthermore in the case of the alfalfa plant bug this is the first report of a behavioural effect of a plant volatile compound. The results imply that olfactory stimuli may be important in host finding of these species. The combination of the two compounds did not affect the number of individuals attracted, thus they did not convey a stronger stimulus together than separately. Attraction to the compounds showed dose-dependence.

2. To my knowledge no previous bioacoustic studies have been previously performed in relation to the sexual behaviour of the European tarnished plant bug. During the experiment both male and female bugs produced vibrational signals by tapping on the surface. There were no significant differences between males and females in the basic signal characteristics. The production of vibrational signals suggest that the sexual behaviour of the European tarnished plant bug is more complex than previously thought and signals transmitted through plants are also important.

3. In field experiments males of *Chrysopa formosa* Brauer, 1850 and *C. pallens* Rambur, 1833 were attracted to aphid sex pheromone baits. This was not affected by adding floral volatile baits. Currently there is no obvious explanation why females were not attracted to the baits. It is possible however that there is a similarity between aphid sex pheromone compounds and (yet unknown) pheromone compounds of these lacewing species.

4. Adults of the *Chrysoperla carnea* species complex were attracted by the floral bait, however aphid sex pheromone baits did not evoke a similar response, furthermore when applied in combination, the number of individuals attracted decreased significantly. This phenomenon is especially interesting bearing in mind that aphids are preferred food for larvae of these species. A possible explanation could be that the effect is due to the similarity of aphid sex pheromone compounds to pheromones of other (e.g. *Chrysopa*) lacewing species. An other possibility is that the effect is due to the strong smell of the defensive secretions of *Chrysopa* adults attracted to aphid sex pheromone baits.

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FURTHER PUBLICATIONS IN THE SUBJECT OF THE THESES

Scientific papers

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