Project final report

Project title: Study of the effect of infochemical stimuli in neuropteran insects

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Chemical ecology is an interdisciplinary field of science at the interface of chemistry and biology and it deals with the chemical mechanisms involved in intra- and interspecific interactions between living organisms. The chemicals involved in these interactions are termed infochemicals or semiochemicals.

The current research project focused on chemical ecology of neuropteran insects, primarily green lacewings (Chrysopidae), which have special importance in biological control of pests as their larvae are predators of several soft-bodied pestiferous insects, especially aphids.

Among green lacewings *Chrysoperla* species are of special importance in respect of biological control (Pappas et al. 2011). The adults of these species are not predatory, but feed on pollen, nectar or honeydew.

Thus, the oviposition of females is of crucial importance. According to previous reports green lacewing females prefer to lay their eggs on protruding parts of plants. Furthermore *Chrysoperla carnea* species-complex females were reported to lay their eggs in the vicinity of a synthetic, ternary floral bait developed at the Plant Protection Institute, Centre for Agricultural Research (Tóth et al. 2009). Behavioral observations and field experiments were conducted to ascertain whether this effect could be further increased with appropriate visual or tactile stimuli. Since some previous reports suggested the presence of oviposition-deterring compounds in green lacewings, the effect of the presence of previously laid eggs was also tested. Our results have shown that with appropriate surface *C. carnea* species-complex females laid more eggs in the vicinity of the baits, however neither tested colour, nor previously laid eggs seemed to have an effect on the attraction or oviposition. The results were published in Journal of Pest Science, an international peer-reviewed journal (Koczor et al. 2017).

Since *Chrysoperla* spp. lacewings are not predatory as adults, from the practical point of view, attraction of predatory adults (*Chrysopa* spp.) could be beneficial. Thus in the course of the project some compounds were selected for further behavioural tests, with special respect to those which have previously been reported to attract green lacewings in different geographic regions (e.g. North America). For these tests, field experiments were chosen to ensure that infochemicals were tested in natural, uncontrolled conditions.

Among the compounds selected for field tests, squalene, a compound previously reported to be attractive to a Nearctic *Chrysopa* species, was found to be attractive to males of *Chrysopa formosa* Brauer, 1850, a species common in agroecosystems in Hungary. When tested in combination with a ternary bait highly attractive to *C. carnea* species-complex, no interaction was found. This is of special interest, since in our previous experiments, an aphid sex pheromone component, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, attractive to *C. formosa* was found to considerably decrease attraction of *C. carnea* species-complex lacewings (e.g. Koczor et al.

2015). Since the combination of the ternary floral bait and squalene did not interfere with attraction of either taxa, this finding may open new perspectives for developing lures attracting multiple species. Our experiments have shown that attraction of *C. formosa* to squalene is dose-dependent. To our knowledge this is the first report on the attraction of any palaearctic green lacewing species to this compound. Furthermore, the combination of the ternary bait and squalene is attractive to both pollen- and nectar feeding (*C. carnea* species complex males and females) and predatory (*C. formosa* males) green lacewing adults, which is also a novel finding. This bait combination may also provide perspectives for practical applications. Our results have been published in Scientific Reports, an international, peer-reviewed journal (Koczor et al. 2019a). Furthermore, we published a brief summary of perspectives of multi-species lures for green lacewings (Koczor et al. 2019b).

In search for semiochemicals of potential interest, extracts were prepared from field-collected *C. formosa* males. The extracts showed electroantennographic activity. The active compound was identified as (*Z*)-4-tridecene. This compound has previously been identified from some lacewing species, primarily outside the Palaearctic region. According to our results, this compound decreased attraction of both *C. formosa* and *C. carnea* species-complex lacewings to traps baited with semiochemicals otherwise attractive for these species. Despite the previous reports on the identification of the compound from green lacewings in different geographic regions, our study provided the first data on behavioral response of Palaearctic green lacewing species to the compound, as only one record was previously available on a Nearctic *Chrysoperla* species (Zhu et al. 2000). Our results suggest, that this compound may serve as a common alarm signal among different lacewing species. As a potential practical application, the results may be applied in development of synthetic lures in order to decrease mortality of green lacewings as beneficial organisms in semiochemical-baited traps used for monitoring pest species. The research was conducted together with the research group of Dr. Michael Birkett in Rothamsted Research (UK) and published jointly in Journal of Chemical Ecology, an international, peer-reviewed journal (Koczor et al. 2018).

Based on observations of Dr. Geir Knudsen (NIBIO, Norway) field experiments were carried out concerning the chemical ecology of another green lacewing species, *Chrysotropia ciliata* (Wesmael, 1841). The results confirmed the observations that is, the males were attracted to p-anisaldehyde, a floral volatile. The compound was tested in EAG screenings and elicited significant responses from *C. ciliata* antennae. In the field experiments only male *C. ciliata* were attracted, which is an interesting phenomenon, it may suggest a special function of the compound. To my knowledge no reports are available in the literature concerning the chemical ecology of the species, thus these results may provide an important contribution to the chemical ecology of Chrysopidae. We are aiming to publish the results jointly with Dr. Gunda Thöming and Dr. Geir Knudsen (NIBIO, Norway) in an international peer-reviewed journal. The manuscript is in preparation.

A field experiment was carried out in order to study the effect of addition of methyl-salicylate, a common herbivore induced plant volatile to nepetalactol and squalene. The results have shown that methyl-salicylate has a strong synergistic effect on nepetalactol as it attracted significantly more *C. formosa* males, than nepetalactol alone. However, when methyl-salicylate was combined with squalene it did not increase attraction of *C. formosa*.

The synergistic effect of methyl-salicylate in combination with nepetalactol is indicating the potential importance of this herbivory-induced compound in foraging of *C. formosa*, however, interestingly almost only male lacewings were attracted. On the other hand the lack of synergism in combination with squalene is also an interesting finding. We are planning to publish the results in an international, peer-reviewed journal.

Isopropanol and terpenyl-acetate have previously been reported to attract *Chrysopa* spp. in other geographic regions. Thus, field experiments were set up to test for potential attraction of Central European green lacewing species to these compounds. In the field experiments terpenyl acetate did not show any attractive effect, however isopropanol was found to be attractive to *Pseudomallada prasinus* species group lacewings. The attraction of *P. prasinus* group lacewings to isopropanol is an interesting scientific novelty, to my knowledge this is the first result on the attraction of the taxon to a chemical stimulus. As another unexpected finding, the compound attracted an antlion species, *Distoleon tetragrammicus* (Fabricius, 1798) as well. Although relatively few individuals were caught, the effect was significant. This finding is of special interest, since only few records are available on attractive semiochemicals for Myrmeleontidae. We are planning to publish the results in an international, peer-reviewed journal.

In conclusion the research provided interesting, novel details concerning the chemical ecology of Neuroptera. From the practical point of view these findings may provide means for monitoring the respective species. Concerning agricultural practice, attractants for multiple green lacewing species as beneficial insects, may contribute to more effective biological control. As another interesting finding, our results suggested that (*Z*)-4-tridecene may have a repellent-like effect for multiple green lacewing species, and such an effect could be applied to decrease potential mortality of these beneficial insects and to obtain higher selectivity of plant-volatile baited traps at the same time. At the 13th International Symposium of Neuropterology we presented a brief overview of our knowledge on the chemical ecology of Central European Chrysopidae with emphasis on potential scientific and practical applications. The manuscript has been accepted, it is in press (Koczor et al. in press).

In the project period I took part in preparing a paper aiming to give an introduction to the methods in chemical ecology in Hungarian for researchers and students who may be interested in this field of research (Vuts et al. 2018).

In 2018, our department organized the conference of the International Society of Chemical Ecology (ISCE), the major conference of chemical ecology, which has been hosted by Hungary for the first time. Since I took part in organizing the meeting, due to the related tasks I asked permission to extend the project period in order that the planned experiments could be performed.

In the course of the project, altogether 6 presentations/posters were presented at international and 3 at Hungarian conferences. In the project period some other papers have been published with my coauthorship. These latter present new results on the chemical ecology or colour preference of insects, primarily pestiferous species (Jósvai et al. 2016; Tóth et al. 2016; Toshova et al. 2017; Lohonyai et al. 2019; Molnár et al. 2019).

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