Szent István University

Research of the chemical communication channels for the development of baits for trapping pest moths (Lepidoptera) and wasps (Vespidae).

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1. INTRODUCTION

Plant protection practice undergoes significant transformations over the last decades. Wide spectrum insecticides are gradually being replaced by more specific products, which for example are only effective at a certain life stage of pests (juvenile hormone analogues, chitin synthesis inhibitors, etc.). In paralell, cost-effective methods became promoted as well and for successful application, growers need to get accurate information about the life stages of pests (and diseases) on their plantations.

Species-specific pheromone traps are widely used in pest control, they help to detect pest populations, follow population changes during the season and assist in timing sprays. In most cases, these traps contain synthetic sex pheromone components (JUTSUM et GORDON, 1989; WITZGALL et al., 2010), which attract only males. At the same time, they do not give any information about the flight of females, despite the fact that a lure capable also catching females would have significant advantages over the use of traps baited with sex pheromone. For example, prediction of egg laying and peak hatching period of young larvae would clearly benefit from knowing female flight patterns. These baits could improve other alternative methods like mass trapping, lure and kill, push - pull, etc. as well. Moreover, in case of such pests, where sex pheromone based communication is limited (for example Vespinae – D'ADAMO et al., 2000), or the chemical composition of the sex pheromone is too expensive for practical application (e.g. *Popillia japonica* Newmann - KLEIN, 1981) it could be important to know a bait based on a different type of chemical communication channel.

Apart from mating, host plant finding behaviour is the second important behaviour in insects, which is connected with oviposition behavior in females. Therefore, components of the host plant volatile often are supposed to be suitable for attractant.

The dissertation includes research efforts for the development of effective female-targeted baits for some pest moths (Lepidoptera) and wasps (Hymenoptera) in Hungary.

1.1. Pest moths

One of the most important moth pests worldwide is the codling moth [*Cydia pomonella* (L.)]. Efforts for defining a host plant-derived attractant have a long story in this species (e.g. YOTHERS, 1927; HERN et DORN, 2001), but the first compound, which was attractive in field conditions as well, was identified only in the early 2000s. This was the pear ester [ethyl (*E*,*Z*) -2,4-decadienoate (LIGHT et al., 2001) - PE], the volatile compound of ripe pears. As results concerning the field activity of PE were inconsistent in later studies, preliminary investigations were needed to confirm the field activity of PE at each given region.

In subsequent studies, other components were tested to increase the activity of PE, like the sex pheromone component of codling moth [(*E,E*) -8,10-dodecadienol - ROELOFS et al., 1971 – PH-CP] or other plant volatiles. Landolt et al. (2007) showed that the addition of acetic acid (AA) to PE acted synergistically on codling moth catches. AA is a microbial product and it is known to be synergistic combined with a food attractant in other insect groups (e.g. wasps – LANDOLT et al., 1999) as well.

Baits containing host plant volatiles are not species-specific, as they are often consist of general plant volatiles that are present in many plants and can be used by many insects for host plant finding (BRUCE et PICKETT, 2011). In case of PE, other pest moths than codling were caught in traps as well (CORACINI et al., 2004; SCHMIDT et al., 2007). These species were the green budworm moth [*Hedya nubiferana* (Haw.)], the beech moth [*Cydia fagiglandana* (Zeller)], the chestnut tortrix [*Cydia splendana* (Hub.)] and the chestnut leafroller [*Pammene fascia* (l.)] from Tortricidae. Each of these are listed as pests (MÉSZÁROS et REICHART, 1995), so beyond the scientific importance, these results were interesting from a practical point of view as well.

From 2007 on intensive research has been carried out in connection with the attractiveness of pear ester and acetic acid bait (PEAA) in Hungary at the Plant Protection Institute CAR HAS. The results of these experiments showed that PE did not, or only weakly attracted male and female codling moth in Hungary, and neither the dose of PE nor the use of different types of dispenser had any effect on catches. However, baits containing both PE and AA have always attracted significantly more moths than PE or AA alone. PEAA traps attracted 5-30% of codling moths as compared to the catch of PH-CP traps and the combination of the two types of bait did not have any benefitial effect on catches (TÓTH et al., 2009a). Traps baited with PEAA attracted other pests and non-pest species apart from the codling moth in significant numbers as well. These were the green budworm moth, the pear moth [*Cydia pyrivora* (Dan.) (Lepidoptera: Tortricidae)] and the apple clearwing moth [*Synanthedon myopaeformis* (Borkh.) (Lepidoptera: Sesiidae)] (TÓTH et al., 2009b; 2009c). What is more, a nymphalid butterfly, the pearly heath [*Coenonympha arcania* (L.) (Lepidoptera: Nymphalidae)] was also caught in PEAA traps. Results in connection with the last two species were particularly surprising because formerly PE attracted only closely related moths belonging to the family Tortricidae.

1.2. Pest wasps

Eusocial pest wasps (Hymenoptera: Vespidae) cause serious economical, ecological and human health problems worldwide. The most significant species belong to the subfamily Polistinae (paper wasps) and Vespinae (yellowjackets). The common characteristics of these species are social behavior, superior competitive and dispersal abilities, high reproductive rates, broad diets and habitat ranges and effective predator defences. Due to the intensive use of world trade networks and globalization, many of these species have spread outside their native distribution area (BEGGS et al. 2011) and nine of them are considered to be highly invasive pests. The two well-known wasp species are German wasp [*Vespula germanica* (F.)] and the common wasp [*Vespula vulgaris* (L.)]. They are originally Palearctic species, but now can be found in nearly all parts of the Earth (MÓCZÁR, 1995).

Most effective method for the control of pest wasps is the continuous reduction of the population at the given area (BEGGS et al., 2011). Two frequently used methods are mass trapping and toxic baiting, which require a strong attractant for successful application. The activity of the widely used natural-based attractants (DEMICHELIS et al., 2014; DVORAK and LANDOLT, 2006) is constantly changing as they ferment, so they need to be replaced frequently, which increases costs. They attract many useful or unwanted insects such as bees (e.g. D'ADAMO and LOZADA, 2003). The aim of the research is to develop a synthetic bait that is shelf-stable, relatively cheap and easy to maintain (RUST et al., 2010).

Similar to codling moth, research on wasp attractants began with the study of volatiles emitted by fermenting fruits or molasses and the systemic screening of these compounds (DAVIS et al., 1967). The most commonly used synthetic attractants are heptil butyrate (HB) and isobutanol (IB). The addition of AA to IB significantly increased the attractiveness of wasps both in North American (LANDOLT, 1999) and European experiments (LANDOLT et al., 2007).

Based on observations in connection with the predatory behavior of the wasps, volatiles from prey insects (kairomones) have been discovered as attractants. Pheromone components of the American spined soldier bug (*Podisus maculiventris* Say – Hemiptera) weakly attracted a North American wasp species, *Vespula maculifrons* (du Buysson) (ALDRICH et al., 1986). Number of wasps caught (*V. maculifrons* and *V. germanica*) could be increased by adding these components to the mixture of IB and AA (ALDRICH et al., 2004). Attractivity to the smell of the prey insects is presumably connected with the learning ability of wasps and not an innate behaviour. For example, pheromone components of *P.*

maculiventris did not attract the members of the *V. germanica* in New Zealand (SPURR, 1995) or in Europe (JÓSVAI et al., 2012). Learning ability can contribute to the successful invasion of wasps worldwide.

Similarly to the above, the synthetic pheromone component [(*Z*) -9-tricosene] of the housefly (*Musca domestica* L.) (Diptera, Muscidae) significantly increased the catches of *V germanica* and *V vulgaris* in European experiments when added to IBAA (JÓSVAI et al., 2012), however, it was not known, whether it could be repeated in other regions invaded by these species.

2. OBJECTIVES

2.1 Experiments on pest moths (Lepidoptera)

I have joined the research project in connection with female-targeted lures for moths of the Plant Protection Institute CAR HAS in 2010. The main target of this project was to reveal the role of PEAA at those moth species, where the attractivity of PE or PEAA was known, or at moths, where the attractivity of PE was known, but the synergistic effect of AA was not investigated. We also wanted to reveal which other microlepidopterans are attracted to the PEAA lure, therefore experiments in various biotopes were conducted.

In case of the green budworm moth, apple clearwing moth, chestnut tortrix and pearly heath electrophysiological tests were conducted with compounds found active in the field to see whether they were evoking responses from isolated antennae of the given species, which would suggest that they readily detected these compounds at the peripheral sensory level.

The effects of different factors on the attractivity of PEAA were investigated as well, which could be important in practical application. These were: dose of PE, dispenser type, formulation of the components, trap design, placement of the trap.

At those species, where the sex pheromone were known (codling moth, apple clearwing moth, green budworm moth, chestnut tortrix) the performance of traps baited with PE or PEAA with that of the conventional pheromone-baited traps were compared and evaluated. The advantage of the application of these two types of lures (PE/PEAA and synthetic sex pheromone) in the same trap was also investigated at the different species.

The synergistic effect of N-butyl sulfide and (*E*)-4,8-dimethyl-1,3,7-nonatriene next to PEAA were tested in case of codling moth, green budworm moth and apple clearwing moth.

These compounds significantly increased the attractivity of PEAA in case of codling moth in North American experiments (LANDOLT et al., 2014; KNIGHT et al., 2011).

Starting from published experiences in case of the feeding attractant of the spotted wing drosophila [*Drosophila suzikii* (Matsumura)] (LANDOLT et al., 2012) we have investigated the effect of the addition of wine - as a natural attractant - to PEAA at the different species. In case of a positive result, next step of the research could be the identification of the particular volatiles, which were responsible for this increase.

2.2. Experiments on pest wasps (Hymenoptera: Vespidae)

One aim of our experiments was to increase the attractiveness of the well-known synthetic bait, IBAA. Therefore the effect of the addition of 2-phenylethanol was investigated, as in North American experiments this component increased the catches of the IBAA bait (Landolt, unpublished).

For a similar reason as in case of pest moths, the effect of the addition of a natural attractant (wine) to IBAA was investigated on wasps as well.

Both the German and common wasps were introduced to New Zealand, where they cause problems both in urban areas and gardens, but especially in the indigenous *Nothofagus* beech forests, where they reach enormous population sizes (BEGGS, 2001). In our tests the attractivity of IBAA combined with the sex pheromone component of the housefly (IBAATR) was investigated in these two different area of New Zealand. In previous experiments, well known wasp attractants were not working in beech forests.

In New Zealand, synthetic wasp lures have been developed by a New Zealad team, which were active to wasps living in beech forests. The components of these lures were identified from green mussel (*Perna canaliculus* Gmelin., Mollusca: Mytilidae), fermented brown sugar and honeydew. With the combination of the components of these sources, a complex, six-component lure was evaluated, which outcompeted known attractants (UNELIUS et al., 2016). As wasps feed on honeydew in Hungary as well, I investigated the bait consisting of the components from honeydew in Hungary. Moreover, I tested the highly attractive New Zealand complex bait as well.

3. MATERIAL AND METHODS

3.1 Field trapping experiments

Field trapping experiments were conducted in different plantations and habitats (e.g. commercial or mating disrupted apple, chestnut, oak, etc.) with randomized complete block design. Traps within blocks were separated by 10-15 m, and blocks were located 20-25 m apart. Traps were suspended in 1,5-2 m or 4-5 m height from branches in the crowns of trees and shrubs. Traps were inspected twice weekly and captured insects were identified, recorded and sexed if needed.

Between 2010 and 2015, a total of 32 field experiments were conducted. Out of these 7 were conducted by co-operating research teams from the SZIE Department of Entomology (Katalin Hári) and the Institute of Plant Protection, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen (Dr. István Szarukán, Dr. Imre Holb, Zsófia Kecskés, Ildikó Drogán-Zsuga, László Krakkó). A particular experiment is usually performed in parallel, in multiple locations, to obtain more reliable results.

The details of the experiments can be found in the 6-12. tables of the dissertation.

3.2. Electroantennograms (EAG)

The objective of electroantennography tests was to determine whether selected synthetic compounds evoke responses from isolated antennae of the different species and are readily detected at the peripheral sensory level. In addition to the attrative components (PE, AA), a standard component was also tested (with medium EAG activity), and solvent control and pure air (control with no chemical stimulus) as well. If it was known, sex pheromone components of the different species were tested as well (e.g. green budworm moth, chestnut tortrix, apple clearwing moth). Tested insects were collected from the field. Tests were performed according to the protocol of this method (ROELOFS, 1977).

3.3 Statistic

Mean moth catches were calculated for each treatment over the length of each field trapping experiment. Data were transformed using $\sqrt{(x+0.5)}$ to normalize them if needed (Roelofs and Cardé 1977). Treatments with zero catch were separated in order to avoid zero variances (Student's t test, $P>0.05$; REEVE and STROM, 2004). Depending on the number of the remaining treatments, means were analysed by Student's t-test or one-way ANOVA.

Normality of residuals were checked by d'Agostino's test ($P>0.05$). Having significant ANOVA, treatment means were separated by Tukey's or Games-Howell's post hoc test, depending on whether homogeneity of variances was accepted or not (by Levene's, at 0.95 level; DAY and QUINN, 1989).

Response amplitudes of EAG tests were normalized against the means of responses to the standard compound, which was tested before and after the test compounds. A log transformation was used to normalize data if needed. After checking for normality (Shapiro-Wilks normality test, $P > 0.05$), response means were analysed using a one-way ANOVA. As the homogeneity of variances was violated, Games-Howell's post hoc test was applied to separate response means.

All statistical procedures were conducted using the software packages IBM SPSS 22.

4. NEW RESULTS FOR SCIENCE

Pest moths - PEAA was highly attractive in our study not only for the codling moth, but for three other important pest moths: the apple clearwing moth, the green budworm and the chestnut tortrix. Ratio of the active compounds, formulation, performance compared to the adequate sex pheromone lure, trap design and trap placement in orchards were optimized in all of these species. In case of codling moth and apple clearwing moth, a "semi-synthetic" lure was developed, which caught several folds more moths than PEAA alone.

PEAA caught four other moth species in significant numbers, where presence of both PE and AA was necessary for good attraction. Interestingly, these four species belonged to four different Lepidopteran families: *Notocelia trimaculana* (Haw.) to Tortricidae, *Ypsolopha scabrella* (L.) to Ypsolophidae, *Watsonalla binaria* (Hufn.) to Drepanidae and *Coenonympha arcania* (L.) to Nymphalidae. Together with the apple clearwing moth (from Sesiidae), the number of lepidopteran families where field activity of PEAA has been demonstrated by us increased to five. This suggests that PE may play a more widespread role in the chemical ecology of different lepidopterous groups than it was thought before.

Apart from these, 6 other species were detected in significant numbers in PEAA traps, however, these species probably responded only to AA. These species were: *Archips crataegana* (Hübn.), *A. xylosteana* (L.), *A. rosana* (L.), *Ptycholoma lecheana* (L.), *Tortrix viridana* L. from Tortricidae and *Pararge aegeria* (L.) from Nymphalidae. The number of the attracted species raises the need for some taxonomic knowledge on the part of the end users in evaluating captures in PEAA-baited traps.

Pest wasps - No additional synthetic compound to increase the attractivity of IBAA lure to wasps was found, however, a new, semi-synthetic attractant, with a new formulation has been developed for wasps.

Invasive populations of pestiferous European wasp species (*V. vulgaris* and *V. germanica*) living in the indigenous beech forests (*Nothofagus* spp.) of New Zealand could not be attracted by either of the well-known synthetic wasp attractants. We showed that neither the IBAATR was attractive to the wasp population living in this special habitat, however, in other areas of New Zealand, with the same conditions as the natural habitat of these wasps, IBAATR-baited traps caught wasps in significant numbers. On the other hand we showed that the two- or six-component baits, developed by New Zealand researchers – which were highly attractive to wasp populations in beech forests – did not catch any wasps in Hungary. These results support that learning has a crucial part in the behavior of these wasps and plays an important role in their successful establishment in foreign areas.

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