Possibilities in development of environmentally friendly control of fruit moths in Hungary

DOCTORAL THESIS

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The applicant met all of the requirements of the Corvinus University of Budapest PhD regulations. During the revision of the Thesis all remarks and recommendations given by the opponents were taken into consideration, thus the revised Thesis is accepted for the defence process.

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1. SCIENTIFIC BACKGROUND AND AIMS OF THE STUDY

There have been a significant number of changes in the practise of insect pest control against fruit moths during the past decades. Procedures aiming to spare the natural enemies of the pests have come to the front. At the same time there has been a change in the approach of the application of pesticides as well.

Sex pheromone traps are essential devices in monitoring the flight activity of fruit moths. With the help of those traps it becomes feasible within the monitored area to follow the emergence and the changes in the number of adults regarding the examined species. Regular (daily in the beginnings) collection of data proves to be inevitable to predict the mass emergence of fruit moth larvae and to determine the timing of insect pest control. It is essential to read the sex pheromone traps regularly, even daily, and to record the temperature data in order to adjust crop protection treatments with insecticide bearing selective effect to the time when the insects are at a vulnerable developmental stage. Pest prediction based on the changes in the number of adults caught by the species-specific sex pheromone traps is usually left unused due to the lack of regular readings and the lack of data recording. Since reading the traps is a time- and labour-consuming task, my aim was to realize the readings with the help of web cameras and the transmission of data.

The production of synthetic sex pheromones offered the possibility to develop a new way of chemical control, that is the method of mating disruption. Mating disruption has been used successfully in many countries so far. In recent years, mating disruption as a way of protection against fruit moths has also been in the focus of interest in our country. As with any new crop protection methods, where the experience is not quite wide-ranging, detailed studies are needed to determine the effectiveness and risk factors. That is why I aimed to monitor the flight pattern of peach moth and oriental fruit moth inside the plantation and their migration outside, and an assessment of the reliability of mating disruption as a means of protection within Hungarian conditions.

In the plantations protected by the method of mating disruption the sex pheromone traps are not suitable for monitoring the flight activity of male adults, therefore, it became necessary to research other types of lures, fragrances which can be used to observe the flight pattern of male adults or even that of females, as well. The research of lures suitable for the monitoring of the flight activity of females is especially desirable as their use would allow a more precise determination of the timing of pest control treatments when the insect is in the most vulnerable stage of development. Landolt et al. (2007) discovered recently that the attractant effect of pear-ester can be increased by adding acetic acid in the case of codling moth. Tóth et al. (2009) confirmed the result as well with a series of domestic research, in addition they found to their surprise that apple clearwing moth were flying into the traps beside the codling moth. Joining this research, my objective was to develop a lure trap which is suitable to observe both male and female adults.

2. MATERIALS AND METHODS

2.1. Mating disruption examinations against *Grapholitha molesta* and *Anarsia lineatella* in an apricot orchard

I made mating disruption examinations against the peach twig borer and the oriental fruit moth in Soroksár, from 2007 to 2010 in a one-hectare apricot plantation. I observed the two adults flight pattern inside the plantations and their migration outside, furthermore I collected larvae during the assessment of fruit damage, in order to determine the damaging species. During the examinations, I used dispensers manufactured by Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). Against the oriental fruit moth *Grapholita molesta* Isomate OFM rosso (dose 600 / ha), against the peach twig borer *Anarsia lineatella* Isonat A (dose 1,000 / ha) type dispensers were placed out in the plantation.

In the mating disruption plantations I placed out 2-2 sex pheromone traps to observe the emergence of the oriental fruit moth and the peach twig borer, while for the observation of the flight activity outside the mating disruption area traps were sited in a distance of 50 and 100 metres from the two edges of the plantation. During the examinations CSALOMON® oriental fruit moth and peach twig borer (manufactured by MTA ATK Plant Protection Institute, Budapest, Hungary), and Deltastop oriental fruit moth (manufactured by PROPHER, Czech Republic) type traps were used. For the observation of the codling moth emergence I also placed out the CSALOMON® trap manufactured by MTA ATK. Readings were performed twice a week, and according to the standard I exchanged the lures every 4-6 weeks within the examination period.

To determine the major species causing damage to the fruit, I carried out examinations on fruit fallen from the trees in the years of 2007 and 2008, whereas in the following years, in 2009 and 2010 I examined fruit fallen as well as fruit picked from the trees. I nurture the larvae which were found in the fruit in BCE laboratory of the Department of Entomology and identified the adults. The nurtured parasitoids from the larvae were also identified.

2.2. Attracting female and male moths with the use of bait in an apple plantation

I carried out the trapping of the codling moth and apple clearwing moth female and male adults by a mixture of pear ester + acetic acid in the outskirts of Tordas in an intense, 10-acre apple orchard between 2009 and 2011. Pest control against codling moth had been realised by the method of mating disruption since 2007.

Chemicals:

- pear ester (ethyl-2,4-decadienoate) (Bedoukian, Danbury, CT, USA),
- acetic acid (CH3-COOH) (Sigma Aldrich, Budapest, Hungary),
- *Synanthedon myopaeformis* pheromone (3Z,13Z)-3,13-octadecadienyl acetate (CSALOMON ®, Plant Prot. Inst., HAS, Budapest, Hungary),
- *Cydia pomonella* pheromone ((E,E)-8,10-dodecadien-1-ol) (CSALOMON ®, Plant Prot. Inst., HAS, Budapest, Hungary).

Attractant dispensers:

- *Polyethylene bag (PE bag) dispensers* (for acetic acid and acetic acid with pear ester): A 1-cm piece of dental roll (Celluron®; Paul Hartmann, Heidenheim, Germany) was placed in a tight PE sachet (ca. 1,5 x 1,5 cm) made of 0,02-mm linear PE foil.
- *Polyethylene vial (PE vial) dispensers* (for acetic acid and acetic acid with pear ester): A 0,5-cm piece of dental roll (Celluron®) was placed in a 0,7-ml PE vial with lid (no. 730; Kartell, Noviglio, Italy).
- *Rubber dispensers* (for synthetic sex attractant of codling moth and apple clearwing moth): Lures for the tests were prepared by using pieces of rubber tubing (no. MSZ 9691/6; Taurus, Budapest, Hungary).

Traps:

- CSALOMON® sticky delta traps (**RAG)** (produced by Plant Prot. Inst., HAS, Budapest, Hungary): sticky inserts of the traps is 10×16 cm.
- CSALOMON® modified funnel traps (**VARs+**) (produced by Plant Prot. Inst., HAS, Budapest, Hungary).
- large sticky delta traps (**nagyRAG**): 2 pieces sticky inserts of the traps are 10×16 cm.
- large sticky delta traps (**nagy2011**): sticky inserts of the traps is 23×35 cm.
- **Deltastop** green sticky delta traps (produced by PROPHER, Czech Republic): sticky inserts of the traps is 12×20.

Traps were suspended at a height of 1.0–1.5 m from branches in the crowns of trees. Traps were arranged as experimental blocks so that each block contained one replicate of each treatment. Traps within blocks were separated by 5–8 m, and blocks were sited 30–40 m apart. Traps were inspected twice weekly. In order to separate the gender the taxonomic key for adults by Laštůvka and Laštůvka (2001) for apple clearwing moth and Danilevszkij and Kuznyecov (1968) for codling moth were used. Separation was carried out in the laboratory of Department of Entomology, Corvinus University of Budapest.

In 2009, my aim was to compare the luring effect of the mixture of pear ester and acetic acid which was used in previous experiments, to that of *Synanthedon myopaeformis* the sex

pheromone in RAG and VARs+ type traps. I also investigated whether the pear ester and acetic acid attracts other species. Based on the results of the analysis of previous and prior years, in 2010 I examined the luring ability of pear ester and acetic acid in the cases: used with different characteristics dispensing vessels (PE bag, PE vial), different amounts of pear ester (6, 18, 60 mg) and larger aperture trap (nagyRAG). In 2011 the aim was to increase the numbers of adults caught by the pear ester + acetic acid-based trap with the variation of dose (3 × 6 mg) and trap shape (nagy2011). In addition, I compared the catching results of the raised (10 mg) dose codling moth sex pheromone available commercially to the results of the pear ester and acetic acid mixture.

2.3. Observation of the development pest trap system used to serve pest prediction

Structure of a sex pheromone trap mounted with a web camera (distant trap)

In the trap design, the traditional and commercially available sex pheromone traps served as a sample. The trap parts, the electronics, and the colour camera (ITM-C-328) are fixed to a metal frame. The electronic panels and the batteries necessary to operate the camera are in a waterproof plastic box. The sticky inserts of the trap was placed on the box shaded by a plexiglass-cover to protect against the rain and insecticide shower, as well as to protect the insects stuck in the sticky inserts of the trap. We fixed the dispenser between the sticky inserts of the trap and the plexiglass. On fixed height above the grip, a hidden camera is placed giving a total size picture of the sticky inserts of the trap. The trap can be fixed at a certain number of points on the trellis or the trees in the plantation.

The operating mechanism of the distant trap and the picture transmitter system

The system is composed of two types of units. The central unit has got a camera, a radio transceiver and is connected to the GSM network. Its task is to forward the pictures taken by its own camera and by those of the satellite units through GSM/GPRS to a defined server. The satellite units are supplied with camera and radio transceiver. Their task is to take and send pictures to the central unit. Both central and satellite units are set up in the observed orchard, in less than 50 m distance to each-other. The central unit’s parameters can set via SMS messages. The pictures are collected on the server of the Corvinus University of Budapest.

With the help of the internet these were available from anywhere at any time. The pictures appear on a website surface, which can be reached as *rcsapda.uni-corvinus.hu*. Visitors are supposed to know the login name and the password to enter the website. If one clicks on the small image displayed on the website, zooming is an option, or one can download the full-resolution image for archiving on the PC.

Assessment of the reliability of the distant trap based on the results of the catching

To determine wether the distant trap is suitable for the observation of the emergence of *Grahpolitha molesta* and *Anarsia lineatella*, I compared the trap with the commercially available CSALOMON® trap manufactured by the MTA ATK Plant Prot. Institute. I carried out the examinations in 2008 and 2009. I sited in the privately owned apricot plantation one distant trap system (central unit and satellite units), a CSALOMON® oriental fruit moth and a CSALOMON® peach twig borer trap. I filled the central unit and the connected satellite unit with dispenser used in the CSALOMON® traps. Traps were suspended in the crowns of trees, 30-40 metres apart. CSALOMON® traps were inspected twice weekly in 2008, while in 2009 I read them once a week. Sticky inserts of the traps were exchanged based on their number of catchings or the degree of contamination. Lures were exchanged every 4-6 weeks.

The ratio of oriental fruit moth and plum moth in the trap for oriental fruit moth

The study was carried out in an apricot orchard and in a peach orchard, Pomáz, Hungary from April to October, 2009. In order to trap oriental fruit moth, CSALOMON® and Deltastop sex pheromone traps of oriental fruit moth were used. For trapping the plum moth, CSALOMON® sex pheromone traps were placed in the orchards. In the apricot orchard, 2-2 CSALOMON® and Deltastop traps of oriental fruit moth and 1 CSALOMON® trap of plum moth were placed. In the peach orchard 2-2 CSALOMON® and Deltastop traps of oriental fruit moth, and 1 CSALOMON® trap of plum moth were placed, too. The lures were changed every 4-6 weeks, while sticky inserts of the traps were replaced weekly during the examination period.

The abdomenal part of trapped males was cut off and immersed in 10% KOH solution. In order to separate the investigated species the taxonomic key for adults by Seprős (1971) was used. Separation on the basis of male genitalia was carried out in the laboratory of Department of Entomology, Corvinus University of Budapest.

3. RESULTS AND DISCUSSION

3.1. Mating disruption examinations against oriental fruit moth and peach twig borer

During a series of examinations carried out from 2007 to 2010 traps sited in the apricot plantation protected by mating disruption caught oriental fruit moth or peach twig borer males very small numbers or none at all. Evidence of their presence was proved by the traps placed outside the plantation. I drew the conclusion from the catching results of those traps that the interruption of male orientation was successful within the orchard. During the examination, the peach twig borer occurred most frequently in the traps from among the pest. Based on the fact that the traps caught peach twig borer males from May to July in the adjacent areas, I conclude that the first and second generations of the peach twig borer occurred in a significant number. This is also supported by the results of the collection of larvae, as peach twig borer larvae were collected in June and July. Based on the catches of the traps and the number of larvae caught, I observed that the presence of oriental fruit moth was minor in the plantation during the first years of the examination period. However, half of the larvae collected in the last year were oriental fruit moth, and the traps in the nearby apricot plantation of 2006 caught more males in August and September as opposed to the previous years. Based on the trap catches I concluded that the oriental fruit moth is present in the orchard and it has a positive impact on the lifecycle of this insect pest that the ripening period of the apricot cultivars and varieties present in the area last from June to September. In spite of the long-lasting harvest period, the level of crop damage was minor, less than 2 percentage. Most of the insects nurtured of the damaged fruit were oriental fruit moth and peach twig borer, besides there were codling moth very small numbers. Within the procedure of nurtured I managed to observe the parasitoid species of the peach twig borer (*Paralitomastix varicornis*) as well. That species of chalcid wasp parasitised as much as one-third of the peach twig borer larvae collected in 2008. It was the peach twig borer larvae in the group of pest species that caused the main crop damages in June and July. Since the parasitoid of that insect pest is also present in the plantation, the chalcid wasp might serve as a valuable alternative in the control of peach twig borer population, providing that pest control is carried out with consideration of the beneficial insects. Based on my results, I may state that the examined young apricot planting – despite of its small size – has been protected against fruit moths with the technique of mating disruption since 2007. Having these results, I assume that the application of mating disruption in against fruit moths might serve as a successful alternative in other domestic apricot orchards as well, taking that conditions are alike. The method may be an efficient part of the integrated pest control.

3.2. Attracting female and male moths with the use of bait in an apple plantation

During investigation the flight pattern of adult *Synanthedon myopaeformis* could successfully be monitored with traps baited with the pear ester-based lures all three years. Based on the catches I determine the catch in pear ester and acetic acid-baited traps amounted to ca. 20% of that in sex attractant-baited traps. The pear ester and acetic acid lures attracted females of the apple clearwing moth. When the sex pheromone of *Synanthedon mypaeformis* is added to the lure consisting of pear ester and acetic acid, the number of adults caught does not increase.

There was only one pest species in greater numbers in the trap namely *Cydia pomonella* besides *S. myopaeformis*. When the sex pheromone of *Synanthedon mypaeformis* is added to the lure consisting of pear ester and acetic acid, the number of codling moth adults caught does not increase, thus the sex pheromone of *Synanthedon mypaeformis* does not attract the codling moth.

There was no significant difference in the evaluation of the results between the RAG and VARs+ type of sex pheromone traps, although, among the two of them VARs+ proved to be the most effective as I achieved higher ratio of catches in the case of *S. myopaeformis*. Presumably, the rapid contamination of the RAG traps could cause that the number of males caught were lagging behind the results of the VARs+ type. When using pear ester and acetic acid in the lure trap, the VARs+ trap caught fewer male and female adults, although, there proved not to be a significant difference in the case of males. The codling moth catching results also showed a similar picture. I assume that the behavioral responses of the adults to the attractants may be the reason for the fact that the VARs+ trap caught fewer adults. Most likely, pinpoint sex-attractant source cannot be detected when using pear ester in the lure trap, as opposed to the case of the sex pheromone. During my studies conducted in 2011, sticky traps with a larger surface caught significantly more *S. myopaeformis* adults then the smaller sized one. Similar results were gained for the codling moth as well. Although, no significant difference was performed by the two types of traps, the number of the catching was as many times bigger as the difference in the size of the trap surface.

The polyethylene vial tested in order to increase the effectiveness of pear ester and acetic acid lure traps did not prove to be better than the polyethylene bags used previously. Therefore, in the case of the pear ester + acetic acid, the lures should continue to be replaced every three weeks.

There was no significant difference in the number of S. *myopaeformis* adults caught either in the case of traps containing various amount of lures observed during an examination aiming to increase the dose of pear ester, or in the case of trap including three dispensers. It is likely that the pear ester features a stretching, wide-dose optimum, and the doses tested fall

into this range, therefore, attempts to increase the results of catching failed. In the case of codling moth, in the evaluation of dose study I found a significant difference between the 6 mg and 60 mg traps with reference to the catching results. In relation to the increase in the proportion of pear ester, a weak but not impressive growth in the results of catching was observed. The sex pheromone trap commercially available for codling moth did not caught adults during the examination period, which was presumably due to the realisation of mating disruption in the plantation. The 10 mg sex pheromone trap for codling moth, commercially also available, caught significantly less adults than the pear ester + acetic acid trap.

During the lure trap studies with pear ester and acetic acid I observed that apple clearwing moth males and females are attracted by the mixture, while in the case of codling moth the positive impact of the attractant was confirmed. Based on the results of the examinations, currently the large-surface sticky trap with 6 mg pear ester / 400 mg acetic acid dosed polyethylene bag issuer (nagy2011) is considered to be the suitable for lure trapping. In the case of apple clearwing moth, the VARs+ trap with sex pheromone proved to be the most suitable. In addition, I found that the traditional and increased dosed (10 mg) traps available commercially for codling moth perform unreliable data when monitoring the population in the case of mating disruption. In contrast, the pear ester and acetic acid-based traps proved to be adequate for the trapping of the codling moth or the apple clearwing moth.

3.3. Examination of a trap system serving pest prediction

I managed to test the distant trap developed by the Department of Entomology with success in the years 2008 and 2009. Pictures of the sticky inserts of the trap surface taken and transmitted daily with the application of the web camera mounted in the trap enabled the implementation of distant readings. The images are saved and stored on a server, and can be reached at any time with the help of the internet. During the operational testing of the distant trap, problems occurred with photography due to the adherent raindrops on the plexiglass plate and the strong sunlight. Elimination of the matter was successful after a shade has been fixed over the sticky inserts of the trap.

During the operational testing of the distant trap, I observed the flight activity of two species, namely *Anarsia lineatella* and *Grapholitha molesta*. When assessing the reliability of the distant traps, I found that the trap is suitable for the observation of the flight activity of the first and subsequent generations of *Anarsia lineatella*. The distant trap is inappropriate for the observation of oriental fruit moth flight pattern, since the traditional trap caught oriental fruit moth male adults more frequently than the distant trap. Furthermore, plum fruit moth males also flew into the oriental fruit moth trap, which raised a problem, as the two species cannot be separated based on their external morphological markers. During my studies I found that the trap for oriental fruit moth attracts plum fruit moth males as well. Catching results of the

traps cannot be used to predict larva-hatching without undertaking genital examination and to determine the proper time for plant protection treatments. Distant trapping is therefore only applicable to monitor the flight activity of moths when the species can clearly be identified based on their distinctive external morphological characteristics.

In the light of the previous studies I conclude that the distant trap is suitable for the monitoring of moth flight activity, and it is also necessary to involve additional insect pests in the examination in order to extend the scope of species sensitive to distant trapping.

4. NEW SCIENTIFIC RESULTS

In our research the following new scientific results have been achieved:

1. I have concluded that the method of mating disruption against *Anarsia lineatella* and *Grapholitha molesta* may be an effective process in our domestic apricot orchards.

2. I have founded that the codling moth as a pest of fruit can appear in apricot plantations protected by mating disruption, however, it will not cause significant harm.

3. I have diagnosed that with the lure consisting of pear ester and acetic acid *Synanthedon myopaeformis* females and males can be trapped. Currently the large-surface sticky trap with 6 mg pear ester / 400 mg acetic acid dosed polyethylene bag issuer (nagy2011) is considered to be the suitable for lure trapping. I have also found that in the case of *Synanthedon myopaeformis*, the VARs+ trap with sex pheromone proved to be the most suitable. I have confirmed that the pear ester and acetic acid lure attracts the *Cydia pomonella* males as well as females. When the sex pheromone of *Synanthedon mypaeformis* is added to the lure consisting of pear ester and acetic acid, the number of codling moth adults caught does not increase, thus the sex pheromone of *Synanthedon mypaeformis* does not attract the codling moth.

4. I have determined that in the mating disruption apple orchard the standard and raised dosed (10 mg) *Cydia pomonella* sex pheromone traps, which are available commercially, can not be used to monitor the emergence. In case of mating disruption we can only use pear ester and acetic acid-based traps to monitor *Cydia pomonella* and *Synanthedon myopaeformis.*

5. I have found that the automated sex pheromone traps (distant trap) developed by us to observe insects is capable for trapping insect pests and monitoring their flight activity, providing that the sex pheromone of the pest species is sufficiently selective or that the pest species can be clearly separated from one another based on their external morphological characteristics.

6. The catching data provided by the distant trap once a day are very useful in the development of an insect pest control prediction system establishing protection against the key pests of plantations. Thus they might serve as an effective means in the reduction of environmental strains delivered by pesticides.

7. I have concluded that the oriental fruit moth pheromone trap attracts plum fruit moth males as well. In the sex pheromone trap the ratio of oriental fruit moth and plum fruit moth males depends on their dominance strength deriving from the patchy occurrence of both species.

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