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PHD THESIS

Environmentally friendly population control of raspberry pests which give rise to cane death

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1. INTRODUCTION AND THE AIMS OF THE RESEARCH

Hungary has a long tradition of growing raspberry. Due to the optimal ecological conditions and the growers' expertise, intensive and exemplary raspberry production can be achieved in the traditional growing regions. Among the most important requirements of competitive, integrated raspberry production the use of pest- and pathogen-free planting material of high quality cultivars grown in appropriate systems and the application of environmentally safe pest management methods have to be emphasised besides the choice of suitable area for raspberry growing. The lack of any of the mentioned elements will cause a decline in the efficiency of production. Hence, the good agricultural practice is indispensable to produce a high value crop.

The main aim of my thesis was to develop the integrated protection of raspberry, which is an important field of research if a high level production is required.

Raspberry has a special insect fauna, most of its pests are host plant specialists causing damage only to *Rubus* species. Consequently, the plant protection methods which have already proved to be suitable for applying against pests in other fruits are only partly adaptable and are needed to be improved. It is inevitable to identify the key pests of raspberry and get detailed information on their biology. This has to be complemented with suitable means of monitoring and the knowledge of plant production and protection methods which make the prevention of problems coming up later possible. A good example for the prevention of damage caused by certain pests is the use of resistant cultivars. Efficient methods of monitoring are also necessary so that the timing and the number of sprayings be optimal. In the development of control methods, the reasonable reduction or even the omission of applying chemical pesticides, and the research for alternative, environmentally friendly control methods are further important targets. With the help of rational plant protection the natural enemies, which may play an important role in the population control of certain pests, can be saved.

Cane death caused by different pests and diseases has always meant a major problem in raspberry protection. As these insects and pathogens may give rise not only to some loss of leaves or fruits but also to the complete death of the affected plants and the rapid conditional decline of plantations, the working out of efficient and environmentally safe plant protection technologies is necessary.

The aims of my research were to examine the biology of two important raspberry cane pests, the raspberry cane midge [*Resseliella theobaldi* (Barnes)] and the rose stem girdler [*Agrilus cuprescens* (Ménétriés)] under Hungarian conditions, to reveal the group of their potential natural enemies and their significance, to develop pest monitoring and to analyse possible environmentally friendly control methods (e.g. the choice of appropriate cultivars).

2. MATERIALS AND METHODS

2.1. Research on the parasitoids of the raspberry cane midge and the rose stem girdler

As a first step, the relevant publications, which give data on the natural enemies of either of the two pests, were collected. All found species names were written in a table according to chronological order of the publications. This process was followed by the check of parasitoid names by using the most modern taxonomical studies and databases with the help of Dr. Csaba Thuróczy. At the same time, I tried to get in touch with those specialists who once identified the species mentioned in papers. In case of old publications, the former workplace (an insect collection of a museum or a research institute) of the author or the specialist where the reared parasitoid specimens could be supposed to have been placed was also tried to be found. In some cases, the specimens were present in these collections, and I could ask for further information on them from present-day curators or other colleagues of the institute. If we deemed it necessary, we asked for a loan of the specimens so as to make their taxonomic status clear. Unfortunately, there was no available information on some parasitoid specimens identified long ago because certain collections have been destroyed or closed. Hence, the present status of some species can not be cleared. (They are marked with the term "species inquirendae" in the thesis.) This research has been carried out within the scope of an international cooperation in which curators and specialists of national insect collections and researchers of plant protection institutes helped the investigations.

Nevertheless, rearing experiments were also made to determine the parasitoids of the raspberry cane midge and the rose stem girdler occurring in Hungarian raspberry plantations. As a part of this study, damaged canes were collected from the region of Nagyréde and Berkenye, between 2002 and 2005 (in case of the raspberry cane midge) and from Nagyréde, Berkenye, Fertőd, Mátraterenye and Romhány, between 2003 and 2007 (in case of the rose stem girdler) to rear adults of parasitoid species. The successfully reared specimens were identified by Dr. Csaba Thuróczy.

2.2. Emergence studies on the overwintering generations of the identified parasitoids of the raspberry cane midge and the rose stem girdler

Examination of the emergence of the first parasitoid generation, which overwinters in larval stage inside the dead body of raspberry cane midge larvae present on raspberry canes, were carried out in 2004, 2006 and 2007. In order to determine the time of emergence, the rate of holed skins of the larvae of the host (already left by the adult of the parasitoid) present on canes collected form Berkenye every second week were studied under laboratory conditions (indirect method). On the

other hand, the number of emerged parasitoid adults in insectaries were counted weekly (direct method).

So as to follow the emergence of rose stem girdler parasitoids, small groups of damaged cane pieces collected from the region of Nagyréde on the 27th of April, 2007, were adequately isolated, and the appearance of parasitoid adults was regularly checked from the middle of May.

2.3. Studies on the occurrence of larvae of the raspberry cane midge and its primary parasitoid on primocanes during the vegetation period

In 2004 and 2005, detailed studies of different raspberry cultivars (*Tulameen*, *Fertődi zamatos*, *Rubaca* and *Autumn Bliss*) were carried out in the growing region of Berkenye to follow the rate of primocane splitting and to check the occurrence of larvae of the raspberry cane midge and its primary parasitoid under the split bark of the examined cultivars. During the vegetation period, samples consisting of 25–25 split primocanes were taken every second week from the time when the rate of split primocanes of the given cultivar reached 10%. In the laboratory, the number of larvae of the pest and its natural enemy were counted in case of each primocane by using a stereomicroscope and a dissecting needle. In this way, data were received every second week on the presence of larvae of both insects found on different cultivars.

2.4. Materials and methods for the monitoring of raspberry cane midge and rose stem girdler adult emergence

Sex pheromone traps developed by the East Malling Research and the Natural Resources Institute (UK) and yellow and white sticky traps were applied to follow the emergence of raspberry cane midge adults. By using the raspberry cane midge sex pheromone trap for the first time under Hungarian conditions as a part of an international study, I could run a test for evaluating its efficiency in raspberry cane midge monitoring. In Berkenye, 2006, all kinds of traps were placed in *Fertődi zamatos* and *Autumn Bliss* plantations treated with chemical insecticides and also in plots of the same cultivars which were left untreated. In each plot, two pheromone traps were fixed at a height of 50 cm within the plantation. In 2007, trials with the pheromone traps were extended to *Fertődi zamatos* plantations treated with insecticides in the growing region of Nagyréde. The catches of the pheromone traps were checked in weekly intervals from April to October in 2006 and 2007, while the lures were changed monthly.

The monitoring of rose stem girdler adult emergence was carried out in the same way as described in the case of its parasitoids.

2.5. Examination of host plants and egg-laying of the raspberry cane midge under laboratory conditions

For the study of host plants and egg-laying of the raspberry cane midge, artificially cut primocanes of raspberry (*Rubus idaeus*) and shoots of quince (*Cydonia oblonga*) and willow (*Salix alba*) together with reared adults of the pest (the larvae of which were collected from Berkenye) were used under laboratory conditions of the Department of Entomology, Corvinus University of Budapest. The aim of the experiment was partly to examine whether the females lay eggs and the eggs hatch under the split bark of quince. With this trial, the uncertain status of quince as a host plant of the pest was targeted to be cleared. Raspberry and willow were the positive (host) and negative (non-host) controls, respectively, in the experiment. Further studies were made with artificially cut willow shoots the splits of which were treated with an extract containing the volatile components of raspberry primocanes in n-hexane gained after distillation. In this trial, the attractiveness of the raspberry primocane extract to the female midges or its stimulating effect on egg-laying in the treated willow splits were tested. For statistical analysis the software "Ministat" was used.

2.6. Identification of volatile components of raspberry primocanes and quince shoots

In 2004, the volatile components of raspberry primocanes and quince shoots were examined in the laboratory of the Department of Food Chemistry and Nutrition Science, Corvinus University of Budapest, so as to find compounds which may play a role in the host plant choice of the raspberry cane midge. For the identification of the aroma spectra of the mentioned parts of the plants, quince shoots (*Cydonia oblonga*) and defoliated raspberry primocanes (*Rubus idaeus* cv. *Autumn Bliss*) were collected for the analysis at the end of June, 2004. There were two types of raspberry samples: one of them consisted of primocanes with an intact bark before prepared for the distillation process, while the other sample consisted of primocanes with splits on the bark. After the distillation process, the extracts of raspberry primocanes and quince shoots in n-hexane were stored in small, glass tubes in a freezer. The chromatograms of the plants and the mass-spectrum of each component were measured by Dr. Kornél Korány using a Hewlett Packard 5890/II GC-5971/A MSD GC-MS equipment. If the reliability of identification of a certain compound in the repeated measurings in case of each plant samples reached 70% at least in one case per plant sample, the compound was accepted to be a volatile component of the plant. The identified components were marked with their PTRI number (**P**rogrammed **T**emperature **R**etention **I**ndex) on the basis of the mean of measurings and the reliability of identification.

2.7. Study on the causes of resistance of different raspberry cultivars to the raspberry cane midge

In 2004 and 2005, the degree of resistance of four raspberry cultivars (*Tulameen*, *Fertődi zamatos*, *Rubaca* and *Autumn Bliss*) to the raspberry cane midge was studied, and the possible causes of resistance were also investigated. The main questions of this research topic were as follows:

1. Is there a difference among cultivars in the number of raspberry cane midge larvae feeding under the split bark of their primocanes?
2. Is there a difference among cultivars in the cumulative split length or the extent of bark peeling of their primocanes?
3. Is their any correlation between the number of larvae and the cumulative split length or the extent of bark peeling?

Primocanes of each cultivar were collected from the same growing area (Berkenye) and at the same sampling dates within a year for this study so that the cultivars could be adequately comparable. Samples were taken every second week in both years for laboratory work, and consisted of 25, randomly cut, split primocanes of the first flush in case of each cultivar. For statistical analysis the software "Ministat" was used.

3. RESULTS AND DISCUSSION

3.1. Potential parasitoids of the raspberry cane midge and the rose stem girdler

As far as the parasitoids of the raspberry cane midge referred to in the literature are concerned, it can be established that the number of species which indeed play an important role in the control of the populations of the pest is small. Among these species, *Aprostocetus epicharmus* (Walker) has to be mentioned first, which was the only one parasitoid species found during my studies. On the basis of my rearing experiments, *A. epicharmus* can be said to be the most important natural enemy of the pest in major raspberry plantations of Hungary. The species is a solitary endoparasitoid developing in the late instar larva of the raspberry cane midge. Monoembryony is typical of this species. The sex ratio of the reared specimens was 82:1 (816 females, 10 males), on the basis of which the possibility of parthenogenesis can not be excluded.

Among further parasitoids of the raspberry cane midge mentioned in earlier publications, *Tetrastichus vincius* (Walker) is undoubtedly, while, on the basis of their biology, *Tetrastichus inunctus* (Nees) (species inquirendae) and a *Tetrastichus* sp. are almost certainly identical with *A. epicharmus*. According to my results, further possible parasitoids of this pest are *Piestopleura catilla* (Walker), *Sigmophora brevicornis* (Panzer), an *Aphanogmus* and a *Leptacis* species and perhaps *Synopeas craterus* (Walker). However, the exact status of these species as raspberry cane midge parasitoids has to be investigated by further rearing experiments. Obviously misidentified, incorrectly cited or ambiguous parasitoids mentioned in the literature in relation to the pest are *Tetrastichus flavovarius* (Nees) (species inquirendae), *Aprostocetus lycidas* (Walker), *Aprostocetus roesellae* (Nees) and *Aprostocetus tymber* (Walker).

On the basis of my studies on the natural enemies of the rose stem girdler, *Tetrastichus heeringi* Delucchi can be established to be an important parasitoid of the pest in Hungary. Specimens of this beneficial insect were reared from cane samples collected at Nagyréde, Berkenye, Mátraterenye and Romhány. No information on *T. heeringi* in relation to the pest was found in the Hungarian literature. The parasitoid was proved to be responsible to an 11% of the total, 79% mortality of the rose stem girdler experienced at the beginning of 2007. *T. heeringi* is a gregarious, idiobiont parasitoid developing in the late instar larva of the rose stem girdler. Polyembryony is supposed to be typical of this species. The sex ratio of the reared specimens was approx. 2:1 (226 females, 96 males).

In the Hungarian literature, *Tetrastichus agrilorum* (Ratzeburg), and *Tetrastichus agrili* Crawford are mentioned as natural enemies of the rose stem girdler. However, on the basis of a short description of their biology and a comparison with my own insect rearing results, it can be assumed that these publications are either about *T. heeringi* or the quite variable *Baryscapus* sp., nr. *endemus* (Walker), which also occurred in my experiments in very small numbers. Unfortunately, the location of the specimens of the described species is unknown, hence the previous supposition can not be proved. On the other hand, for this very reason, the occurrence of *T. agrilorum* (synonymised as *Baryscapus agrilorum* (Ratzeburg)) in the Hungarian fauna as a parasitoid of the rose stem girdler can not be said to be proved, while the presence of *T. agrili* in Hungary can be excluded as this latter species is distributed in the Nearctic region. According to my results, further possible parasitoids of this pest are *Eupelmus vesicularis* (Retzius), *Heterospilus rubicola* Fischer, a *Tetrastichus* and a *Xylophrurus* species, some *Eurytoma* species belonging to the *Eurytoma rosae* species group and, in North-America, *Baryscapus rugglesi* (Rohwer) and *Cubocephalus annulatus* (Cresson). However, the exact status of these species as raspberry cane midge parasitoids has to be investigated by further rearing experiments. The mention of *Platygaster cottei* Kieffer as a natural enemy of the pest is probably a misidentification or a mistake in the publication concerned.

3.2. Emergence of the overwintering generation of *Aprostocetus epicharmus* and *Tetrastichus heeringi*

According to the results of a three-year study, the mean time of emergence of first generation *A. epicharmus* adults (parasitoid of the raspberry cane midge) is between the middle of May and the middle of June under Hungarian conditions. However, the emergence of the parasitoid is elongated, and the adults may appear earlier in the season in case of mild winters or hot springs as it happened in 2007.

T. heeringi emerges about two weeks after its studied host, the rose stem girdler. Due to the mild winter and the hot spring in 2007, adults of the pest left the canes in which they had overwintered relatively soon, and most of them appeared in the second part of May. This was followed by the emergence of *T. heeringi*.

The described methods for the monitoring of the appearance of first generation parasitoids make it possible to carry out an environmentally safe plant protection technology by appropriate timing of sprayings against the pests but saving their natural enemies. The suitable protection of habitats where the parasitoids overwinter has to be emphasised, too.

3.3. The occurrence of raspberry cane midge and *Aprostocetus epicharmus* larvae on primocanes during the vegetation period

On the basis of a two-year study (2004–2005) on the occurrence of larvae present on primocanes, both the raspberry cane midge and its primary parasitoid seemed to have at least three generations per year in Hungary. However, the method for the monitoring of larvae described in the thesis is quite labour-intensive, and acceptable results can be gained only if a huge amount of primocanes collected in an appropriate way are used for the investigation of larval emergence of the species.

The rate of parasitism is low at the time of the first larval generation of the raspberry cane midge, but this rate can be high during the second and the third generations of the pest. On the average of two years and the four cultivars, the rate of parasitism of the raspberry cane midge larvae by *A. epicharmus* reached 35%.

3.4. Population dynamics of the raspberry cane midge and the rose stem girdler

As far as the monitoring of the raspberry cane midge adults is concerned, the use of sex pheromone traps (2006–2007), compared with other known monitoring techniques, proved to be the

most suitable method for the following of midge emergence under Hungarian conditions. On the basis of pheromone trap catches, the pest had four generations in both years. In 2006, the first three, while in 2007, which was extremely dry and hot, the first two generations could be clearly distinguished. About 30–40 days were necessary for the devepolment of a generation. By evaluating the catches of the traps, no visible effects of insecticide treatments were seen, but the cultivars with different growing methods might have affected the number of midges to some extent.

The results from 2007 in connection with the emergence of the rose stem girdler were shown in 3.2. in relation to its parasitoid, *T. heeringi*.

3.5. Results on the host plant choice and egg-laying of the raspberry cane midge

By laboratory experiments, it has been proved that females of the raspberry cane midge can lay eggs in the artificial splits of quince shoots. The number of eggs was significantly less than it was in the case of raspberry, but it was more than in the case of willow. On the basis of these results, split quince shoots, besides split raspberry primocanes, can also be presumed to have some kind of stimulating effect on the egg-laying of the midge. Nevertheless, quince can not be said to be a real host plant of the pest as under natural conditions its non-splitting shoots are not suitable for the oviposition of the raspberry cane midge. On the other hand, no eggs were found on split willow shoots treated with raspberry primocane volatile extracts. The reasons for this might be the quick evaporation of raspberry primocane volatiles applied in small quantities, but the identification of the host plant by the pest by other (e.g. visual or tactile) stimulus or the presence of repellent components in the shoots of willow can not be excluded either.

3.6. The aroma spectrum of raspberry primocanes and quince shoots

In case of raspberry primocanes, 113, while in quince shoots, 84 volatile components have been identified. Among these compounds, 59 were present in both plant species, which means that 54 components were found only in raspberry and 25 only in quince. Therefore, it can be supposed that the 59 components identified in both plant species together with some others extracted only from raspberry primocanes may play a role in the attraction of raspberry cane midge females or may stimulate their egg-laying.

3.7. The resistance of different raspberry cultivars to the raspberry cane midge

On the basis of two years' results, the least raspberry cane midge larvae were counted on the split primocanes of *Tulameen* and *Fertődi zamatos*. The extent of bark peeling proved to be smallest also in the case of cultivars mentioned before. On the other hand, the accumulated length of splits reached the highest value in *Tulameen* and the lowest in *Autumn Bliss*, the bark of which, nevertheless, peeled readily. The correlation between the small extent of bark peeling and the small amount of midge larvae on primocanes in case of *Tulameen* and *Fertődi zamatos* was strong. *Rubaca* and *Autumn Bliss* could be characterised by many larvae feeding under their readily peeling bark. The results show that the accumulated length of primocane splits, compared with the extent of bark peeling, might have a smaller effect on the number of larvae feeding on primocanes.

4. NEW SCIENTIFIC RESULTS

1. The potential parasitoids of two important raspberry cane pests, the raspberry cane midge [*Resseliella theobaldi* (Barnes)] and the rose stem girdler [*Agrilus cuprescens* (Ménétriés)] have been determined. By the study of samples collected in different raspberry growing regions of Hungary, it has been established that the most important natural enemy of the raspberry cane midge is *Aprostocetus epicharmus* (Walker) (Chalcidoidea: Eulophidae), while in case of the rose stem girdler *Tetrastichus heeringi* Delucchi (Chalcidoidea: Eulophidae) plays a major role in the population control of the pest. This is the first mention of these parasitoid species as natural enemies of the studied pests in Hungary. New data have also been given on their biology, the host - parasitoid interaction and their population dynamics. Recommendations were made to develop the environmentally friendly plant protection methods to save these beneficial insects.
2. The first field trials under Hungarian conditions for testing the raspberry cane midge sex pheromone trap (developed in the UK) have been carried out within the scope of my work. The emergence of the pest was investigated in two growing regions, with two cultivars (*Fertődi zamatos* and *Autumn Bliss*) and in case of different growing and plant protection methods. The raspberry cane midge sex pheromone trap used in the described way proved to be highly effective for the monitoring of the pest.
3. The volatile components (aroma spectrum) of raspberry primocanes and quince shoots have been identified. In laboratory experiments, significantly more eggs laid by the raspberry cane midge were found in artificial splits of raspberry primocanes than in the splits of quince and willow shoots. However, the number of eggs in splits was significantly higher in quince shoots compared to willow shoots. These results may serve as important data for further studies in the research of host plant choice by the raspberry cane midge.
4. The susceptibility of four raspberry cultivars, *Fertődi zamatos*, *Tulameen*, *Rubaca* and *Autumn Bliss* to the raspberry cane midge has been determined. The correlation between the number of midge larvae feeding under the split bark and the extent of bark peeling of primocanes has been shown to be stronger than that of between the larval numbers and the accumulated length of primocane splits. *Tulameen* and *Fertődi zamatos* have been established to show a good resistance to the raspberry cane midge.

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