Thesis of Doctoral Dissertation

The monitoring, daily activity, and development of forecasting methods of raspberry cane midge (*Resseliella theobaldi* (BARNES))

Kitti Sipos

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Name: Doctoral School of Horticultural Sciences

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Head of Ph.D. School: Prof. Dr. Magdolna Tóth
Doctor of the Hungarian Academy of Sciences
Head of Department of Fruit Sciences
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

Supervisor: Prof. Dr. Béla Pénzes
Professor
Head of Department of Entomology
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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Prof. Dr. Magdolna Tóth	Prof. Dr. Béla Pénzes
Head of Ph.D. School	Supervisor

1. Introduction and aims of research

The raspberry cane midge (*Resseliella theobaldi* BARNES), is a major pest of raspberry. The larvae develop and feed under the skin of primocanes, this is the direct damage. Also the pest along with numerous fungal pathogens (e.g. *Leptosphaeria coniothyrium*) causes a syndrome known as midge blight. Chemical control is only possible against the first generation's, at mass appearance of adults, as the other generations flight at the bloom and harvest of raspberry.

The timing of control against raspberry cane midge is very important in the integrated management of raspberry. My aim was to elaborate a method based on temperature summation which could be used to predict the emergence of the raspberry cane midge. This work required to study the biology and lifestyle of the pest.

Thorough research into the biology of the pest started about 50 years ago, but the identification the raspberry cane midge's sex pheromone and the release the trap allow new possibilities to study the pest. This trap was tested by the researchers of Department of Entomology of Corvinus University in Hungary. They found it suitable for monitoring the males.

The aim of my research was to predict the time of protection against the raspberry cane midge. For the prediction of pests, including the raspberry cane midge, knowing their biology and lifestyle in detail is essential. Open-field studies and also laboratory experiments were carried out during my PhD work. The raspberry cane midge males were monitored with sex pheromone traps with which the daily activity of males was followed. The daily activity of the females was observed only indirectly by determining the time of oviposition. For this investigation, artificially wounded primocanes were used. In the laboratory experiments the lower developmental threshold (LDT) and the sum of effective temperature (SET) of some developmental stages of the raspberry cane midge were determined. It was concluded that the data (LDT and SET) of pupa are necessary for the prediction of the mass appearance of adults.

The aim of my research was:

- to observe the daily activity of raspberry cane midge.
- to test and develop the automated sex pheromone insect trap combined with meteorological meter.
- to determine the lower developmental threshold (LDT) and the sum of effective temperature (SET) of the raspberry cane midge.
- to time of control against the overwintering population of raspberry cane midge based on temperature summation.
- to describe the annual flight activity based on temperature.

2. Materials and Methods

My investigations (monitoring the flight activity and recording the temperatures) were done in an *Autumn Bliss* and *Fertődi zamatos* raspberry plantation owned Berkenye Faluszövetkezet in Berkenye (Nógrád County) in 2006-2010.

2.1. The daily activity of raspberry cane midge adults

To determine the time of oviposition, artificially wounded primocanes were used in the plantation of *Autumn Bliss*. The primocanes were incised with a knife lengthwise at a length of 10 cm, and the epidermis was divided from the tissues underneath, thus making the wound suitable for oviposition. Based on literature data, the wounds were incised in the lower 50 cm-zone of the plants. In order to count the eggs laid, the wounded primocanes were removed at certain intervals. In case primocanes could not be evaluated right after the removal, the wounded parts were kept in nylon bags until assessment.

Out attempt was to provide raspberry cane midge females with both fresh (incised max. 1 hour previously) and older wounds (incised 1-13 hours previously).

In the first part of the experiment, 20 primocanes were incised hourly from the morning hours onwards (9 am), and primocanes were removed at twilight (8-9 pm) in July 2007. In August 2007 and in 2008 I repeated the study, but only with 10 primocanes hourly.

Furthermore, information on the suitability of primocanes wounded in the morning for oviposition in the evening hours was also obtained. The time of evening oviposition was monitored in 2007-2008. In both years 50 primocanes were incised in the afternoon (17:35–17:50), 10 of which were removed every half hour to twilight.

In the second part of the experiment, I examined the attractant effect of odours released during primocane incision. After incising primocanes at the same time in the morning hours and removing first 20 (July 2007) then 10 (August 2007 and 2008) every hour, they were evaluated: primocanes where eggs were laid into wounds were counted, and the number of eggs was determined. In 2008, the egg-laying activity of females was examined. 130-140 primocanes were incised between 7 and 8 am, and 10 primocanes were removed hourly until twilight (8-9 pm). Thus, the test of previous year was repeated by elongating the observation time.

The data of study:

13-15 July 2007, 17-18 July 2007, 20 July 2007, 3-5 August 2007

9 May 2008, 11 May 2008, 16 June 2008, 20 June 2008, 23 June 2008, 11 August 2008, 13 August 2008, 21 August 2008

The daily activity of the raspberry cane midge males were studied by sex pheromone traps checked every hour (AgriSense Ltd.) in 2007 and an automated trap with a built-in meteorological meter (Madomat Kft.) in 2008-2009.

In my preliminary studies in 2007 four traps were placed 30 m far from each other, in 50 cm height in the plantation of *Autumn Bliss*. The sticky sheets were changed every day. Adult males caught on the sticky sheets were counted every hour from 8 am to 5 pm on the first observational day (13 July 2007), and from 8 am to 8 pm on the following test days (14-15 July 2007, 3-6 August 2008).

In July 2008 an automated trap with a built-in meteorological meter (developed by Madomat Kft.) was placed in the plantation of Fertõdi zamatos. The equipment took photographs of the sticky sheets every hour and the number of trapped males was defined based on the photos. The flight activity of males was monitored for 24 hours throughout July.

An automated sex pheromone trap combined with a built-in meteorological meter (developed by Madomat Ltd.) was used to monitor the raspberry cane midge continuously. The trap is also equipped with a computer unit placed in a waterproof box as well as a camera. It takes photos of the specimens stuck on the sticky sheets at intervals set in advance (by hour, day, week etc.), and the information is saved on its hard disc. The meteorological meter is supplied with an internal and external thermometer, a hygrometer, a barometer, an anemometer and a rain gauge. The equipment sends the photos taken and the recorded meteorological data to a central server via internet connection. The energy necessary for the operation of the trap is provided by a solar cell and stored in a battery; therefore no external energy source is needed. The automated trap system is fixed on a frame sinkable into the soil: the solar cell is placed on the top of the frame, whereas the anemometer and rain-gauge are placed on the cross-bar 50 cm away from the vertical axis in both directions. This makes it suitable for collecting rain water coming from any direction. The built-in frame allows using the trap in practically any culture (with or without trellis system; horticultural and agricultural plants). There’s an insect trap that opens on two sides applied to the vertical axis, where the pheromone dispenser as odour source, the sticky sheet and the camera with lighting unit for night-time exposure are placed. The insect trap can be adjusted to the frame at different heights, allowing the collection of insects flying at different vertical levels (0-3 m).

In my study the insect trap was adjusted to the frame that the sex pheromone capsule could be in 50 cm height. The test of the trap was in a *Fertődi zamatos* plantation (Berkenye) between 9 May-26 June 2008. In 2009 the trap was in an *Autumn Bliss* plantation (Berkenye) from May to October.

There were two pesticide (Cyper) treatments (3 May and 15 May) in the area. The sticky sheets were changed weekly, and capsules monthly. The camera took photo about the sticky sheet hourly.

2.2. Determining the lower temperature threshold and effective accumulated temperature

In order to determine the lower temperature threshold, the generations of the species were reared at five constant temperatures of 18 °C, 20 °C, 23 °C, 25 °C and 30 °C. The experiment was started with eggs of the same age collected from open field. For this, the results of our previous open field studies were used, according to which oviposition takes place from early afternoon until dusk. To collect eggs the method of artificially wounding canes was used: 100-200 canes per measuring were wounded in the afternoon, then cut out at dusk and transported to the laboratory. The number of eggs per cane was counted under stereomicroscope, followed by cutting out the wounded parts and placing them on wet paper (180 µl water) in a plastic tube. The wet paper provided suitable humidity for the canes and the developing larvae. The Petri dishes were placed in an environmental test chamber (12 hours light, 12 hours dark), and the developmental stages of the larvae were checked and recorded daily (24 hours). Egg stage, developed larva stage leaving the cane (prepupa stage), pupa stage and adult stage was differentiated. As larvae were present en mass on the canes, differentiating each larval stage was not reliable, thus the L1 and L2 stages were not taken into account when determining the lower temperature threshold. When the L3 larvae left the canes, they were individually separated. Larvae were placed on wet paper (75 µl water) of the same size in Eppendorf tubes. Following this, they were kept in dark, because these stages develop in the soil. They were observed until the death of the adults. At each temperature and at each developmental stage the numbers of days needed for the development were recorded. First the development time (day) was determined, from which the developmental rate (1/day) was counted, and then the developmental rate was plotted in relation to the temperature. The values of the lower developmental threshold (LDT) and the sum of effective temperatures (SET) were calculated with the equation (y = a+bx) obtained by linear regression analysis.

The threshold temperature of each developmental stage was calculated with the formula of LDT= –a/b and SET=1/b.

y = a+bx (linear regression equation)

x – temperature
y – developmental rate
b – slope of regression

a – offset of regression

LDT= -a/b
LDT – lower developmental threshold

SET= 1/b
SET – sum of effective temperature

The pest overwinters in the soil in larvae stage. In order to predict the overwintering generations flight peak, the lower developmental threshold and the development rate of the pupa stage is needed.

2.3. Monitoring the raspberry cane midge males with sex pheromone trap

The results obtained in the laboratory experiment were compared with the data gained in open-field studies of five years (2006-2010) where temperatures were recorded and emergence was monitored with sex pheromone traps in a raspberry plantation (Berkenye) from April to the end of September or the beginning of October. Each year of the experiments we operated two traditional sex pheromone traps in the plantation 30 m far from each other, and in 50 cm height. In the traps, sticky sheets were changed weekly, and capsules monthly. Catches were recorded weekly. The specimens stuck on the sticky sheets were identified with a stereomicroscope.

2.4. Open-field temperature recording

During these years, temperatures were recorded every half hour with an automatic soil and air thermometer (TGP-4510) in 2006-2010. The soil thermometer was placed 10 cm deep in the soil, and the air thermometer in a cardboard box with airing was attached to the trellis at a height of 50 cm. Daily average was calculated from the temperatures recorded. Following this, the day was determined when the soil temperature raised above the lower temperature threshold recorded in the laboratory. From this day onward, the values of the soil temperature above the lower temperature threshold were added daily to prediction the mass appearance of the first generation.

2.5. Statistics methods

The data of catches of the traditional and automated traps were analysed by SPSS programme. First, the testing of normality was carried out (Kolgomorov-Smirnov Test), followed by t-test (Paired Sample Test).

3. RESULTS

3.1. Testing of the automated insect trap

According to my observations, the automated sex pheromone trap with a built-in meteorological meter, combined with a computer system and a camera makes it possible to observe the raspberry cane midge males. The gadget is multi-purpose: it can be used for emergence of the males, as well as for gaining thorough knowledge of the biology of the species (such as daily activity, emergence, determining reproduction time, vertical location).

The catches of the sex pheromone trap (AgriSense Ltd.) and the automated trap were compared, and based on the statistical analysis, no significant difference was found (t=1,389; df=25; SL=0,177). Based on this, the design of the insect trap house is suitable, its openings are the right size and the spread of the pheromone is good. The resolution of the camera and the quality of the photos transmitted are suitable for identifying the specimens. The energy provided by the solar cell is enough to operate the computer, the camera and the lighting.

Based on the daily catches the average caught was 20-60 males a day, but the number of males fluctuated in the different months. In May, when pesticide was used against adults, the fewer males were caught. After this month the number of males increased month by month. The most males flew to the trap in August.

Based on the daily catches it has been found that the sticky sheets catch males at a high level of efficiency for 2–3 days after being changed. After this, the number of males decreases or they often stop flying to the traps. This phenomenon was also observed when the surface of the sticky sheets was not full of specimens caught in the traps. This requires continuous monitoring because the automated change of sticky sheets has not been solved yet. According to my experience, in the case of the species observed change of the sticky sheets is necessary every 3–4 days, irrespective of the density of the catch.

3.2. The daily activity of adults

The daily activity of the raspberry cane midge males were studied by sex pheromone traps checked every hour (AgriSense Ltd.) and an automated trap with a built-in meteorological meter (Madomat Kft.) in open-field. On the basis of the number of specimens caught hourly it was found that in daytime the raspberry cane midge males fly in low numbers but continuously to the pheromone. Two peaks of emergence can be separated: one in the morning, and a stronger one early in the evening. The males fly to the sex pheromone from 16 p.m. to twilight. The morning peak is often missing, whereas the strong emergence in the evening always takes place.

Analysing the meteorological data it was concluded that in rainy weather and strong winds the males do not fly to the traps.

The time of oviposition was studied on artificially wounded canes. It was found that eggs were laid from late afternoon to twilight. Before twilight the numbers of eggs are decreased one a half hour.

Since the mass flying of males to sex pheromone trap was in the evening similar to oviposition time, the copulation of males and females could be in the evening.

Based on my examinations of artificially wounded canes, it has been concluded that the wounds of the canes incised were most probably dried out in a non-irrigated plantation in a dry year, but not in a normal year. In an average year a wound can be suitable for oviposition in the evening.

3.3. Determining the developmental time of raspberry cane midge

The development of the raspberry cane midge was studied under laboratory conditions. Based on these data the lower developmental threshold and the sum of effective temperature of some developmental stages of midge (egg-3rd stage of larvae; prepupa; pupa; adult) were determined.

The development times at each temperature were studied. 60 days at 18°C, 36 days at 20°C, 26 at 23°C, 23 days at 25°C, and 19 days at 30°C are needed for one generation to develop. The larvae leave the primocanes and fall to soil surface after 25 days at 18°C, 18 days at 20°C, 13 at 23°C, 11 days at 25°C, and 9 days at 30°C. The prepupa and the pupa spend 37 days at 18°C, 19 days at 20°C, 13 at 23°C, 12 days at 25°C, and 15 days at 30°C. The adults live 4 days at 18°C, 3 days at 20°C and 2 days at 23, 25 and 30°C.

Based on the regression equation obtained, the effective accumulated temperature necessary for the development of one generation is 345 degree-days (above 12,3 °C). Out of this figure, on average 175 degree-days are spent in the soil (prepupa, pupa) and 170 degree-days above ground (adult, egg, feeding larval stages).

According to these data the pest had three generations in open field in Hungary 2006-2010, but a fourth generation may have developed in 2007.

The developmental stages living in the wounds of canes (egg-3rd stage of larvae) spend 159 day degree above 12,3 °C (R^2=0,99) on the canes. For pupation of the overwintering larval stage (prepupa) 59 day degree above 16 °C (R^2=0,90) is necessary. The lower temperature threshold of the pupa stage is 7,3°C and the effective accumulated temperature is 116 degree days (R^2=0,90). . The adults live 33 day degree above 10,8 °C (R^2=0,97).

3.4. The timing of control against raspberry cane midge

The results obtained in the laboratory experiment were compared with the data gained in open-field studies of five years (2006-2010) where temperatures were recorded and emergence was monitored with sex pheromone traps in a raspberry plantation (Berkenye). Daily average was calculated from the temperatures recorded in 2006-2010. Following this, the day was determined when the soil temperature raised above the lower temperature threshold of the pupa stage recorded in the laboratory. From this day onward, the values of the soil temperature above the lower temperature threshold were added daily. In the years of the investigation, the average daily temperature of the soil raised above +7,3 °C in third decade of March. The 116 degree-days accumulated in every years in third decade of April, accept in 2010. In this year, the effective degree-days accumulated only in first decade of May.

The time of mass appearance of the males determined on the basis of data obtained in the lab tests, as well as the actual time of emergence observed in the open field. In 2006, the mass emergence was between 1-9 May (131-176 degree-days), 2 days later than I calculated (29 April 2006). In 2007, the most males flew to the trap between 19-26 April (107-164 degree-days). The calculated day was 21 April. In 2008, the mass appearance was between 2-9 May (132-186 degree-days). The 116 degree-days accumulated on 30 April. In 2009, the mass emergence was waited on 20 April, and the trap caught the most males between 19-24 April (108-145 degree-days). In 2010, the mass appearance of males observed between 5-12 May (109-156 degree-days), and the calculated day was 7 May.

It has been found that our method based on calculating degree-days is suitable for predicting the first flight of males, thus suitable for the timing of control against the overwintering population.

Based on my prediction method, the daily soil temperature is measured after winter. After reaching 116 degree-days above 7 °C, the mass appearance of males can be expected, and the chemical control against the adult males is recommended.

4. NEW RESULTS

1) I have developed an automated sex pheromone trap with a built-in meteorological meter, combined with a computer system and a camera. It makes possible to observe continuously the raspberry cane midge males.

2) Based on monitoring with sex pheromone trap hourly, the raspberry cane midge males fly in the evening (4-9 pm).

3) The time of oviposition studied on artificially wounded canes shows an evening peak (from late afternoon to twilight).

4) The lower developmental threshold and the sum of effective temperature of one generation (345 degree-days above 12,3°C), egg-3rd stage of larvae (159 degree-days above 12,3°C), prepupa (59 degree-days above 16°C) and pupa (116 degree-days above 7,3°C) were determined.

5) Based on effective accumulated temperature (above lower developmental threshold) necessary for the development of one generation, the pest generally had three generations in open field in Hungary 2006-2010, but a fourth generation may have developed in 2007.

6) I determined and tested the precise time of control against raspberry cane midge (116 degree-days above 7°C).

7) Based on my examinations of artificially wounded canes, it has been concluded that the wounds of the canes incised were most probably dried out in a non-irrigated plantation in a dry year, but a normally year no. In an average year a wound can be suitable for oviposition in the evening.

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