Biological control of the cherry fruit fly, *Rhagoletis cerasi* L. (Diptera, Tephritidae) by use of entomopathogenic nematodes: first experiences towards practical implementation.

Erste Erfahrungen bei der Anwendung entomopathogener Nematoden zur biologischen Bekämpfung der Kirschfruchtfliege in der Praxis.

Annette Herz[1], Kirsten Köppler[1,4], Heidrun Vogt[1]; Ellen Elias[2], Peter Katz[2]; Arne Peters[3]

Abstract

The use of entomopathogenic nematodes (EPN) is a promising approach to control the cherry fruit fly, *Rhagoletis cerasi* L.. We already demonstrated the high potential of EPN to infect larvae after leaving the cherry for pupation in the soil in laboratory and field experiments. For practice, an application technique is needed, that is both, grower- and EPN friendly. We tested a tractor mounted spray boom for treatment under the canopy area. The achieved rate of EPN in the soil met the expectations. The activity of EPN in soil samples was high after application, but dropped to 60% of the initial activity within one week. Exact forecasting of larval drop from cherries is another major challenge. To obtain basic data, we recorded the phenology of infestation and larval emergence on trees which were not harvested. Sequential infestation on the same cherry variety was observed and larvae dropped from individual trees for several weeks.

Keywords: *Rhagoletis cerasi*, biological control, entomopathogenic nematodes, application technique, nematode persistence, phenology

Introduction

Entomopathogenic nematodes (EPN) are efficient biological control agents and are currently used against a number of main pests in field crops, turf and ornamentals as well as vegetables and fruits (GREWAL et al. 2005). According to their soil living habitat they are most effective when applied against soil dwelling pests, especially root feeders as *Diaprepes abbreviatus* on citrus or *Otiorhynchus sulcatus* on strawberry. EPN-applications for control of fruit damaging pests aim at soil pupating or hibernating developmental stages to suppress their populations in a preventive manner (VINCENT & BELAIR 1992, LACEY & UNRUH 1998, YEE & LACEY 2003). We recently demonstrated the high efficacy of EPN against larvae of the European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera, Tephritidae) when entering the soil for pupation in laboratory and field tests (Koeppler et al. 2003, 2004).

Rhagoletis cerasi is the key pest of sweet cherries in Europe. Beside the use of traps and crop netting, no other means are currently registered for control of this pest in organic cherry growing. Hence, high infestations levels can often be observed and severe yield loss can be a consequence because infested cherries are refused by the market. The situation is expected to become even worse in the near future, because the current product of choice - Dimethoate - in conventional cherry growing has been removed from registration and fruit fly populations in a given area will probably raise to high levels when not controlled adequately during the next years. The exploration and development of alternative control strategies is therefore urgent. As beneficial organisms, EPN do not require a registration for use and can be applied in organic growing. EPN are commercially available and are offered in formulations which are easy to use and compatible to the orchard envi-

[1] Institute for Plant Protecion in Fruit Crops, Federal Biological Research Centre for Agriculture and Forestry, Schwabenheimer Str. 101, 69221 Dossenheim
[2] Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth
[3] e-Nema GmbH, Klausdorfer Str. 28-36, 24223 Raisdorf
[4] Dep. of Zoology, University of Heidelberg, Neuenheimer Feld 230, 69120 Heidelberg

ronment. Thus, if their control potential for the cherry fruit fly can be proved also under practical conditions, an implementation of their use in organic and convential cherry growing can be fast and direct.
Beside the general infection potential for the particular target pest and the quality management during the production process, proper application techniques, application rate and frequency as well as timing are crucial for the efficacy of EPN under field conditions and were main objectives of our research in 2005. For practice, an application technique is needed, that is both, grower- and EPN friendly.
We tested a tractor mounted spray boom for treatment under the canopy area and monitored nematode persistence in the field after application. The coordination of applicaton time and occurrence of the susceptible insect stage in the field is another major challenge. We recorded the phenology of infestation and larval emergence on trees which were not harvested to obtain basic data for exact forecasting of the larval drop from cherries.

Material and Methods

Experimental field site
All field trials reported here were performed at the experimental area of the Institute for Plant Protection in Fruit Crops, Dossenheim. There are several cherry plantations of different varieties and age. No insecticide treatments against the Cherry Fruit Fly have been applied since 2002.

Nematode application and estimation of field persistence
The nematode product nemaplus® (e-nema GmbH, Raisdorf), containing the nematode *Steinernema feltiae*, was dissolved in water according to producer's instruction. We used a tractor mounted spray boom to treat the canopy area of two tree rows, each consisting of 5 large cherry trees (variety "Van"). Eight nozzles (Type Albuz® 117APG 80°, flat fan nozzle) were fixed on four po sitions along the spray boom. The nozzles are recommended for hop-culture and have a flow-rate of 10.48 l/min at 3.5 bar at 20°C according to the manufacturer. O ne row was treated once with a rate of 500.000 EPN/m² on 23rd of June and another row was treated with a rate of 250.000 EPN/m² on 23rd and on 30th of June. The spraying was carried out at a pressure of 3.3 bar and tractor velocity of 1.5 km/h to achieve the application of 1 l spray liquid per m². Pre-application and post-application irrigation was also done at a rate of 1 to 2 l water per m² in order to obtain optimum soil moisture and to rinse any nematodes sticking on plant surfaces into the soil. No further irrigation was performed afterwards. Four soil samples (10x10x10 cm) were taken under the canopy area of different trees at particular intervals after the application. In the laboratory, activity of nematodes in the soil samples was evaluated by placing 10 last instar *Galleria mellonella* larvae into the soil samples and evaluating larval mortality after 7 days of incubation at 25°C.

Phenology of infestation
We monitored the infestation by *R. cerasi* on medium-sized cherry trees (variety “Hedelfinger“) in an adjacent plantation. From 16 trees, we collected 40 cherries per tree (10 from each direction) on each sampling date. The cherries were examined in the laboratory for emergence holes, then broken and suspended in a 10% salt solution to force emergence of larvae from cherries. After two hours, emerged larvae were collected, the cherries were washed and the water was poured through gaze to collect also small larvae. Larvae were sorted according to size in young, mid and old larvae. Eggs were not recorded by this method. The trees were not harvested and sampling continued until the cherries on the tree started to decay and fall down. A part of these trees was also treated by one to several applications of 250.000 EPN/m².

Monitoring of larval drop
We estimated the duration of larval drop in the non-harvest situation on an adjacent tree row, consisting of 5 large cherry trees of the variety “Hedelfinger“. Four plastic boxes (0.25 m² surface area)

were placed under the canopy of each tree and filled with a layer of 2 cm sand to provide a place for pupation for dropped *R. cerasi*-larvae. Access of predators was prevented. Boxes were emptied in regular intervals and the sand was sieved to collect all pupae inside. Boxes were placed from 23rd of June until 27th of July when no larvae were collected anymore.

Results

The tractor mounted spray boom proved to be suitable for the application of EPN, because the quality of EPN was maintained and the desired liquid volume of 1l/m² was achieved (HERZ et al. 2005). But nematode acitivity in soil samples taken on 23rd of June was less than expected (Figure 1). This was probably caused by exposure of EPN to sunshine, because the application was done in the early hours of a sunny day. The second treatment in the evening of 30th of June preserved the activity of EPN better. Nevertheless, the activity of EPN dropped down to approximately 60% of the initial value after one week.

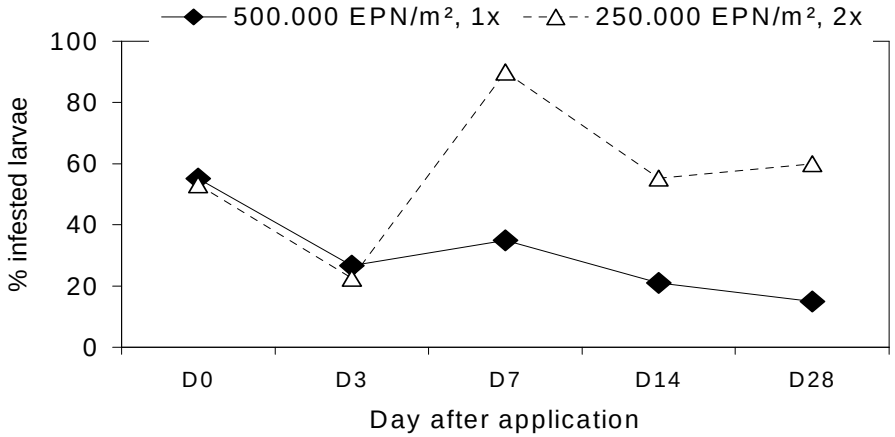

Figure 1: Activity of *Steinernema feltiae* in soil samples taken after soil application of 500.000 EPN/m² (on 23rd (D0) of June) or 250.000 EPN/m² (on 23rd (D0) and 30th (D7) of June) of cherry trees ("Van"). Activity was determined as infection of wax moth larvae in laboratory bioassays.

The infestation by *R. cerasi* on the highly susceptible cherry variety "Hedelfinger" raised within one week from 8 to 44 % (Figure 2). Larvae developed rather quickly and started to emerge from cherries end of June, the usual date for harvest of this variety at Dossenheim. Both infestation and larval drop continued for two weeks after this date.

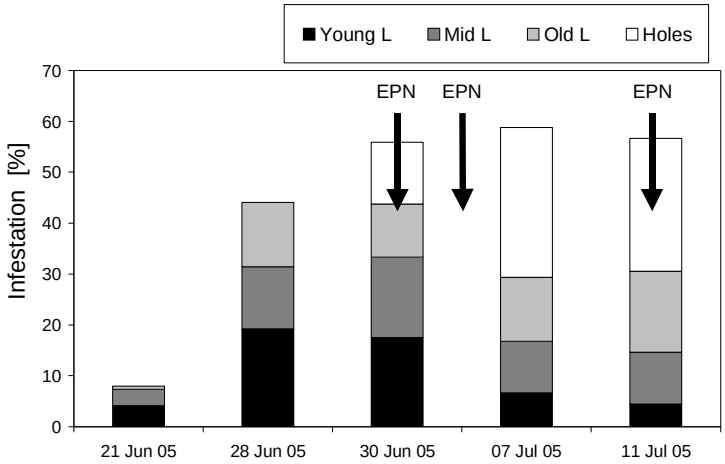

Figure 2: Phenology of infestation by *Rhagoletis cerasi* on "Hedelfinger"-cherries. Arrows indicate applicaton of EPN (250.000/m²) at several trees (30th June, 4th and 11th July).

Drop of larvae from the older “Hedelfinger“ trees nearby even started some days earlier (Figure 3) and larvae could already be observed on 23rd of June after placing the collecting boxes in the field. In former years, harvesting time of these trees was around 27th of June. In the non-harvest situation in 2005, larval drop extended to more than 4 weeks.

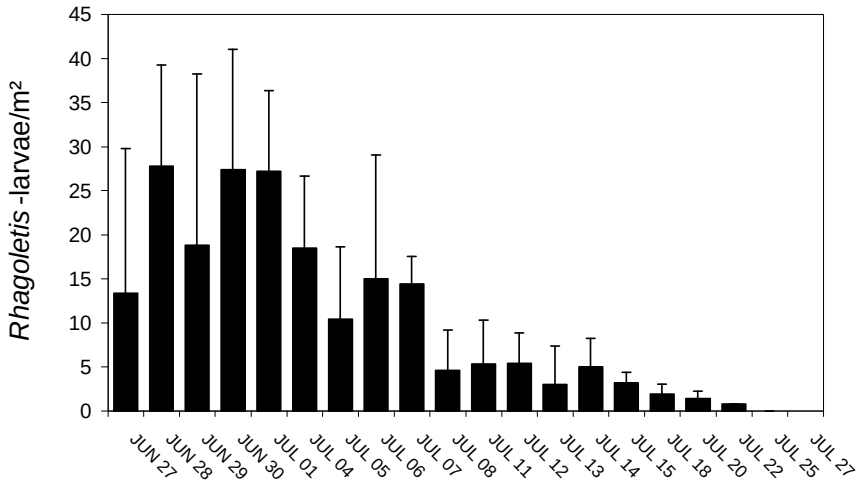

Figure 3: Number of collected *Rhagoletis cerasi* - larvae per m² soil surface after dropping from single cherry trees (“Hedelfinger“). Collection was done from 23rd of June to 27th of July 2005. Trees were not harvested.

Discussion

In general, EPN are compatible with most spraying equipment as long as used with low pressures (recommended at 5 bar) and nozzles with large orifices to let the nematodes pass without damage. It is also known that EPN should be applied on moist soil and with a high volume of water. In contrast, modern spraying equipment in fruit growing like axial fan sprayers etc. is often developed towards a minimum of spraying liquid. In our study, we found satisfying results regarding applied volume and nematode quality with the tractor mounted spray boom usually used for herbicide treatments and field application of EPN using this equipment was also performed at several commercial cherry plantations during July 2005 (HERZ et al. 2005). Whether such a system is accepted will depend on the availability for cherry growers. In organic growing, farmers do not apply herbicides and therefore may not possess this equipment. Hence, further exploration of other tools for EPN-application is needed to reach also the requirements of this target group.

After the experience gained in 2005, the major difficulty is the exact timing of the EPN-application. On the one hand, the treatment has to be early enough in order to catch also the first larvae which leave the cherries. Depending on the cherry variety, the usual harvest time is not the suitable indicator date. In the field trial on “Hedelfinger“ (Figure 2), the first EPN-application was timed too late, as part of the population already had left the cherries and pupation usually occurs within hours after emergence from the cherries. On the other hand, larval drop from trees can extend to weeks. This probably depends on the temperature as well as synchronisation of larval development. But we also found that subsequent attack on the same variety can be observed for several weeks. This situation is rather critical in the case of large trees which are not completely harvested and where a part of the cherries is usually left in the upper part of the canopy. Hence, a sufficient persistence of nematodes in the soil is required. The results obtained so far let us focus on two major tasks for the next years of research: first the forecasting of larval drop has to be improved by the development of a feasible but rigid sampling schedule, taking into account larval development on different cherry

varieties and weather conditions, and second improving nematode persistence in the field by post-application irrigation or application regimes which consider several EPN-treatments.

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