Field applications of entomopathogenic fungi against *Rhagoletis cerasi*.

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Abstract

*Two myco-insecticides, Naturalis-L (*Beauveria bassiana*) and PreFeRal®WG (*Paecilomyces fumosoroseus*), were applied against adult* R. cerasi *(Diptera: Tephritidae) in two orchards in north-western Switzerland in summer 2006. Both products were applied at a concentration of 5.75x10^4 CFU / ml. With four applications at seven day intervals the whole flight period of* R. cerasi *was fully covered. During this period (5th June – 6th July) the temperature averaged 19.2°C, total precipitation was 60 mm. Under these conditions Naturalis-L significantly reduced the number of damaged fruit (efficacy: 69-74%), whereas damage was not significantly reduced with PreFeRal®WG (efficacy: 27%). Fungus infested flies were found on the yellow sticky traps proving that the myco-insecticides worked as predicted.*

Climatic conditions were different in 2007: temperature averaged 15.9°C (16th May – 4th July), total precipitation was 281 mm. In 2007 only Naturalis-L was tested: with five applications an efficacy of 73% and 78% was obtained on the cherry varieties Langstieler and Dolleseppler, respectively. However, for the latest ripening and most infested variety Schauenburger only an efficacy of 18% was observed. In these cherries a high number of young larvae were found indicating that the efficacy was low at the end of the treatment period. In conclusion a control of R. cerasi *with myco-insecticides seems possible. However, application regime still has to be improved and should not only be adapted to the flight period of* R. cerasi*, but also to the cherry varieties.*

Keywords: *Rhagoletis cerasi,* Biocontrol, *Beauveria bassiana, Paecilomyces fumosoroseus*.

Introduction

The European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), is a highly destructive pest of sweet cherries in Europe. Without treatment up to 100% of the fruits can be infested. The use of yellow sticky traps and crop netting are currently used to control this pest in organic cherry production. However, both strategies are labour-intensive and do often not provide sufficient control. The use of micro-organisms as biological control agents against *R. cerasi* might be an alternative. In previous laboratory experiments the virulence of different entomopathogenic fungi on different life stages of *R. cerasi* was evaluated. Adult flies were found to be the only life stage susceptible to fungus infection, whereby *Beauveria bassiana* and *Paecilomyces fumosoroseus* were most virulent. Therefore these two fungus strains, which are formulated in two commercial products Naturalis-L and PreFeRal®WG, were tested in field trials. The aim of these trials was to transfer the good laboratory results into a viable field application strategy.

Material and Methods

In 2006 the trials were conducted in two 6-year old, organically managed cherry orchards in Wintersingen and Sissach-Isleten. The orchard in Wintersingen consisted of 30 cherry trees arranged in a long row at intervals of 10m between trees. The experiment was arranged in a randomized block design with 5 replicates (3 trees per plot).

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The orchard in Sissach-Isleten consisted of 21 cherry trees arranged in 5 rows with 3 to 7 trees each at intervals of 10 m in each direction. The trial was arranged in a randomized block design with 7 replicates (1 tree per plot). This orchard was treated with sulphur on 26th May 2006. No other pesticide treatments were applied.

In 2007 the following three experiments were conducted: (A) the experiment in Wintersingen was replicated with the same experimental design as in 2006. (B) The orchard in Sissach-Voregg consisted of 28 8-year old trees arranged in two rows at intervals of 10 m between trees. The trial was arranged in randomized block design with 4 replicates (2 trees per plot). (C) In a 30 year old orchard in Eptingen the trial was arranged in a block design with 7 replicates (1 tree per plot) on 26 trees arranged in 6 rows with 2 to 8 trees each at intervals of 7 to 14 m in each direction.

Beauveria bassiana (product: Naturalis-L; Intrachem Bio Italia S.p.A.) was applied in all experiments. *Paecilomyces fumosoroseus* (product: PreFeRal®WG, Biobest, Belgium) was used only in the trial in Sissach-Isleten. Both fungus strains were applied at a concentration of 5.75 x 10^4 CFU / ml to runoff (3 l per tree; Eptingen: 15 l per tree) using a high-pressure hand held gun. Concentrations for the products were adjusted according to the concentrations given in the package instructions (250ml Naturalis-L per 100l; 2.88g PreFeRal®WG per 100l). The beginning of the flight period was determined using yellow sticky traps. Within 5 days after first catches of flies, the first application was conducted. Four treatments at 7 day intervals were applied in Sissach-Isleten, Wintersingen and Sissach-Voregg. In Eptingen two application strategies were compared: intensive application of Naturalis-L at 7 day intervals (5 treatments) and extensive application of Naturalis-L at 14 day intervals (2 treatments). The last application was conducted 7 to 14 days before harvest. Untreated trees served as control.

Flight period of *R. cerasi* was monitored with one yellow sticky trap per tree. The assessment of treatment efficacy was made at harvest. A sample of cherries was taken from each tree. Sample size varied depending on the total yield of the trees. Date of sampling varied depending on harvest date of different cherry varieties. The cherries were dissected to estimate damage caused by all larval stages of *R. cerasi*.

Normality of data and homogeneity of variance were tested before conducting an ANOVA. If necessary, data were transformed. Means were compared using Tukey HSD post hoc tests (α=0.05). Correlation between efficacy (%, Abbots formula) and number of days between last application and sampling was calculated.

Results

<u>Climatic conditions</u>

During the experimental period in 2006 (5th June – 6th July), the relative humidity averaged 68.1%, with high humidity levels during the nights (RH_{max}: 99%), appearance of dew in the early morning and low humidity during the afternoons (RH_{min}: 29.0%). Temperature averaged 19.2°C, with low temperatures during the nights (T_{min}: 6.8°C) and high temperatures during the afternoons (T_{max}: 31.0°C). Over the whole experimental period a total precipitation of 59.8 mm was measured. Average global radiation was 6.6 kWh / m^2 per day. The flight of adult *R. cerasi* started in the first week of June and strongly increased in Sissach-Isleten during the following warm and sunny days. In orchard Wintersingen flight activity remained at a low level which might be due to intense wind in this location. The first application of fungus products was accomplished on 05th June 2006. The following days were sunny and increasingly warm. Seven days later a second application was conducted at hot and sunny weather. A week later after two rainy days the third application was made on 19th June 2006.

The last application was conducted on 26th June 2006 after 15 mm of rain. With these four applications the whole flight period of *R. cerasi* was fully covered.

In 2007 (16th May – 4th July) less sunshine and more rain were registered. The relative humidity averaged 81.0%, with high humidity levels during rain and during the nights (RH_{max}: 100%) and low humidity during the afternoons (RH_{min}: 40%). Temperature averaged 15.9°C, with low temperatures during the nights (T_{min}: 2.6°C) and high temperatures during the afternoons (T_{max}: 28.1°C). Over the whole experimental period a total precipitation of 281.2 mm was observed. Average global radiation was 5.1 kWh / m^2 per day. In Wintersingen and Sissach-Voregg the flight of adult *R. cerasi* started in mid-May and remained at a very low level until end of June. In Eptingen flight period started one week later, reached a peak in mid-June and decreased at the end of June. The first application of fungus products was accomplished on 16th June 2006 in orchard Sissach-Voregg. One week later on 23rd May, first treatments were conducted in Wintersingen and Eptingen. The whole experimental period was very wet and rainy, however, all treatments were conducted in dry conditions, no rain occurred during applications and five hours after. Frequently, heavy rain occurred one day after application.

Flight activity and mycosis of flies

The cumulative number of flies per trap caught during the experimental periods is given in Figure 1. No differences between treatments were found in any experiment. However, in all experiments traps hanging in treated trees tended to catch fewer flies. Pooling the data for treatment Naturalis-L and control over all five experiments, the differences in number of flies per trap were significant (three-way-Anova, data transformed [$\log_{10}(x)$]; treatment: $F_{1,49}$ = 5.56, p= 0.02; orchard: $F_{3, 49}$ = 143.41, p < 0.0001; year: $F_{1, 49}$ = 35.17, p < 0.0001). Traps hanging in Naturalis-L treated trees caught significantly fewer flies than traps hanging in control trees. Differences between the orchards and the two years of experiment were also significant. In Eptingen the number of flies per trap was significantely influenced by cherry variety: traps hanging in variety Dolleseppler caught significantly more flies (153.0 ± 16.7 flies per trap) than traps hanging in the varieties Langstieler (72.4 ± 12.6) and Schauenburger (62.0 ± 4.0; two-way Anova [treatment, variety]; $F_{2,\ 16\ variety}$ = 16.90, p< 0.001, Tukey HSD-Test α=0.05; $F_{2,\ 16\ treatment}$ = 0.32, p= 0.23).

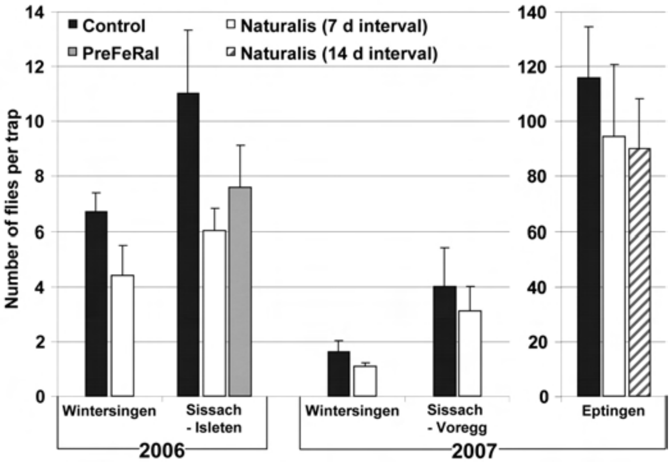

Figure 1: Cumulative number of flies caught by yellow sticky traps in five trials in 2006 and 2007.

The percentage rate of mycosis of flies caught on sticky traps was only examined in 2006. An infection of flies under field conditions was possible: 7 to 23% of flies on yellow sticky traps in the treated plots showed signs of mycosis. However, catching the flies with sticky traps is unsuitable for a quantitative assessment of infestation level. Especially for flies trapped at an early stage of infection, the fungus often failed to develop during incubation as shown in preliminary laboratory tests. Trapped flies probably die too rapidly for the fungus to develop. However, this method allows a qualitative assessment of the pathogenicity of the fungi to *R. cerasi* under field conditions. Morphological comparison between flies caught by yellow sticky traps and mycosed flies from laboratory trials indicated that the mycosis was due to *B. bassiana* and *P. fumosoroseus.*

Damage of fruits

The number of damaged fruits differed considerably between the orchards and years. Pooled data from all experiments for treatment Naturalis-L and control revealed significant influences of the factors treatment, orchard and year (three-way-Anova, data transformed [arcsine√(x)]; treatment: $F_{1, 49} = 20.68$, $p < 0.0001$; orchard: $F_{3, 49} = 13.19$, $p < 0.0001$; year: $F_{1, 49} = 4.12$, $p = 0.047$). Examining all experiments separately, Naturalis-L (applied in seven day intervals) significantly reduced the number of damaged fruits in all experiments showing more than 5% damage in the untreated control (figure 2). No significant differences could be detected in orchards with a low infestation level in control plots (Wintersingen and Sissach-Voregg in 2007). No significant differences were found between the control and the PreFeRal®WG treatment in Sissach-Isleten in 2006 or between the control and the Naturalis-L applied at 14 day intervals in Eptingen in 2007.

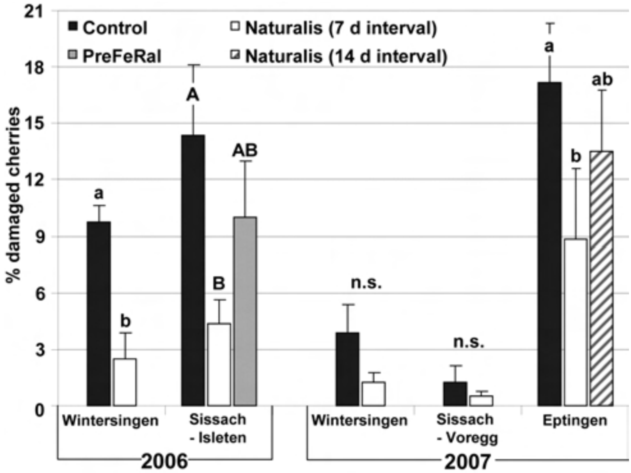

Figure 2: Percent of damaged cherries (± se) (Statistical analysis: data transformed [arcsine√(x)]; Wintersingen 2006: one-way Anova $F_{1, 8} = 16.63$, $p = 0.004$; Sissach-Isleten 2006: two-way Anova $F_{2, 15\,[treatment]} = 6.62$, $p = 0.009$, $F_{1, 15\,[variety]} = 9.60$, $p = 0.007$; Wintersingen 2007: one-way Anova $F_{1,8} = 1.67$, $p = 0.23$; Sissach-Voregg 2007: one-way Anova $F_{2, 9} = 0.26$, $p = 0.78$; Eptingen 2007: two-way Anova $F_{2, 16\,[treatment]} = 7.37$, $p = 0.005$, $F_{2, 16\,[variety]} = 21.05$, $p < 0.001$)

In four out of five experiments the efficacy (Abbott's formula) of Naturalis-L ranged between 62% and 74%. In the experiment in Eptingen (2007) the overall efficacy for Naturalis-L only reached 49%. In this experiment the different cherry varieties showed significantly different rates of damaged fruits: the number of damaged cherries in variety Schauenburger (23.6 ± 2.9% infested fruits) was significantly higher than in variety Langstieler (11.9 ± 6.0%) and Dolleseppler (6.4 ± 3.0%). Calculated for each variety separately, the efficacy of Naturalis-L was 78% in variety Langstieler, 73% in variety Dolleseppler, and 18% in variety Schauenburger. Fruit samples from the three varieties in Eptingen were taken at 7, 12 and 14 days after last application, respectively. Based on the data from all five experiments, a negative correlation was found between the efficacy and the days until harvest (Efficacy calculated according to Abbott; linear regression: [efficacy = 128.7 – 5.9 * days]; R^2 = 0.57; $F_{1,5}$ = 6.60; p = 0.05).

Discussion

Foliar applications of *Beauveria bassiana* (product Naturalis-L) in seven day intervals significantly reduced the number of damaged cherries by 60-70%. The results were obtained from five experiments in two years with considerably different weather conditions and in different orchards with different flight intensities of *R. cerasi*. The other treatments tested (PreFeRal®WG, application of Naturalis-L in 14 day intervals) were less effective. Nevertheless, the level of market tolerance (maximum 2% infested fruits) was exceeded. However, *R. cerasi* was not controlled in the experimental orchards for more than 10 years which led to a high infestation pressure. With consequent applications of Naturalis-L in succeeding years it might be possible to lower the population level below the economic threshold.

Flight activity monitored by yellow sticky traps was only slightly affected by treatments. This result is not surprising since flies remain active during 3 to 4 days post exposure and might be trapped during this time. An infection of flies under field conditions was shown to be possible.

In the experiment in Eptingen in 2007, the efficacy of Naturalis-L treatments in variety Schauenburger (18% efficacy) was considerably lower than in varieties Langstieler (78%) and Dolleseppler (73%). Most evidently, the low efficacy in variety Schauneburger was due to the expanded period between last application and harvest (14 days). A negative correlation was found between efficacy and number of days from last application until harvest. The shorter the time between last treatment and harvest, the better was the efficacy. This observation is supported by the distribution of larval stages found in variety Schauenburger: more than 50% of larvae found in Naturalis-L treated cherries were L_1 or L_2 stage, whereas only 4% of larvae in treated cherries of the variety Dolleseppler were in L_1 or L_2 stage. Therefore, applications until shortly before harvest seem necessary for a good efficacy. Application regime has to be improved and should not only be adapted to the flight period *of R. cerasi,* but also to the different the developmental stages of cherry varieties.

Climatic conditions have major impact on the efficacy of myco-pesticides. A temperature of 20 to 25 °C is considered to be the optimum for a development of entomopathogenic fungi. Above 35 °C and below 15 °C development is limited (Dimbi *et al*., 2004). In our field trials the temperature averaged 19.2 °C (2006) and 15.9 °C (2007). High levels of humidity are needed for germination of conidia and for sporulation on cadavers. The germination of conidia was found to be reduced at relative humidity levels less than 95% (Walstad *et al.*, 1970).

However, the microclimate is considered to be more important than the ambient environment. Shipp *et al*. (2003) assumed that a zone of high humidity (boundary layer) exists immediately next to the leaf surface facilitating conidia germination. In our laboratory trials a high infestation level was observed at 60% relative humidity in cages without host plants. Inglis *et al.* (2001) stated that sufficient moisture even exists within the microenvironment of the host's body surface. In our field trials the relative humidity averaged 68.1% (2006) and 81.0% (2007). Another crucial factor is sunlight (Daoust & Pereira, 1986). Especially UV-B radiation has a detrimental effect on micro-organisms (Smits *et al*., 1997). Leaf samples taken from experimental trees in 2006 and 2007 showed a rapid degradation of conidia: Within 24 hours after application 52 to 70% of the original inoculum was lost and 3 days after treatment 90 % was degraded. However, living fungal propagules could still be detected 14 days after treatment. In the experiments in 2006 a faster degradation of CFU per leaf was observed than in 2007. This might be due to the higher global radiation observed in the experimental period in 2006 (6.6 kWh / m^2 per day; 2007: 5.1 kWh / m^2 per day). No rain occurred in the leaf sampling period in 2006. Therefore, conidia were not washed off but, rather degraded by UV. Thus, the formulation of Naturalis-L is suitable to keep the conidia of *B. bassiana* viable during more than 7 days. Nevertheless, repeated applications seem appropriate.

Fungal propagules applied onto phylloplanes are exposed to pesticides. In organic agriculture only sulphur is likely to be applied during the critical period. This pesticide was found to be compatible with entomopathogenic fungi, whereas synthetical fungicides can be toxic to entomopathogenic fungi. Thus, the integration of myco-insecticides for cherry fruit fly control in an organic plant protection system might be possible.

Further research is needed to examine (1) if applications in subsequent years are able to lower the infestation at a tolerable level; (2) if the formulation of the entomopathogenic fungi might be improved and (3) if better adapted application regimes can increase efficacy.

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