Pilot field studies on insect pathogenic fungi to control mirid pests of apples in Norway

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Abstract

In a pilot field study conducted in an apple orchard in Hardanger (Western Norway) in two succesive years both ***Beauveria bassiana*** and Metarhizium anisopliae *were applied to apple trees just after hatching of mirid nymphs in spring. Both predatory, omnivorous and principally plant-pathogenic species of mirids were collected, and all groups of species were infected by* B. bassiana *or* M. ansiopliae *in treated plots.* B. bassiana *and* M. anisopliae *were also found on some individuals in non treated control plots the second year*. M. anisopliae *was more predominant in 2006,* B. bassiana *in 2007. Mirids were also observed for natural occurrence of parasitoids, and parasitoids were found both years.*

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, biological control, insect pathogenic fungi, Miridae, Norway, parasitoids

Introduction

Insect pathogenic fungi have shown to be effective in controlling important insect pests both in conservation biological control and as bio-based insecticides (Copping, 2004; de Faria & Wright, 2007). Earlier work has shown that several species of insect pathogenic fungi can cause death in mirids (Hemiptera: Miridae) (Liu *et al*., 2003; McGuire *et al*., 2006; de Faria & Wright, 2007).

Mirids are important pests in apple and pear production in both Europe and the United States (Wheeler, 2001; Alford, 2007). In organic production the degree of fruit damage can exceed 40 % (Røen *et al*., 2003). Mirid bugs feed on fruit sap in shoot tips, flower buds and fruitlets, resulting in deformation and stony pits in the fruit (Wheeler, 2001). Mirids damaging fruit are usually both polyphagous and omnivorous, and it can be difficult to assess whether a species should be considered as a pest or as a beneficial insect. To be able to use insect pathogenic fungi for the biocontrol of mirids it is important to evaluate their effects on fruit damage as well as against various species of mirids.

The objective of this study was to conduct a pilot study to evaluate the effect of field application of Norwegian isolates of the two insect pathogenic fungi *Beauveria bassiana* (NCRI 12/96) and *Metarhizium anisopliae* (NCRI 250/02) on (1) mortality and fungal growth in the most abundant mirid species found in the field, and (2) mirid damage on fruit.

Material and Methods

Trials were conducted over two successive years in an apple orchard in Hardanger, (Western Norway). Two different fields in the same orchard were used in 2006 and 2007. In both years experiments were set up as a randomized block design with five replications, and in each plot there were 3 trees cv ‘Aroma’, and a minimum of 2 border trees between each plot.

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The field used in 2006 was planted in 1994 and had a tree height of 2-2.5 m, whereas in 2007 a field planted in 1993 with a tree height 2.5-3 m was used. Both fields had a tree planting distance of 4.5 x 1.5 m. In both years no pesticides were used from the application of pathogens onwards until 2 weeks after the last sampling of insects.

The two isolates were grown in Petri dishes on potato dextrose agar (*B. bassiana*) and sabouraud dextrose agar (*M. anisopliae*) for 25 days at room temperature in the dark until good sporulation was obtained. About 15 ml autoclaved aqueous Tween solution (0.05%) was added to each Petri dish, followed by separation of spores from the mycelium with a sterile glass spatula. 25 ml of the spore suspension were then injected by a sterile syringe into a polypropylene bag (20 x 30 cm) with 200 g of Jasmine rice and 85 ml of water previously autoclaved for 5 minutes in 121 ^{o}C and cooled. Inoculated bags were stored at room temperature in the dark for at least 2 weeks and shaken gently every 2-3 days.

B. bassiana and *M. anisopliae* growing on rize were diluted in water and 0.05% Tween, poured through a sieve (mesh size 1 mm) to obtain a spore suspension whithout rize debris, and applied with a hand-held Hardy wheelbarrow sprayer until run-off. Samples from each suspension were collected to determine the applied spore concentration. Two applications were performed in both 2006 and 2007, the first just before flowering (first flower open) and the second at petal fall. Application time, application volume and concentration of spores (spores/ml and spores/ha) for both years are given in Table 1.

Table 1. Application time, volume (l/100 m row) and spore concentration (spores per ml and per ha) for first and second applications of *B. bassiana* and *M. anisopliae* in 2006 and 2007.

Year	Application time	Species	Spores/ml	l/ha	Spores/ha
2006	30 May	*B. bassiana*	6×10^{6}	1362	8×10^{12}
		M. anisopliae	6×10^{6}	1778	1×10^{13}
	12 June	*B. bassiana*	5×10^{6}	1680	8×10^{12}
		M. anisopliae	5×10^{6}	1778	1×10^{13}
2007	21 May	*B. bassiana*	7×10^{7}	2549	2×10^{14}
		M. ansiopliae	5×10^{7}	2351	1×10^{14}
	29 May	*B. bassiana*	2×10^{7}	2589	5×10^{13}
		M. anisopliae	3×10^{7}	2687	7×10^{13}

To investigate the incidence of fungal infection, insects were collected by beating three random branches on every tree in each plot with a stiff rubber hose both one and two weeks after the last spray application. Each branch received three quick beats, and dislodged insects were collected by a beating tray. All insects were identified and counted, but only mirids and *Anthocoris nemorum* (L.) were incubated. All mirids collected were nymphs, most of them in the third or fourth instar. Both adults and nymphs of *A. nemorum* were collected. Individual mirids were placed on an apple shoot embedded in 1.5 % water agar in a 25 ml vial. Mirids were incubated at 20°C, 70% relative humidity and 16 hours light in both years. Mortality was recorded daily? for seven days. Dead mirids were transferred to a new 25 ml vial with 1.5 % water agar for observation of fungal growth. Fungi were identified by observing characteristic fungal growth patterns on the mirids and by microscopic examination (x600). Mirids were also examined for parasitoids by observing for emerging larvae and by dissection of dead nymphs. Parasitoid species were not identified.

Data presented are numbers of infected mirids and *A. nemorum* in the different treatments. An analysis of variance for mirid damage on fruits was done with treatment and block as explanatory variables (SAS Institute, 2003).

Results

In treated plots mirids were found infected with *B. bassiana* and *M. anisopliae* in both years. In addition, some individuals in the control plots were infected in 2007 (Table 2 and 3). In 2006 only *Lygocoris pabulinus* (L.) and *Psallus ambiguus* (Fallén) were infected with *B. bassiana*, but only *P. ambiguus* also with *M. anisopliae* (Table 2). These two species were also the most abundant mirids in 2006. A higher number of mirids were found infected in the first sampling compared to the second sampling (data not shown). In 2006, the number of mirids with naturally occurring parasitoids was higher than mirids infected with fungi, and both *Orthotylus marginalis* (Reuter), *L. pabulinus* and *P. ambiguus* were found with parasitoids (Table 2).

Table 2: Total number of mirids and *Anthocoris nemorum* (N), and number of individuals infected by *Beauveria bassiana*, *Metarhizium anisopliae* or found with parasitoids in different treatments in 2006. Data from first and second samplings are combined.

Treatment	Species	N	Number with *B. bassiana*	Number with *M. anisopliae*	Number with parasitoids
Control	*Lygocoris pabulinus*	26	0	0	2
Control	*Orthotylus marginalis*	2	0	0	1
Control	*Psallus ambiguus*	17	0	0	6
Control	*Phytocoris* sp.	9	0	0	0
Control	*Anthocoris nemorum*	30	0	0	0
Beauveria	*Lygocoris pabulinus*	14	0	0	0
Beauveria	*Orthotylus marginalis*	0	-	-	-
Beauveria	*Psallus ambiguus*	16	4	0	1
Beauveria	*Phytocoris* sp.	18	0	0	0
Beauveria	*Anthocoris nemorum*	30	0	0	0
Metarhizium	*Lygocoris pabulinus*	21	0	1	2
Metarhizium	*Orthotylus marginalis*	4	0	0	1
Metarhizium	*Psallus ambiguus*	25	0	7	4
Metarhizium	*Phytocoris* sp.	18	0	0	0
Metarhizium	*Anthocoris nemorum*	26	0	0	0

The degree of infection by *B. bassiana* and *M. anisopliae* was generally higher in 2007 than in 2006 (Table 3). All of the presented insect species were found to be infected with both fungi in 2007. A *B. bassiana* infection was found in plots treated with *M. anisopliae*, and in the second sampling infection by *B. bassiana* was higher than *M. anisopliae* in plots treated with *M. anisopliae* (data not shown). One species, *P. ambiguus,* was found to be infected with *M. anisopliae* in plots treated with *B. bassiana*. Further, infections with both pathogens were found in the control. The number of insects carrying parasitoids was low in 2007, only *P. ambiguus* parasitism.

Treatment with the selected entomopathogens did not reduce crop damage by mirids in this study. Neither treatment nor block had a significant effect on damage in either year (Table 4) (2006: df = 6, 8, F = 1.14, p = 0.42; 2007: df = 6,8, p = 1.47, p = 0.30).

Table 3: Total number of mirids and *Anthocoris nemorum*, and number infected by *Beauveria bassiana*, *Metarhizium anisopliae* or found with parasitoids in different treatments in 2007. Data from first and second samplings are combined.

Treatment	Species	N	Number with *B. bassiana*	Number with *M. anisopliae*	Number with parasitoids
Control	*Lygocoris pabulinus*	5	1	0	0
Control	*Orthotylus marginalis*	4	0	0	0
Control	*Psallus ambiguus*	35	3	0	2
Control	*Phytocoris* sp.	11	0	1	0
Control	*Anthocoris nemorum*	9	0	0	0
Beauveria	*Lygocoris pabulinus*	7	2	0	0
Beauveria	*Orthotylus marginalis*	6	3	0	0
Beauveria	*Psallus ambiguus*	20	11	1	0
Beauveria	*Phytocoris* sp.	12	9	0	0
Beauveria	*Anthocoris nemorum*	12	3	0	0
Metarhizium	*Lygocoris pabulinus*	0	-	-	-
Metarhizium	*Orthotylus marginalis*	8	4	1	0
Metarhizium	*Psallus ambiguus*	36	3	11	5
Metarhizium	*Phytocoris* sp.	9	0	1	0
Metarhizium	*Anthocoris nemorum*	15	3	4	0

Table 4: Percent capsid damage on apples at harvest in 2006 and 2007. 100 apples per plot were investigated for damage (n = 5).

Year	Treatment	Total number of apples	Average % damage
2006	Control	500	1.4
	B. bassiana	500	2.0
	M. anisopliae	500	1.0
2007	Control	500	19.4
	B. bassiana	500	15.8
	M. anisopliae	500	13.0

Discussion

Both *M. anisopliae* and *B. bassiana* were found to infect the species of mirids collected in this trial. *M. anisopliae* was predominant in 2006, whereas in 2007 *B. bassiana* was more common. These differences might be caused by a higher concentration of *M. anisopliae* spores at both applications in 2006 and a higher concentration of *B. bassiana* spores in the first application in 2007. Generally, a higher degree of infection and more species being infected in 2007 might be caused by a higher concentration of spores and a higher spray volume used in that year. Fungal infections were also found in control plots in 2007. This might indicate a natural infection of *B. bassiana* and *M. anisopliae* in the field, although a more likely explanation is that spores were transported by wind or water during spraying or by flying insects shortly after application. In future work, distance between plots should be increased to rule out these factors. More work is needed to investigate the natural fungal infection on mirids in Norway.

Predatory species (*A. nemorum* and *Phytocoris sp*.), omnivorous species (*O. marginalis* and *P. ambiguus*) and clearly pest species (*L. pabulinus*) were infected in this study. As far as we know, previous work on the effect of *B. bassiana* and *M. anisopliae* on mirids has focused on pest species (Liu *et al*., 2003; McGuire *et al*, 2006).

However, to evaluate the net benefit of a pathogen as a control method against insects, the effect on both pests and beneficials are needed.

The mirid *P. ambiguus* seem to be susceptible to both fungal isolates tested. Based on our data it also seemed to be more attractive to naturally occurring parasitoids. Both *P. ambiguus* and *O. marginalis* are mainly regarded as predators, but might occasionally cause damage because they are able to feed on plants (Taksdal, 1983; Wheeler, 2001; Edland, 2004). However, it is likely that *P. ambiguus* is causing fruit damage more often than assumed earlier. Unpublished data show that number of *P. ambiguus* is positively correlated to damage in apples (G. Jaastad, N. Trandem, S. Mogan & B. Hovland, unpublished). Further, Taksdal (1983), showed that *P. ambiguus* can cause stony pits in pears in an isolation bag trial. Lastly, in this study more individuals of this species were found in 2007 (total of 93) compared to 2006 (total of 52), and more damage was recorded in 2007 compared to 2006.

Treatment with *B. bassiana* and *M. anisopliae* did not affect the degree of damage caused by mirids in this study. Visual damage by mirids is dependent on the time-point of feeding during the phenological development of fruits. According to a review by Wheeler (2001) mirid feeding on flowers and fruitlets between the bloom stage and two weeks after petal fall is most likely to cause visible damage on apples. Thus, if mirids do not die shortly after they have become infected by fungi, damage might occur. In summary, this study shows that both beneficial, omnivorous and pest species of mirids can be infected by *M. anisopliae* and *B. bassiana* after field application. However, a clear effect of pathogen infection on mirid damage to apples was not found. More work on insect pathogenic fungi and mirids in fruit orchards is needed to evaluate their potential effect as control agents against this group of pests.

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