Beauveria bassiana strain ATCC 74040 (Naturalis®), a valuable tool for the control of the cherry fruit fly (*Rhagoletis cerasi*)

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Abstract

Naturalis® is a bioinsecticide based on living conidiospores of the naturally occuring Beauveria bassiana *strain ATCC 74040. The entomopathogenic fungus acts primarily by contact: once attached to the insect's cuticle, the conidiospores germinate producing penetration hyphae, which enter and proliferate inside the insect's body. The fungus invades and feeds on its host, causing its death due to dehydration and/or depletion of nutrients. Several years of laboratory, semi-field and field studies showed that also Tephritid flies (*Ceratitis capitata*,* Rhagoletis cerasi*,* Bactrocera oleae*) are susceptible to infection by B. bassiana strain ATCC 74040. The results of efficacy trials conducted in 2004-05 are reported. Naturalis was tested both alone and in an integrated pest management strategy. The product showed high efficacy in controlling* R. cerasi*, comparable to or higher than that of the chemical reference treatment. The* B. bassiana*-based product Naturalis can thus be considered an efficient tool for the control of the cherry fruit fly.*

Keywords: *Beauveria bassiana*, *Rhagoletis cerasi*, cherry, organic farming, integrated pest management

Introduction

Beauveria bassiana (Balsamo) Vuillemin (Deuteromycetes, Moniliales) is an entomopathogenic fungus, recognized in 1835 by Agostino Bassi as the causal agent of the white muscardine disease of the silk worm. It can affect a wide range of arthropod pests, such as coleopterans, mites, and hemipterans, and all their developmental stages, but different *B. bassiana* strains differ in their host range (Knauf, 1992; Lacey *et al*., 1999; Mayoral *et al*., 2006; Talaei-Hassanloui *et al*., 2007).

Naturalis® is a bioinsecticide based on living conidiospores of the naturally occuring *B. bassiana* strain ATCC 74040, isolated from *Anthonomus grandis* (Boheman), the cotton boll weevil, in the Lower Rio Grande valley, Texas, USA (BCPC, 2004). The formulated product contains at least 2.3 x 10^7 viable spores / ml. Since the bioinsecticide is a dispersion of living conidiospores in vegetable oil, it can be used also in organic farming. In 2005, Intrachem Bio International S.A. (Geneva, Switzerland) acquired the intellectual property of Naturalis from Troy Biosciences, and the commercial product is now manufactured in Europe by Intrachem Production S.r.l (Grassobbio, Italy).

B. bassiana strain ATCC 74040 acts primarily by contact: once attached to the insect's cuticle, the conidiospores germinate producing penetration hyphae, which enter and proliferate inside the insect's body. The fungus feeds on its host, causing its death due to dehydration and/or depletion of nutrients.

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Infection can take between 24 and 48 hours, depending on the temperature (BCPC, 2004). The proliferation of the fungus inside the insect's body leads to the insect's death within 3-5 days.

Several years of laboratory, semi-field and field studies showed that also Tephritid flies, *Ceratitis capitata* Wiedemann, *Rhagoletis cerasi* Loew, and *Bactrocera oleae* (Gmelin), are susceptible to infection by *B. bassiana* strain ATCC 74040 (Ladurner *et al*., 2007; Daniel *et al*., 2008). The European cherry fruit fly, *R. cerasi*, is the major pest on sweet cherries throughout Europe and in parts of temperate Asia (Fischer-Colbrie & Busch-Petersen, 1989). If left uncontrolled, severe fruit damage may occurr, especially on medium to late ripening cherry varieties. Furthermore, *R. cerasi* control is becoming increasingly challenging not only in organic and in integrated farming, but also in conventional production due to ecotoxicological and residue issues (Kovanci & Kovanci, 2006), and microbial control agents may thus represent valuable tools for the containment of this pest.

The two trials conducted in 2004-05 aimed at verifying the efficacy of the *B. bassiana*-based bioinsecticide Naturalis, applied both alone and in an integrated pest management strategy, in controlling the cherry fruit fly on sweet cherry in the open field.

Material and Methods

The trials were conducted in 2004-2005 by a certified testing facility (G.Z. S.r.l., Italy) in Pavullo (Modena, Italy) in a 5-6-year old cherry cv Durone Nero orchard. Distance between rows and plants was 4 m, and plant height was approximately 2.5 m. The efficacy in reducing the percentage of fruits damaged by *R. cerasi* of the *B. bassiana*-based bioinsecticide Naturalis, applied alone at two different rates (130 and 250 ml/hl, respectively) and in an integrated pest management strategy, was compared to that of an untreated control and of a chemical reference insecticide. The different treatments, the number of applications per treatment, and the application dates are reported in Table 1.

Table 1: Treatments, number of applications per treatment, and applications dates in the trials conducted in 2004-2005 (applied spray volume: 1500 l/ha).

Treatment no.	Active substance	Formulated product (% a.i.)	Applied rate (ml/hl)	No. applic.s	Application dates (dd/mm)
		Trial no. 1-2004			
1	*B. bassiana* strain ATCC 74040	Naturalis (7.16%)	130	5	28/05, 04/06, 11/06, 17/06, 23/06
2	*B. bassiana* strain ATCC 74040	Naturalis (7.16%)	250	3	28/05, 11/06, 23/06
3	Dimethoate + *B. bassiana* strain ATCC 74040	Rogor L40 (38%) + Naturalis (7.16%)	50 + 130	1 + 2	28/05 17/06, 23/06
4	Dimethoate	Rogor L40 (38%)	50	1	04/06
5	Untreated control	-	-	-	-
		Trial no. 2 - 2005			
1	*B. bassiana* strain ATCC 74040	Naturalis (7.16%)	130	5	07/06, 13/06, 17/06, 22/06, 28/06
2	*B. bassiana* strain ATCC 74040	Naturalis (7.16%)	250	3	07/06, 17/06, 28/06
3	Dimethoate + *B. bassiana* strain ATCC 74040	Rogor L40 (38%) + Naturalis (7.16%)	50 + 130	1 + 2	07/06 22/06, 28/06
4	Dimethoate	Rogor L40 (38%)	50	1	13/06
5	Untreated control	-	-	-	

Treatments were applied from beginning of fruit colouring (BBCH 81; target stage: mostly eggs) to fruit ripe for consumption (BBCH 89; target stage: mixed). To compare the different treatments, a fully randomized block design was used with 4 replicates per treatment, and with 2 trees per plot.

To assess percent fruit damage at harvest (28/06/04 and 30/06/05), in each plot, 5 kg of cherries were harvested, damaged and healthy fruits were counted, and the percentage of fruits damaged by *R. cerasi* was determined. Furthermore, the efficacy according to Abbott (1925) of the different treatments in reducing fruit damage at harvest was calculated.

In each trial, the percentage of damaged fruits at harvest was compared across treatments using one-way ANOVA, followed by the Student-Newman-Keuls test for posthoc comparison of means. Levene's test was used to test for homogeneity of variances.

Results

In both trials significant differences among treatments in the percentage of fruits damaged by the cherry fruit fly emerged (Table 2): fruit damage was always significantly higher in the untreated control plots than in the treated plots. In 2004 no significant differences among treated plots were recorded, while in 2005 fruit damage was significantly lower in the plots treated with Naturalis at 130 ml/hl, with the IPM strategy, and in those treated with the reference insecticide dimethoate than in those treated with Naturalis at 250 ml/hl.

The mean efficacy of Naturalis applied at 130 ml/hl ranged from 87 to 90%, and was always comparable to that of the IPM strategy (80-89%), and higher than or comparable to that of the chemical reference product applied alone (39-90%) (Table 2).

Table 2: Percent fruit damage in the different treatments and trials and efficacy according to Abbott of the different treatments in reducing fruit damage at harvest. Different letters within the same column and trial indicate statistically significant differences (SNK-test: $P<0.05$).

Treatment no.	Active substance	Applied rate (ml/hl)	No. applic.s	Fruit damage (%) (m±s.d.)	Efficacy (%) (m±s.d.)
			Trial no. 1-2004		
1	*B. bassiana* strain ATCC 74040	130	5	0.6 ± 0.6 b	88.6 ± 11.2
2	*B. bassiana* strain ATCC 74040	250	3	1.5 ± 0.8 b	72.9 ± 16.7
3	Dimethoate + *B. bassiana* strain ATCC 74040	50 + 130	1 + 2	1.2 ± 0.4 b	80.4 ± 5.4
4	Dimethoate	50	1	3.2 ± 1.6 b	39.0 ± 36.9
5	Untreated control	-	-	6.1 ± 2.0 a	-
			Trial no. 2 - 2005		
1	*B. bassiana* strain ATCC 74040	130	5	1.3 ± 0.7 c	90.4 ± 5.1
2	*B. bassiana* strain ATCC 74040	250	3	4.4 ± 1.4 b	66.6 ± 9.9
3	Dimethoate + *B. bassiana* strain ATCC 74040	50 + 130	1 + 2	1.6 ± 1.2 c	88.5 ± 8.2
4	Dimethoate	50	1	1.2 ± 1.0 c	90.6 ± 8.4
5	Untreated control	-	-	13.2 ± 1.3 a	

Discussion

In none of the trials a significant dose response effect was observed for the *B. bassiana*-based bioinsecticide. In both trials, instead, the efficacy of the product applied alone in reducing fruit damage was higher when it was used at a low rate (130 ml/hl) at 5-7-day intervals (5 applications in total) than when it was applied at a higher rate (250 ml/hl) at 10-14-day intervals (3 applications in total). Germination ability and spore viability of different *B. bassiana* strains are strongly linked to their tolerance to UV exposure (Bidochka *et al*., 2002; Fernandes *et al*., 2007), and conidial survival in epigeal habitats decreases over time due to exposure to UV-radiation (Inglis *et al*., 1993, 1995; Ulevicius *et al*., 2004). The results indicate that, for an effective control of this target pest, not exceeding the time interval of 7 days between successive applications is more important than increasing the field rate of the bioinsecticide. In order to efficiently control *R. cerasi* with Naturalis alone, the product should thus be applied from the beginning of fruit colouring up to harvest (period of highest susceptibility of sweet cherry to cherry fruit fly attack), at a recommended rate of approx. 130 ml/hl and at a 5-7-day time interval between applications.

In 2004, the IPM strategy (one application of the standard reference product followed by two applications of Naturalis at 130 ml/hl at a 6-7-day interval just prior to harvest) provided good and consistent control of *R. cerasi* (efficacy: 80.4±5.4%), while the dimethoate-based product showed high variability in suppressing cherry fruit fly damage (efficacy: 39±36.9%). In this trial, for the chemical reference treatment, the time interval between last application and harvest was 24 days. Pre-harvest interval instructions for the active ingredient dimethoate were followed (20 days on cherry in Italy in 2004-05), but cherry fruit fly control was highly variable among plots, and can thus not be considered satisfactory. This is probably due to the fact that close to harvest dimethoate levels on the vegetation were not sufficient any more to provide adequate and consistent crop protection.

In 2005, the efficacy of the chemical reference treatment was comparable to that of the IPM strategy, but pre-harvest interval requirements were not met (time interval between last application and harvest: 17 days), which translates to a high probability of undesired residues in fruit. Naturalis, instead, does not have any pre-harvest interval restrictions and minimum detectable residue level. The bioinsecticide can therefore be used up to harvest without any risk of undesired residues in the final production.

In conclusion, the *B. bassiana*-based bioinsecticide, applied both alone and in an IPM strategy, showed high efficacy in reducing *R. cerasi* fruit damage on sweet cherry. The product can be considered an important and valuable tool for the control of this target pest not only in organic farming but also in any other pest management strategy, because it shows good efficacy in suppressing cherry fruit fly infestations and because it can help to avoid the presence of inadequate levels of residues in fruit.

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