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Evaluation of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 for the management of *Ceratitis capitata*

Salvatore Ortu, Arturo Cocco, Riccardo Dau
Dipartimento di Protezione delle Piante - Sezione Entomologia agraria, Università degli Studi di Sassari, Italy

Abstract

The effectiveness of the bioinsecticide Naturalis based on the *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina Hyphomycetes) strain ATCC 74040 against the medfly *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) was evaluated in laboratory and field experiments. Under laboratory conditions, the bioinsecticide (150 ml/l) protected fruits from medfly ovipositions more effectively when they were covered uniformly with a spray volume of 5.4 ml/fruit. In laboratory choice bioassays medfly females laid respectively 5 and 3 times more eggs on untreated fruits and on fruits sprayed with inert co-formulants of the bioinsecticide than on fruits treated with the bioinsecticide. However, females did not prefer to oviposit on untreated fruits over fruits sprayed with co-formulants, suggesting that inert co-formulants did not affect oviposition behaviour of *C. capitata* females. Adults landed indifferently on both untreated fruits and fruits sprayed with the bioinsecticide and medflies did not appear to be repelled by chemical compounds or fungal structures of *B. bassiana*. However, in no-choice laboratory tests significantly more punctures per fruit were observed on untreated fruits than on fruits treated with the bioinsecticide, suggesting that medfly females prefer untreated over treated fruits to effectively oviposit. Preliminary field trials were conducted to confirm the effectiveness of the *B. bassiana*-based bioinsecticide for medfly control. The bioinsecticide was as effective as a pyrethroid in reducing adult medfly populations and protecting orange fruits in the field. However, our results suggest that the bioinsecticide could be a valuable additional tool for the management of *C. capitata* in both integrated and organic groves.

Key words: biological control, Mediterranean fruit fly, citrus, entomopathogenic fungus, peach, apple.

Introduction

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) is a key pest of fruits in the Mediterranean basin. The medfly is distributed worldwide and attacks more than 250 species of fruits and vegetables (Morales *et al*., 2004), causing large economic losses. Its economic importance is due to the direct damage caused by females that oviposit into the host fruit and larvae feeding on the fruit flesh. In addition to direct losses, severe quarantine policies are imposed by importing countries to avoid importation and establishment of exotic pests. This tephritid fly finds optimal climatic conditions in the warmer areas of the Mediterranean basin, mainly in heterogeneous agricultural landscapes with mixed-fruit groves where ovipositing females can find host fruits to lay eggs throughout the year.

The repeated use of conventional insecticides against *C. capitata* larvae and adults could cause serious ecological problems, including detrimental effects on non-target arthropods, environmental pollution, food contamination, and development of insecticide resistance (Magaña *et al*., 2008). These problems have raised concern about the sustainability of chemical approach for pest control. Recently, ecologically compatible strategies to manage *C. capitata* populations were developed, including the use of entomopathogenic viruses, fungi, nematodes, protozoa, and bacteria as biological control agents (Castillo *et al*., 2000; Lacey *et al*., 2001).

More than 700 species of fungi have been reported to be entomopathogenic (Hajek and St. Leger, 1994). Under laboratory and greenhouse conditions, various isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina Hyphomycetes) were active against adults and pupae of *C. capitata* (Lacey *et al*., 2001; Ekesi *et al*., 2002; Dimbi *et al*., 2003; Ekesi *et al*., 2005; Konstantopoulou and Mazomenos, 2005; Quesada-Moraga *et al*., 2006; Almeida *et al*., 2007). *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Deuteromycotina Hyphomycetes) has been reported to reduce fecundity and fertility of the Mediterranean fruit fly (Castillo *et al*., 2000). Some commercial products based on different strains of these fungal species are currently available for biological insect control (Lacey *et al*., 2001). However, most published studies report the susceptibility of arthropod pests to wild strains of *B. bassiana*, for which limited information on the modes of action are available. Furthermore, few experiments to evaluate the pathogenicity of *B. bassiana* to insect pests under grove conditions have been conducted (Santamaria *et al.*, 1998; Godonou *et al.*, 2000).

The aims of the present study were to investigate the effectiveness of a commercial product based on *B. bassiana* strain ATCC 74040 against adults of *C. capitata* under laboratory conditions, and to preliminarily evaluate whether the fungus might have indirect mode(s) of action in reducing the oviposition rate of the medfly on treated fruits. In addition, we aimed at evaluating the efficacy of *B. bassiana* strain ATCC 74040 against adults of the Mediterranean fruit fly under grove conditions. This strain has been tested against the cherry fruit fly, *Rhagoletis cerasi* L., and the olive fly, *Bactrocera oleae* (Gmelin) (Diptera Tephritidae) (Anagnou-Veroniki *et al*., 2005; Benuzzi *et al*., 2007; Ladurner *et* *al.*, 2008). The mechanism by which this entomopathogenic strain is toxic to *C. capitata* has not yet been adequately investigated. Benuzzi and Santopolo (2001) report that *B. bassiana* strain ATCC 74040 acts mainly by contact, infesting the host through the cuticle and thereby causing its death by dehydration or depletion of nutrients, rather than by producing mycotoxins such as beauvericin, bassianolide, or destruxin.

Materials and methods

Laboratory experiments

Insects

The adults of *C. capitata* were obtained from a stock colony maintained at the Department of Plant Protection, University of Sassari, Italy, at 25 ± 1 °C, 65% RH, under a 14:10 (L:D) photoperiod. Larvae were reared on a diet of water (50.5%), sucrose (16.2%), bran (24.2%), torula yeast (8.0%), citric acid (0.6%), and benzoic acid (0.5%). Adults were provided with water and a solid diet consisting of sucrose, yeast, and enzymatic yeast hydrolysate in a 6:2:1 ratio (Cavalloro and Girolami, 1969).

Evaluation of different bioinsecticide spray volumes

Prior to conducting choice and no-choice tests we verified whether the applied spray volume and thus percent fruit surface coverage with the spray solution would affect the efficacy of the bioinsecticide in reducing fruit infestation. The experiment was carried out at the Department of Plant Protection, University of Sassari, Italy, in a climatic room maintained at 23.5 °C, 65% RH, under a 14:10 (L:D) photoperiod. "Tarocco" oranges from an organic citrus grove were harvested with their peduncle and placed on top of a plastic cylinder (6 cm diameter, 3 cm height) as a pedestal to facilitate their handling after the bioinsecticide application. Pre-existing oviposition punctures on fruits and/or skin damage that could facilitate *C. capitata* egg oviposition during the bioassay were marked under a stereomicroscope (Papaj *et al.*, 1989).

Three spray volumes of the bioinsecticide based on the *B. bassiana* strain ATCC 74040 (Naturalis, Intrachem Bio Italia S.p.A.) at the recommended highest label rate (HLR) (150 ml/hl) were tested: 1.8, 5.4, and 9 ml/fruit, covering respectively 60, 90, and 100% (until run-off) of the skin surface. Fruits were sprayed from a distance of ca. 40 cm using a hand-held sprayer and air-dried for about 1 h. One fruit per treatment was placed inside a Plexiglas cage (30 x 30 x 30 cm) provided with 2 mesh-covered windows (27 x 27 cm) to allow displacement of fruits and good air flow. Fruits were placed on vertexes of an ideal triangle and were not moved during the experiment to avoid the risk of medflies flying away from the cage. Treatments were replicated in 5 different cages. Five ovipositing *C. capitata* females and 2 males at an age of approx. 10 days were aspirated from the stock colony with a manual aspirator and released inside the cages for 48 h. Adults were fed with water and the same diet used for the stock colony. At the end of the bioassay, medfly punctures were scored under a stereomicroscope and dissected with a scalpel to confirm egg presence.

Choice tests

After the laboratory test described above had indicated that the optimal spray volume of the bioinsecticide solution was 5.4 ml/fruit, several laboratory choice tests were conducted to investigate the effectiveness and the mode of action of the bioinsecticide by comparing the following paired treatments:

Choice test 1 (bioinsecticide versus untreated control):
The volume of 5.4 ml/fruit of bioinsecticide at the recommended HLR (150 ml/hl) and an untreated control were compared. Protocol and climatic conditions used for Choice test 1 were the same as described above for the evaluation of different spray volumes, except that fruits were sprayed using a different hand-held sprayer for each treatment and treatments were replicated 3 times in 6 cages. Furthermore, in order to investigate whether the *B. bassiana*-based commercial product had a repellent effect on *C. capitata* females, behavioural observations were carried out during Choice test 1. The number of medfly females that landed on treated and untreated fruits for at least 5 sec was recorded by visual inspection of the cages. Observations were repeated 29 times during the bioassay with time intervals of at least 30-45 min;

Choice test 2 (bioinsecticide versus co-formulants):
The volume of 5.4 ml/fruit of bioinsecticide at the recommended HLR (150 ml/hl) and 5.4 ml/fruit of co-formulants (a blend of vegetable oils used as inert ingredients) (150 ml/hl) were compared, using "Royal Gala" apples;

Choice test 3 (inert co-formulants versus untreated control):
The volume of 5.4 ml/fruit of co-formulants (150 ml/hl) and an untreated control were compared, using "Naveline" oranges.

The same protocol used for the Choice test 1 was used in Choice test 2 and 3, except that after 24 h fruits were inspected under a stereomicroscope and oviposition punctures were marked. Afterward, fruits were placed again inside the cages for additional 24 h. At the end of the bioassays, fruits were examined under a stereomicroscope and punctures were dissected with a scalpel to verify egg presence. The 24 h inspection allowed to determine whether *C. capitata* punctures were done within 24 h (*period 1*) or between 24 and 48 h (*period 2*).

The experimental design intended to (i) reduce possible chemical cue interferences that could modify the medfly behaviour, (ii) point out potential side effects of bioinsecticide co-formulants, and (iii) evaluate the efficacy of the bioinsecticide in reducing the number of punctures/fruit on different *C. capitata* hosts.

No-choice test

To test the responses of *C. capitata* females to *B. bassiana*-treated fruits, the medfly-ovipositing behaviour was investigated without choice options on fruits treated with the bioinsecticide and untreated fruits. No-choice conditions are stricter than choice conditions and might be useful to evaluate the effectiveness of the bioinsecticide against the medfly.

The protocol previously described for Choice tests 2 and 3 was used, except that "Redhaven" peaches were used and treatments (5.4 ml/fruit of bioinsecticide at the HLR [150 ml/hl] versus untreated control) were put in cages placed side by side. Each treatment was replicated 3 times in 4 different cages.

Field experiments

Field experiments were conducted in a clementine (*Citrus clementina* Hort. ex Tan.) grove in Posada (Italy), a northeastern Sardinia citrus area (40°38'26"N, 9°42'15"E) under climatic and agronomic conditions favorable to *C. capitata*-population buildup (Ortu, 1995). Moreover, clementine fruits are very susceptible to medfly attack, due to their early ripening time and their thin skin. The experimental grove consisted of two clementine plots (later defined as "biological" plot and "treated" plot) of 1000 m^2 each with 5 adjacent rows of 12 trees each, separated by a row of olive trees. The trees were 18 years old and spaced 6 m apart between the rows and 3 m within the rows.

To evaluate the efficacy of the *B. bassiana*-based compound against *C. capitata* adults, (A) weekly sprays (starting on October 10, 2005) with the commercial product at the recommended HLR (150 ml/hl) and (B) sprays of a pyrethroid (esfenvalerate, Sumialfa, Basf Agri) at 30 ml/hl were compared.

The bioinsecticide application carried out on November 17 was washed off by severe rainfalls and therefore repeated on November 21. Treatment B was used as chemical reference treatment because growers in that area used this product as a standard strategy to control *C. capitata* in clementine groves. The grower applied the pyrethroid autonomously on October 10, 22, and 30, 2005. To ensure thorough wetting of plants, a spray volume of 600 l/ha was used for each treatment application.

Population dynamics of *C. capitata* during the experiment were monitored with white plastic discs (26 cm in diameter) baited with liquid glue and 2.5% of liquid trimedlure (Ortu, 1995). Three traps were placed in each plot 1.5 m above ground in the exterior of the tree canopy. Traps were replaced weekly and the number of adults captured per trap was recorded.

A pre-treatment sample of 50 fruits was randomly collected from the central rows of each plot to assess the infestation of *C. capitata* at the beginning of the experiment. Fruits were inspected under a stereomicroscope and the number of infested fruits was recorded. Subsequently, on October 27, November 9 and 22, the infestation levels on fruits were evaluated using the same methodology as described above for the pre-treatment assessment.

Data analysis

All statistical analyses were performed using the statistical software SAS (SAS Institute, 2002). For the evaluation of different bioinsecticide spray volumes, data were checked for homogeneity of variances using Bartlett's test. Then, the number of punctures/fruit was compared across treatments using one-way analysis of variance (ANOVA) (PROC GLM), followed by the Fisher's Least Significance Difference (LSD) test for post-hoc comparison of means ($P < 0.05\%$).

The number of medfly punctures and landings per fruit on choice and no-choice tests were preliminarily analyzed with Mann-Whitney *U* test, a non parametric test, using PROC NPAR1WAY to evaluate potential significant differences among cages. Then, if no differences were observed, treatment means were pooled and compared with the same test. The non parametric test was used to avoid assumptions on normal distribution and equal variances for the observations (Steel and Torrie, 1980).

Differences in fruit infestation between plots were evaluated using exact logistic regression because some observations had zero value or separation of the data set occurred (PROC LOGISTIC) (Heinze and Ploner, 2003).

Results and discussion

Laboratory experiments

Evaluation of different bioinsecticide spray volumes

Orange fruits sprayed with the lowest volume of the bioinsecticide (1.8 ml/fruit) were significantly more vulnerable than fruits treated with 5.4 or 9 ml/fruit of bioinsecticide ($F = 10.98$; $df = 2$; $P = 0.0019$) (table 1). However, increasing the bioinsecticide volume from 5.4 to 9 ml/fruit did not significantly decrease the number of punctures per fruit, suggesting that the volume of 5.4 ml/fruit appears to be best from an ecological and economic point of view. Dissection of fruits with the scalpel revealed that 90% of punctures contained eggs. The statistical analysis on the number of punctures with eggs/fruit confirmed results previously obtained for the number of punctures/fruit ($F = 9.40$; $df = 2,4$; $P = 0.0079$) (table 1).

Table 1. Mean total number of *C. capitata* punctures/fruit and mean number of *C. capitata* punctures with eggs/fruit on "Tarocco" oranges sprayed with different volumes of the *B. bassiana*-based bioinsecticide solution.

Treatment	Mean total no. of punctures/fruit (mean ± SE)	Mean no. of punctures with eggs/fruit (mean ± SE)
Volume 1 (1.8 ml/fruit)	4.0 ± 0.6 a	3.8 ± 0.7 a
Volume 2 (5.4 ml/fruit)	0.6 ± 0.4 b	0.6 ± 0.4 b
Volume 3 (9.0 ml/fruit)	1.4 ± 0.6 b	1.0 ± 0.6 b

Treatments means were analyzed using PROC GLM, significant differences were separated using Fisher's LSD test ($P < 0.05$). Treatments followed by the same letter within the same column are not significantly different.

Table 2. Mean number of *C. capitata* punctures and landings per fruit on untreated "Tarocco" oranges and on oranges sprayed with the *B. bassiana*-based bioinsecticide under choice laboratory conditions.

Treatment	Mean total no. of punctures/fruit (mean ± SE)	Mean no. of punctures with eggs/fruit (mean ± SE)	Mean no. of female landings/fruit (mean ± SE)
Untreated	1.1 ± 0.3 a	1.0 ± 0.3 a	0.10 ± 0.01 a
Bioinsecticide	0.2 ± 0.1 b	0.2 ± 0.1 b	0.07 ± 0.01 a

Treatments means were analyzed with Mann-Whitney *U* test (PROC NPAR1WAY), values followed by the same letter within the same column are not significantly different ($P < 0.05$).

Choice tests

Choice test 1 (*C. capitata* oviposition preference on "Tarocco" oranges treated with the bioinsecticide over untreated fruits):

The preliminary Mann-Whitney *U* test showed that differences among cages were not significant, so the data were pooled. The mean number of oviposition punctures/fruit was significantly higher on untreated fruits than on fruits sprayed with the bioinsecticide (1.1 versus 0.2 punctures/fruit) (χ^2 = 6.8384; *df* = 1; *P* = 0.0089) (table 2). Fruit examination under the stereomicroscope revealed that 95% of punctures on untreated fruits contained eggs, while the proportion decreased to 75% on fruit sprayed with the bioinsecticide.

During Choice test 1, behavioural observations indicated that *C. capitata* females landed more frequently on untreated fruits (0.10 females/fruit/observation) than on fruits sprayed with the bioinsecticide (0.07 female/fruit/observation), but differences were not significant (Mann-Whitney *U* test: χ^2 = 1.5557; *df* = 1; *P* = 0.2123) (table 2).

Choice test 2 (*C. capitata* oviposition preference on "Royal Gala" apples treated with the bioinsecticide over fruits treated with co-formulants):

During the 48-h experiment, few punctures were observed both on apple fruits treated with the bioinsecticide and with the co-formulants. After 24 h, only 3 punctures were observed on fruits sprayed with co-formulants, while no fruits treated with the bioinsecticide were attacked (figure 1A). However, there was no significant difference between the treatments (χ^2 = 3.1818; *df* = 1; *P* = 0.0745). At the end of the bioassay, 26 more punctures were observed on co-formulant-treated fruits for a total of 29 oviposition wounds and a mean of 1.7 punctures/fruit, which was significantly higher than the mean punctures detected on fruits treated with the bioinsecticide (0.5 punctures/fruit) (χ^2 = 9.8192; *df* = 1; *P* = 0.0017). When examined under the stereomicroscope at the end of the test, all punctures revealed the presence of eggs.

Choice test 3 (*C. capitata* oviposition preference on "Naveline" oranges treated with co-formulants over untreated fruits):

Medfly females attacked untreated fruits a total of 60 times (3.3 punctures/fruit), while a total of 46 punctures were observed on fruits treated with co-formulants (2.6 punctures/fruit). However, the difference between treatments was not significant (χ^2 = 2.7861; *df* = 1; *P* = 0.0951) (figure 1B). In both *period 1* (0-24 h) and *period 2* (24-48 h), fruits treated with co-formulants showed less punctures (0.9 and 1.6 punctures/fruit, respectively) than untreated fruits (1.3 and 1.9 punctures/fruit, respectively), but differences were not significant (*period 1*: χ^2 = 1.8506; *df* = 1; *P* = 0.1737; *pe-*

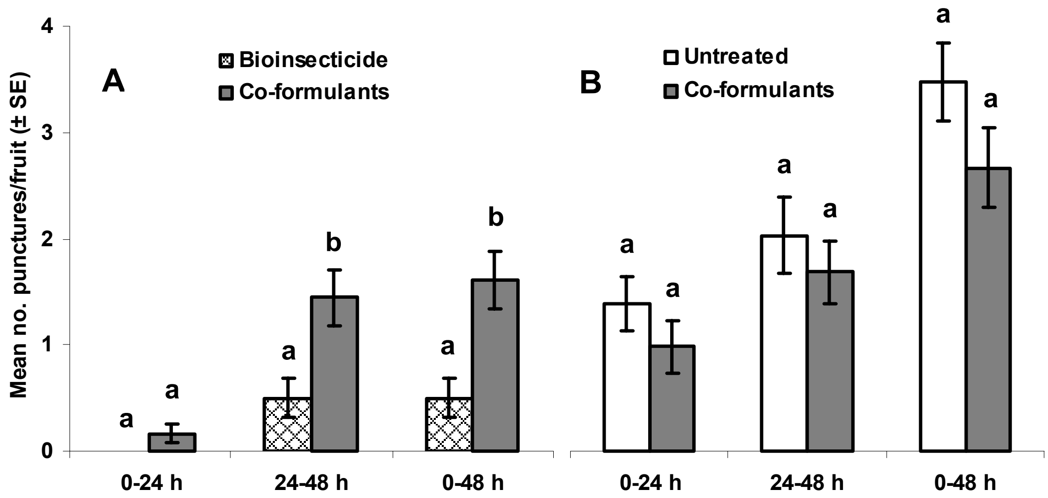

Figure 1. Mean number of punctures/fruit after 24 and 48 h on "Royal Gala" apples (A) and "Naveline" oranges (B) under choice test laboratory conditions. Bars with the same letter are not significantly different by Mann-Whitney *U* test ($P < 0.05$).

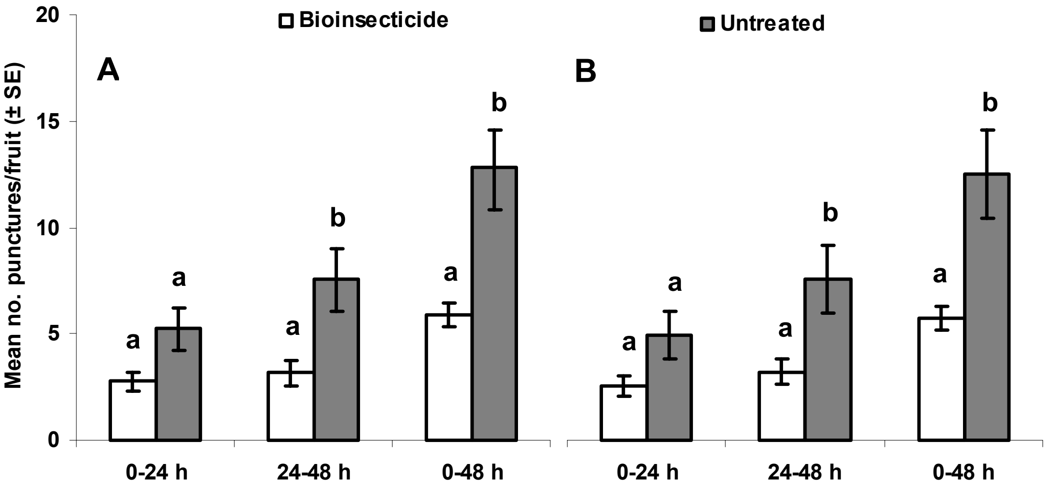

Figure 2. Mean total number of *C. capitata* punctures/fruit (A) and mean number of *C. capitata* punctures with eggs/fruit (B) on untreated "Redhaven" peaches and on peaches sprayed with the *B. bassiana*-based bioinsecticide under no-choice laboratory conditions. Bars with the same letter are not significantly different by Mann-Whitney *U* test ($P < 0.05$).

riod 2: $\chi^2 = 0.2841$; $df = 1$; $P = 0.5940$). Examination of oviposition cavities under the stereomicroscope revealed that 95% of punctures on untreated fruits contained eggs, while 98% of oviposition cavities on fruits sprayed with co-formulants exhibited eggs. Statistical comparisons between treatments using the mean number of punctures with eggs/fruit were consistent with the results obtained for the total number of punctures/fruit (data not shown).

No-choice test

The fruits used in the no-choice test ("Redhaven" peaches) are very attractive to ovipositing *C. capitata* females. As a result, a remarkably high number of punctures was observed on both fruits treated with the bioinsecticide (a total of 71 punctures) and untreated fruits (154 punctures). Fruits sprayed with the bioinsecticide exhibited 5.9 punctures/fruit, which was significantly lower than those observed on untreated fruits (12.8 punctures/fruit) ($\chi^2 = 9.1405$; $df = 1$; $P = 0.0025$) (figure 2A). The mean number of punctures on untreated and treated fruits was not different during the first 24 h of the bioassay (2.8 and 5.3 punctures/fruit, respectively) ($\chi^2 = 3.1307$; $df = 1$; $P = 0.0768$), when *B. bassiana* conidia had not yet germinated. This result suggests that the germination of conidia, the development of mycelial structures, and/or the release of chemical compounds might affect oviposition behaviour of *C. capitata* females. During *period 2* of the test (24-48 h) more punctures than during *period 1* (0-24 h) were observed both on untreated and bioinsecticide-sprayed fruits (7.6 and 3.2 punctures/fruit, respectively), with differences between treatments being significant ($\chi^2 = 5.5373$; $df = 1$; $P = 0.0186$).

Observations of egg cavities under the stereomicroscope revealed that most punctures contained eggs (93 and 94% on fruits sprayed with the bioinsecticide and untreated fruits, respectively). Consistent with the previous results, the mean number of punctures with eggs per fruit was not different after 24 h ($\chi^2 = 3.3113$; $df = 1$; $P = 0.0688$), while treatment means were significantly different during *period 2* ($\chi^2 = 5.1712$; $df = 1$; $P = 0.0230$) and the entire bioassay ($\chi^2 = 10.3028$; $df = 1$; $P = 0.0013$) (figure 2B).

Field experiments

Pre-experiment monitoring of *C. capitata* population showed that in both plots population densities exceeded by far the citrus economic damage threshold of 50 adults/trap/week (Ortu *et al.*, 2005) (figure 3). On October 5, 132 adults/trap/week were captured in the "biological" plot, while 100 adults/trap/week were recorded in the "treated" plot. After spray applications on October 10, the medfly population density in the "biological" plot was lowered under the damage threshold, except on October 27, when 64 adults/trap/week were detected. In the "treated" plot, *C. capitata* population exceeded the damage threshold on October 20, 27, November 3, and 22 (87, 114, 54, and 58 adults/trap/week, respectively). Severe rainfalls from October 19 to 25 washed off both the biological and the traditional insecticide in the experimental plots, compromising the effectiveness of spray applications. During the experiment, the trimedlure-baited traps captured a total of 401 adults/trap in the "biological" plot, while traps in the "treated" plot captured a total of 534 adults/trap.

The clementine fruit samples collected during the study period showed variable infestation levels (table 3). Before the beginning of the experiment, the proportion of infested fruits was significantly higher in the "biological" plot than in the "treated" plot (coefficient = 2.19; Odd Ratio = 8.97; exact $P = 0.0267$). There were no significant differences in the percentage of infested fruits between "treated" and "biological" plots on October 27, November 9, and 22 (exact $P = 1.000$, 0.8097, and 0.3567, respectively). The highest fruit infestation was recorded on November 9 in both "treated" and "biological" plots (12 and 10% infestation, respec-

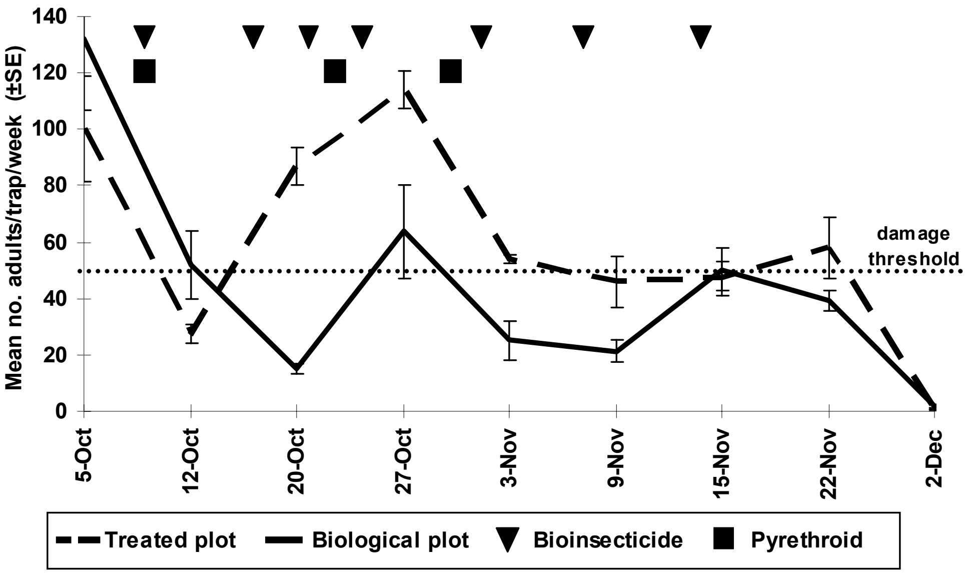

Figure 3. Mean number of *C. capitata* adults captured in white trimedlure-baited traps and pesticide spray schedule in a clementine grove in the biological and in the treated plot, during October-November 2005.

Table 3. Infestation of *C. capitata* on clementine fruits in the biological and in the treated plot, during October-November 2005.

Date	Infestation on clementine fruits (%)		Logistic regression		
	Biological plot	Treated plot	Coefficient	Odd Ratio	Exact *P*
October 5 [a]	6 a	0 b	2.19	8.97	0.0267
October 27	6 a	6 a	0	1	1.0000
November 9	10 a	12 a	-0.23	0.79	0.8097
November 22 [b]	4 a	8 a	-0.78	0.46	0.3567

Significant differences compared with PROC LOGISTIC, treatments followed by the same letter within the same row are not significantly different (exact $P < 0.05$).
[a] Pre-treatment infestation.
[b] Infestation during fruit harvest.

tively). At harvest *C. capitata* females damaged 4 and 8% of the clementine fruits in "biological" and "treated" plots, respectively (November 22).

Conclusions

Previous studies on the effectiveness of *B. bassiana* against *C. capitata* are difficult to be compared with our findings because medfly adults were exposed to fungal colonies (Konstantopoulou and Mazomenos, 2005), fungal suspension (Quesada-Moraga *et al.*, 2006), or dry conidia (Dimbi *et al.*, 2003), while we exposed adults to fruits sprayed with the *B. bassiana*-based bioinsecticide. In recent field trials, the bioinsecticide Naturalis resulted as efficient as chemical insecticides in controlling the cherry fruit fly and the olive fly (Benuzzi *et al.*, 2007; Ladurner *et al.*, 2008).

The results of our laboratory and field experiments suggest that the bioinsecticide based on *B. bassiana* strain ATCC 74040 can reduce *C. capitata* populations and the number of punctures on fruits. Laboratory experiments indicated that co-formulants of the commercial product had no side effects on the oviposition behaviour of the medfly. Fruits treated with co-formulants were punctured significantly more (3 times) than fruits sprayed with the bioinsecticide, while there was no significant difference in the number of punctures per fruit between untreated fruits and fruits sprayed with co-formulants.

The medfly females did not appear to be repelled by bioinsecticide-sprayed fruits, because they landed indifferently on both treated and untreated fruits. However, significantly more punctures per fruit were observed on untreated fruits than on fruits treated with the bioinsecticide, suggesting that medfly females prefer untreated fruits to lay eggs. These results seem to indicate not only a direct effect of the entomopathogenic fungus against *C. capitata* adults (Magnano di San Lio and Vacante, 1989; Wright and Chandler, 1992; Benuzzi and Santopolo, 2001), but also indirect modes of action. The entomopathogenic fungus might produce chemical compounds or fungal structures (e.g. hyphae) that reduce or inhibit medfly oviposition. Moreover, repellent volatile cues might be produced by *B. bassiana*, but the influence of these compounds on oviposition behaviour of *C. capitata* is uncertain. Repellent cues might diffuse inside the laboratory cages, making it impossible for medfly females to discriminate between fruits with or without the fungus. Thus, in our laboratory experiments, the oviposition behaviour of *C. capitata* might be affected only by compounds and/or fungal structures on the fruit surface. A number of fungal species have been shown to produce metabolites repellent to insects (e.g. peramine) (Rowan *et al*., 1990; Daisy *et al*., 2002).

During the field trial, trimedlure-baited traps captured more *C. capitata* adults in the "treated" plot than that in the "biological" plot, while there were no significant differences in the proportion of infested fruits at harvest, even though at the pre-treatment assessment fruit infestation was significantly higher in the "biological" plot than in the "treated" plot. More field experiments will be conducted to confirm our laboratory results. However, our studies suggest that the bioinsecticide based on *B. bassiana* strain ATCC 74040 could be a valuable additional tool to efficiently manage *C. capitata* infestations in both integrated and organic orchards.

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Authors' address: Salvatore ORTU (corresponding author, ortusal@uniss.it), Arturo COCCO, Riccardo DAU, Dipartimento di Protezione delle Piante - Entomologia agraria, Università degli Studi di Sassari, via De Nicola 1, 07100 Sassari, Italy.

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