Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Biocontrol of almond bark beetle (*Scolytus amygdali* Geurin-Meneville, Coleoptera: Scolytidae) using *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes)

Y.A. Batta

Laboratory of Plant Protection, Department of Plant Production and Protection, Faculty of Agriculture, An-Najah National University, Palestine, Israel

Keywords
bark beetles, *Beauveria bassiana*, biocontrol, entomopathogenic fungi, *Scolytus amygdali*.

Correspondence
Y. A. Batta, Laboratory of Plant Protection, Department of Plant Production and Protection, Faculty of Agriculture, An-Najah National University, P. O. Box 425 (Tulkarm), West Bank, Palestine, via Israel.
E-mail: yabatta@najah.edu

2007/0040: received 11 January 2007, revised and accepted 15 February 2007

doi:10.1111/j.1365-2672.2007.03369.x

Abstract

Aims: To formulate the entomopathogenic fungus *Beauveria bassiana* in invert emulsion, then apply it against adults of almond bark beetle (*Scolytus amygdali*) under laboratory and field conditions.

Methods and Results: The effect of formulated *B. bassiana* in invert emulsion against *S. amygdali* adults was shown by comparing the mortality percentage of adults exposed to the formulated fungus using a Petri dish treatment method and by field applications to infested peach trees with mortality of adults exposed to the unformulated fungus or the untreated control. Results obtained from both exposure methods have indicated that treatment of *S. amygdali* adults with the formulated fungus resulted in a significantly higher mean mortality percentage ($P < 0{\cdot}05$) when compared with the treatment with the unformulated fungus or the untreated control. This mortality ranged from 81·2 to 100%, 10 days after treatment with the formulated fungus when compared with 6·7 to 49·6% mortality, 10 days after treatment with the control or the unformulated fungus, respectively. Viability of the fungus conidia in invert emulsion was assessed by calculating the germination percentage of the conidia over time. Results indicated a high storage stability shown by a small loss of germination percentage for the formulated conidia of both strains (5·8 to 8·4% over a 12-week period) *vs* a low storage stability shown by a high loss of germination percentage for the unformulated conidia of the same strains (58·9 to 61·0% over the same period). The presence of *B. bassiana* in the galleries of beetles following the treatment of infested trees was shown in the present research.

Conclusions: The results obtained have demonstrated a significantly higher level of efficacy of formulated *B. bassiana* in invert emulsion against *S. amygdali* adults under laboratory and field conditions. The ingredients of invert emulsion used in the formulation of the fungus had a negligible effect on the viability of formulated conidia when compared with the unformulated.

Significance and Impact of the Study: Results obtained in the present research are promising and may be exploited commercially to control *S. amygdali* adults on various species of stone fruit trees, especially peach trees. This type of biocontrol of this insect may be used as an alternative means to chemical control for management of the insect. No adverse environmental impacts of the fungus or its formulation have been observed during application.

Introduction

Bark beetles are considered one of the most important insect pests on various species of forest and fruit trees because they make galleries and holes in the bark of infested trees (Avidov and Harpaz 1969; Elzinga 1997). The beetles' attack usually destroys the phloem tissues in the inner bark of infested trees and then disrupts the translocation of photosynthetic products causing the death of infested trees when the stem bark is destroyed. The almond bark beetle, *Scolytus amygdali*, is an economic pest insect of many cultivated species of stone fruit trees, especially peach, plum, apricot and almond, grown in the Mediterranean region and Southern Europe. The older trees of these species are also susceptible to beetle's attack and may die because of this attack under hot dry weather conditions (Mahhou and Dennis 1992; Mendel *et al.* 1997).

The almond bark beetle can be managed by preventive application of nonselective insecticides such as Dimethoate and Chlorpyriphos methyl, in addition to pruning dead infested branches or burning dead infested trees (Mendel *et al.* 1997). When the activity of the beetle increases during the dry hot months of summer and fall, the control measure that could be applied to prevent the extensive damage by the insect is the chemical control by insecticides and pheromones such as aggregation pheromones specific to *S. amygdali* (Ben-Yehuda *et al.* 2002; Zada *et al.* 2004). No studies have been reported on control of *S. amygdali* using entomopathogenic fungi like *Beauveria bassiana*. This entomopathogenic fungus has been reported to be used as biocontrol agent of other bark beetles species such as elm bark beetles (*Scolytus scolytus*) (Doberski 1981), spruce bark beetles (*Ips typographus*) (Kreutz *et al.* 2004a,b), in addition to other beetles species such as *Agrilus planipennis* (Coleoptera: Buprestidae) (Liu and Bauer 2006), *Anoplophora glabripennis* (Coleoptera: Cerambycidae) (Hajek *et al.* 2006).

It is noteworthy to mention that, in the above research, *B. bassiana* is often applied as an aqueous suspension of conidia directly to the target insects either by immersion or by spraying (Luz *et al.* 1998; Haraprasad *et al.* 2001; Lecuona *et al.* 2001; Kreutz *et al.* 2004a,b; Behle 2006; Hajek *et al.* 2006; Liu and Bauer 2006). It is well-known that under field conditions, the aqueous conidial suspension of *B. bassiana* showed rapid loss of activity, expressed as a loss of conidial viability and loss of insecticidal activity, during a short period after application (Behle 2006). In addition, it is important to mention that the entomopathogenic fungi may be efficacious if they are properly formulated for specific application. A few formulations of *B. bassiana* have been used effectively against the targeted insect species. For example, nonwoven fabric strip formulation (Shimazu 2004), a dried formulation of the fungus conidia called Boverol (Kreutz *et al.* 2004a,b), a granular formulation of the fungus applied at whorl and pollen-shed stages (Bruck and Lewis 2002), nonwoven fibre bands impregnated with the fungus (Hajek *et al.* 2006), a pure oil formulation of the fungus 'Naturalis-L' (Vidal *et al.* 2003) and BotaniGard EC or WP from Laverlam International, formerly Emerald BioAgriculture and Mycotech company (Hajek *et al.* 2006).

Invert emulsion formulation used in the present research to formulate *B. bassiana* has a milky appearance and can provide the formulated fungus conidia with the water required for germination after application. It has been attempted here to increase the efficacy of *B. bassiana* against the almond bark beetles and to be used as an alternative nonchemical control measure for this pest insect. Therefore, the objectives of the present research were: (i) to formulate the conidia of two strains of *B. bassiana* (149 and Medea) using invert emulsion as a new formulation of this entomopathogenic fungus and to study their viability over time, (ii) to apply the formulated strains of the fungus against the adults of almond bark beetle, *Scolytus amygdali*, under laboratory and field conditions, and (iii) to evaluate the treatment effect of *S. amygdali* adults with the formulated fungus under laboratory and field conditions.

Materials and methods

Peach trees used in the experiments

A naturally infested peach orchard (*Prunus persica* L. Batsch, cv: Redhaven) with almond bark beetles (*Scolytus amygladi*, strain SA5) in Tulkarm area, West Bank, Palestinian territories, via Israel, was used in the field experiments with *B. bassiana* against *S. amygladi* adults.

Fungal strains used in the treatment

Strains 149 and Medea of the entomopathogenic fungus *B. bassiana* were used in bioassay against *S. amygdali* adults. Both strains were obtained from 'The Institute for biological control in Darmstadt, Germany', they were originally isolated from *Rhynchophorus ferrugineus* and *Agrilus planipennis*, respectively. For bioassays, the strains were subcultured on plates with oat meal agar (OMA) medium and then 14-day-old cultures of the two strains were used to conduct the various tests. The concentration of conidial suspensions of both strains prepared from these cultures was adjusted to $15{\cdot}5 \times 10^7$ conidia per ml. Conidial concentrations in the prepared suspensions were counted microscopically using hemocytometer.

Insects used for bioassays

Adult insects of *S. amygdali* (strain SA5) obtained from naturally infested peach trees (See section 'Peach trees used in the experiments') were used in the laboratory bioassays. Entire peach trees infested with the same strain of the insect were used for carrying out the field bioassays.

Treatments and formulations of *Beauveria bassiana* used for bioassays

Six treatments of *B. bassiana* strains were applied in bioassays against *S. amygdali* adults. They were: (i) formulated *B. bassiana* (strain 149) in invert emulsion, (ii) formulated *B. bassiana* (strain Medea) in invert emulsion, (iii) conidial suspension of *B. bassiana* (strain 149) in sterile deionized water, (iv) conidial suspension of *B. bassiana* (strain Medea) in sterile deionized water, (v) control treatment with sterile deionized water and (vi) control treatment with blank formulation of invert emulsion. Ingredients of the invert emulsion used in the tests were identical to those of invert emulsion used by Batta (2003a) [the ingredients (w/w) are: Tween 20 (2·50%), mixture of coconut oil (19·00%) and soybean oil (28·50%), glycerine (4·00%), water-soluble wax (Dehymuls K) (0·75%) and sterile distilled water (45·25%)]. The conidia of *B. bassiana* of both strains harvested from 14-days-old cultures of the fungus grown on OMA plates were introduced into the invert emulsion according to the technique developed by Batta (2003a). Such introduction was accomplished by homogenizing of 45·25 ml of conidial suspension of *B. bassiana* strains taken from a water stock that contained $34{\cdot}2 \times 10^7$ conidia per ml with the other ingredients (totalled at 54·75 ml) to obtain a total volume of 100 ml of prepared invert emulsion. The final concentration of *B. bassiana* conidia of both strains in the prepared invert emulsion was $15{\cdot}5 \times 10^7$ conidia per ml. This concentration was the same as that used in the treatment with the unformulated form of the fungus or its conidial suspension in sterile deionized water.

Method of testing *Beauveria bassiana* conidial viability in invert emulsion

Four treatments of *B. bassiana* (two strains consisting of 149 and Medea, and two forms consisting of the invert emulsion and aqueous suspension prepared from the unformulated strains) were used in the viability test. The stock from which these treatments were taken was stored in tightly closed glass bottles at 25 ± 2°C in dark cabinet and sampled weekly for 12 successive weeks. Samples of 100 μl were taken from the above treatments then spread in a thin layer on the surface of glass slides kept in Petri dishes under humid conditions (to prevent conidial desiccation) at 25 ± 2°C for 24 h, a time needed for conidial germination of the fungus at 25°C. Conidial germination was assessed 24 h after spreading by counting germinated (with a germ tube) and nongerminated conidia of each sample in a randomly chosen field microscope using the magnification 400×. The average germination percentage of counted conidia was calculated for each sample. The storage stability which is the loss of germination percentage was then calculated for samples of formulated and unformulated conidia. Ten replicates representing ten samples were set up for each sampling time of each strain. Unformulated dry conidia of each strain were used for comparison. Aqueous suspensions prepared from unformulated conidia contained the same concentration as that in the formulated conidia in invert emulsion for viability comparison.

Methods for control of *Scolytus amygdali* adults with *Beauveria bassiana*

Petri dish treatment method

This method includes a liquid treatment of *B. bassiana* conidia of both strains to the bottom of Petri dishes containing filter papers (9 cm diameter and 63·6 cm^2 surface area). This treatment was performed by applying two rapid jetting sprays standardized at 1·5 ml per replicate using a small calibrated hand sprayer (1·5-l capacity) equipped with a nozzle suited to low-volume spray application. The application rate was equivalent to 23·6 $\mu l\ cm^{-2}$. Each Petri dish represented a treatment replicate and three replicates were used per experimental treatment. Six experimental treatments were used (See section 'Treatments and formulations of *Beauveria bassiana* used for bioassays'). Ten newly emerged males and females of *S. amygdali* adults (strain SA5, See section 'Peach trees used in the experiments') were introduced into each Petri dish immediately after spraying. The same spray volume (1·5 ml per replicate) of sterile deionized water or blank formulation of invert emulsion was applied in the control treatments. All Petri dishes were kept at 25 ± 2°C for 10 days successively before being evaluated for adult mortality. This mortality was shown either by the lack of movement of treated adults within 5-min period of continuous observation or by the appearance of white mycelial growth with conidiophores and conidia typical to *B. bassiana* on the bodies of dead adults. The mortality percentage of *S. amygdali* adults in each experimental treatment was calculated then compared.

Treatment of peach trees infested with Scolytus amygdali *in the field*

This method includes spraying entire peach trees manifesting heavy natural infestation of *S. amygdali* (strain

SA5, See section 'Peach trees used in the experiments') with the various *B. bassiana* formulations of both strains. Control treatments include the infested trees that were sprayed with water or blank formulation of invert emulsion. The concentration of the conidia in the sprayed liquid treatments of both strains was $15{\cdot}5 \times 10^7$ conidia per ml. A complete coverage of each tree with the sprayed liquid treatment was assured during the spraying process. This is equivalent to 3·5 l of spray volume per tree. A 12-l knapsack sprayer equipped with a nozzle suited to low-volume spray application was used. Each tree is considered a replication and three replicates were used per experimental treatment. Six experimental treatments were used (See section 'Treatments and formulations of *Beauveria bassiana* used for bioassays'). Two evaluation methods for insect mortality (or treatment efficacy) were conducted for the field application: (i) the tree branch sample method collecting branches 10 days after application. The sample collection was carried out by a random cutting of pieces of treated and untreated branches (control) from trees in each experimental treatment, then dissecting carefully these branches to determine the insect mortality. Five pieces of branches of 20 ± 1 cm long and 4 ± 0·5 cm diameter per treatment were randomly cut from each tree for this purpose. Treated and untreated infested trees were included in bioassays for comparison. Temperature and relative humidity prevailing in the field during the experiment were registered and the average daily temperature and relative humidity were calculated. The treatment effect with *B. bassiana* was determined by a careful dissection of the bark of treated and untreated branches to check the adult mortality of *S. amygdali*. This mortality was demonstrated either by the lack of movement of treated adult insects within 5-min period of continuous observation or by the appearance of white mycelial growth with conidiophores and conidia typical to *B. bassiana* on the bodies of dead adults at the time of observation. Mortality percentage of *S. amygdali* adults in each experimental treatment was calculated then compared, and (ii) the tree branch sample method collecting branches 1 day after application represents another evaluation method for insect mortality (or treatment efficacy). Pieces of infested branches (20 ± 1 cm long and 4 ± 0·5 cm diameter) were randomly cut from entire treated and untreated infested trees. These pieces were then incubated at 25 ± 2°C under humid conditions using rectangular plastic trays (30 × 20 cm) covered with thin plastic film for 10 days before dissection of the bark to check mortality of the beetles. Ten pieces of treated and untreated infested branches were used per experimental treatment. The treatment effect with *B. bassiana* was determined as indicated above for the first evaluation method. Mortality percentage of *S. amygdali* adults in each experimental treatment was calculated then compared.

Methods of detecting *Beauveria bassiana*

On the bodies of Scolytus amygdali *adults*

In the treatments with *B. bassiana* under laboratory and field conditions, killed beetles of *S. amygdali* without manifestation of white mycelial growth with conidiophores and conidia typical for *B. bassiana* were washed with tap water then disinfected with 70% ethyl alcohol by dip for 10 s before being rinsed three times with sterile distilled water. The beetles were then incubated in Petri dishes under humid conditions at 25 ± 2°C for 10 days before being evaluated for the appearance of white mycelial growth together with the conidia and the conidia bearing structures (conidiophores) typical for *B. bassiana* on their bodies. Each Petri dish is considered a replication and three replications were used per experimental treatment. Each Petri dish contained ten beetles.

In the galleries of Scolytus amygdali *adults*

In the field experiments, a white mycelial growth with conidiophores and conidia typical to *B. bassiana* was frequently observed in the galleries of *S. amygdali* adults while dissection of the treated bark. This indicates a saprophytic growth of the fungus on the saw dust in the galleries. To demonstrate the presence of the fungus in the galleries of *S. amygdali*, a microscopical examination and an isolation method of the fungus on OMA medium amended with chloramphenicol (250 mg per litre added to the autoclaved liquid medium before pouring into plates) was used. After dissection of the infested bark treated with formulated and unformulated fungus conidia, a part of saw dust present in the galleries was diluted in sterile distilled water [1 : 10 (w/v) of saw dust to water]. After homogenization then filtration of the mixture, 100 μl of the resulted filtrate was spread on the surface of OMA + chloramphenicol plates. The plates were then incubated at 25 ± 2°C for 10 days before being examined for the appearance of white mycelium with conidiophores and conidia typical to *B. bassiana*. Five replications representing five plates of OMA + chloramphenicol were used per experimental treatment. Microscopical examination was also used to verify the presence of the conidia in samples of saw dust collected from galleries.

Statistical analyses

Data obtained on means of the mortality percentage of *S. amygdali* adults in the different experimental treatments were analysed by ANOVA and means were separated by Duncan's Multiple Range Test (DMRT).

Table 1 Effect of treatment with *Beauveria bassiana* on the adult mortality of almond bark beetle, *Scolytus amygdali*, using Petri dish treatment method and incubation at 25 ± 2°C under humid conditions

Strains and forms of *B. bassiana*	Mean mortality percentage and numbers ( ) of *S. amygdali* adults	
	4 days after the treatment*	10 days after the treatment†
Formulated *B. bassiana* (strain 149) in invert emulsion§	43·3 c‡ (13/30)	100 c‡ (30/30)
Formulated *B. bassiana* (strain Medea) in invert emulsion§	66·7 d (20/30)	100 c (30/30)
Conidial suspension of *B. bassiana* (strain 149) in sterile deionized water	16·7 b (5/30)	60·0 b (18/30)
Conidial suspension of *B. bassiana* (strain Medea) in sterile deionized water	20·0 b (6/30)	66·7 b (20/30)
Control treatment with sterile deionized water only	0 a (0/30)	6·7 a (2/30)
Control treatment with blank formulation of invert emulsion only	10·0 ab (3/30)	16·7 a (5/30)

*Each replicate represents one Petri dish with five males and five females of *S. amygdali* treated with one of the six treatments. Observed dead adult 4 days after the treatment have manifested no movement especially when excited with a needle prick.

†Each replicate represents one Petri dish with five males and five females of *S. amygdali* treated with one of the six treatments. Observed dead adults 10 days after the treatment have manifested white mycelial growth typical to *B. bassiana* covering partially or completely the outer surfaces of dead adults.

‡Means in the same column followed by different letters are significantly different ($P < 0·05$) using ANOVA and DMRT.

§Formulation of invert emulsion used in the experiment contained the following ingredients (w/w): Tween 20 (2·50%), mixture of coconut oil (19·00%) and soybean oil (28·50%), glycerine (4·00%), water-soluble wax (Dehymuls K) (0·75%), and sterile distilled water (45·25%).

Results

Control of *Scolytus amygdali* adults using treatment in Petri dishes

Significant differences ($P < 0·05$) were obtained between the treatments of *S. amygdali* adults with *B. bassiana* in comparison with the control using Petri dishes treatment technique (Table 1). The treatments with formulated *B. bassiana* (strains 149 and Media) in invert emulsion resulted in a higher significant mortality percentage ($P < 0·05$) of *S. amygdali* adults than the treatments with conidial suspension of both strains in sterile deionized water and the control (Table 1). The mean mortality percentage of *S. amygdali* adults ranged from 43·3 to 66·7, 4 days after the treatment with formulated *B. bassiana* strains in invert emulsion, and was 100%, 10 days after the treatment with the same strains of the formulated fungus. In addition, significantly lower means of mortality percentage of *S. amygdali* adults were obtained in the other treatments (Table 1).

Control of *Scolytus amygdali* adults using treatment on infested trees

Significant differences ($P < 0·05$) within treatments were obtained when *B. bassiana* formulations were applied against *S. amygdali* adults on infested peach trees when compared with the unformulated fungus or the control (Table 2). Treatments with *B. bassiana* strains (149 and Medea) formulated in invert emulsion resulted in a higher significant mortality percentage ($P < 0·05$) of *S. amygdali* adults infesting intact and detached branches when compared with the other treatments, especially the control (Table 2). This mortality percentage ranged from 81·2 to 86·5, 10 days after the treatment with formulated *B. bassiana* strains in invert emulsion when compared with 12·8–20·2% for the control treatments at the same time period. These results confirm a higher efficacy of treatment with formulated *B. bassiana* in invert emulsion against *S. amygdali* adults infesting peach trees.

Viability of *Beauveria bassiana* conidia in invert emulsion

For both strains of *B. bassiana* used in the experiments, the germination percentage of the conidia in invert emulsion decreased from 90·4 to 82·0% for the strain 149 and from 94·2 to 88·4% for the strain Medea over a 12-week sampling period (Table 3). The loss of germination percentage relative to the initial germination percentage or the storage stability was amounted to 8·4 and 5·8% for the strains 149 and Medea, respectively. Such loss is considered small when compared with that occurred in the unformulated form of the fungus strains over the same sampling period (58·9 and 61·0%, respectively). These results indicate the negligible effect of invert emulsion ingredients on viability of the fungus conidia in invert emulsion when compared with the unformulated conidia subjected to great loss of viability because of conidial desiccation or loss of water content. Thus, the formulation of invert emulsion improved the storage stability of formulated conidia when compared with the unformulated (dry) conidia.

Table 2 Effect of treatment with *Beauveria bassiana* on the adult mortality of almond bark beetle, *Scolytus amygdali*, infesting peach trees (Field conditions during experiment: 25 ± 8°C, 75 ± 10% RH)

Strains and forms of *B. bassiana*	Mean mortality percentage and numbers ( ) of *S. amygdali* adults 10 days after the treatment	
	Intact infested branches on peach trees (25 ± 8°C and 75 ± 10% RH)*	Detached infested branches of peach trees incubated at 25 ± 2°C and humid conditions†
Formulated *B. bassiana* (strain 149) in invert emulsion§	81·2 c‡ (50/62)	82·1 c‡ (90/109)
Formulated *B. bassiana* (strain Medea) in invert emulsion§	84·2 c (48/57)	86·5 c (105/121)
Conidial suspension of *B. bassiana* (strain 149) in sterile deionized water	33·2 ab (20/60)	39·2 b (47/118)
Conidial suspension of *B. bassiana* (strain Medea) in sterile deionized water	43·1 b (24/56)	49·6 b (72/145)
Control treatment with sterile deionized water only	19·5 a (10/51)	12·8 a (16/124)
Control treatment with blank formulation of invert emulsion only	20·2 a (10/50)	19·5 a (25/128)

*Each replicate represents a piece of infested and treated branches that has been randomly chosen on infested and treated or untreated peach tree. Five replicates per experimental treatment were used. Each piece was dissected carefully to check the adult mortality 10 days after the treatment.

†Each replicate represents a piece of branch that has been randomly chosen and cut from infested and treated or untreated peach tree (dimension: 20 ± 1 cm long and 4 ± 0·5 cm diameter). Ten replicates per experimental treatment were used. The bark of each branch piece was dissected carefully to check the adult mortality 10 days after incubation.

‡Means in the same column followed by different letters are significantly different ($P < 0·05$) using ANOVA and DMRT.

§Formulation of invert emulsion used in the experiment contained the following ingredients (w/w): Tween 20 (2·50%), mixture of coconut oil (19·00%) and soybean oil (28·50%), glycerine (4·00%), water-soluble wax (Dehymuls K) (0·75%), and sterile distilled water (45·25%).

Table 3 Viability of *Beauveria bassiana* (strains 149 and Medea) conidia in invert emulsion stored in tightly closed glass bottles at 25 ± 2°C for 12 successive weeks

Time (in weeks) after introduction of *B. bassiana* conidia into the invert emulsion or keeping them in unformulated form	Mean germination percentage of formulated *B. bassiana* conidia in invert emulsion*		Mean germination percentage of unformulated conidia of *B. bassiana*†	
	Strain 149‡	Strain Medea‡	Strain 149‡	Strain Medea‡
0	90·4 ± 1·6	94·2 ± 2·1	93·6 ± 3·2	95·8 ± 4·1
1	88·2 ± 2·1	92·3 ± 1·7	88·2 ± 4·8	91·4 ± 3·5
2	87·5 ± 1·3	91·8 ± 1·1	82·6 ± 1·7	87·3 ± 5·1
3	85·9 ± 0·9	90·7 ± 0·9	80·5 ± 2·5	82·7 ± 4·6
4	85·1 ± 0·8	90·1 ± 0·3	73·7 ± 5·2	77·8 ± 3·8
5	84·7 ± 1·1	89·9 ± 0·4	65·6 ± 5·1	73·1 ± 2·6
6	83·8 ± 1·7	89·6 ± 0·4	59·9 ± 4·6	69·6 ± 3·4
7	83·2 ± 1·0	89·3 ± 0·7	55·2 ± 3·2	65·3 ± 3·1
8	82·7 ± 0·5	89·1 ± 0·4	53·4 ± 2·1	59·8 ± 5·3
9	82·4 ± 0·3	89·1 ± 0·3	49·7 ± 3·9	53·7 ± 3·8
10	82·0 ± 0·4	88·6 ± 0·8	42·8 ± 4·3	48·1 ± 6·4
11	82·1 ± 0·2	88·5 ± 0·5	35·5 ± 4·8	39·3 ± 2·9
12	82·0 ± 0·3	88·4 ± 0·3	32·6 ± 2·8	36·9 ± 2·3

*Formulation of invert emulsion used in the experiment contained the following ingredients (w/w): Tween 20 (2·50%), mixture of coconut oil (19·00%) and soybean oil (28·50%), glycerine (4·00%), water-soluble wax (Dehymuls K) (0·75%), and sterile distilled water (45·25%).

†Unformulated form of *B. bassiana* was in form of dry conidia harvested directly from the fungus culture.

‡Ten replicates representing 10 samples of of 100 μl of formulated and unformulated fungus conidia per sample time. Means of germination percentage of *B. bassiana* conidia were presented here as mean ± standard error of the mean (SEM).

Detection of *Beauveria bassiana* in the galleries of *Scolytus amygdali*

In addition to observing white mycelial growth with conidiophores and conidia typical to *B. bassiana* in the galleries of *S. amygdali* adults in the treated bark with the fungus, the isolation method on OMA + chloramphenicol plates and microscopical examination have also demonstrated the appearance of white mycelium with conidiophores (single, irregularly grouped or in verticillate

clusters) and conidia (subhyaline, rounded to ovoid, 1-celled, dry and borne singly on small denticles) typical to *B. bassiana* on the plates' surface. The conidia and conidiophores of *B. bassiana* were identified under the microscope using the magnification 400×. By these methods, the fungus was successfully detected. This indicates the presence of the fungus in the galleries of *S. amygdali* adults after the treatment and from these galleries, the conidia borne by the saprophytic fungus mycelium are able to infect beetles and kill them.

Detection of *Beauveria bassiana* on bodies of infected *Scolytus amygdali* adults

Incubation of treated *S. amygdali* adults with *B. bassiana* in Petri dishes under humid conditions at 25 ± 2°C for 10 days has revealed the appearance of white mycelium with conidiophores and conidia, as described in section 'Detection of *Beauveria bassiana* in the galleries of *Scolytus amygdali*', typical to the fungus on the bodies of infected beetles in comparison with the uninfected. The conidia and conidiophores of *B. bassiana* were identified under the microscope using the magnification 400×.

Discussion

The treatment with *Beauveria bassiana* (strains 149 and Medea) formulated in invert emulsion has shown a high efficacy against the adults of almond bark beetle, *Scolytus amygdali*, using Petri dishes treatment method and by field applications to infested peach trees. A high mortality percentage of *S. amygdali* adults reaching up to 100% was obtained during bioassays. In addition, the formulation of the fungus in invert emulsion has the following important characteristics that may explain the efficacy of the formulated fungus against *S. amygdali* adults: (i) it can be applied without being diluted in water through the direct spraying of infested peach trees. There was no observation of harmful side-effects on treated trees during application, (ii) it has no adverse side-effects either on the consumer's health or on the environment because the formulation's ingredients are used as food additives or in manufacturing of cosmetics, (iii) the present ingredients of invert emulsion were the same as that used in formulating and testing of the efficacy of another entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). The formulated *M. anisopliae* in invert emulsion has been used effectively in biocontrol of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Tetranychus cinnabarinus* (Acari: Tetranychidae) (Batta 2003a,b), and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) (Batta 2005), (iv) it has a negligible reducing effect on germination percentage of the formulated fungus conidia in comparison with a great reduction in germination percentage of the unformulated conidia. Thus, it improved the storage stability of the formulated fungus conidia when compared with the unformulated, and (v) it provides adequate quantities of water and humidity needed for germination and penetration of the applied *B. bassiana* conidia into the target insects especially under hot dry field conditions. Behle (2006) reported that, under the hot dry field conditions, there was a rapid loss of conidial viability of unformulated *B. bassiana* when applied in form of an aqueous conidial suspension against *Trichoplusia ni*. In addition, the effect of temperature and relative humidity prevailing under field conditions on the efficacy of the unformulated conidia of *B. bassiana* was confirmed by Luz *et al.* (1998); Luz and Fargues (1999); Haraprasad *et al.* (2001); Lecuona *et al.* (2001).

A high mortality percentage in treated *S. amygdali* adults ranging from 81·2 to 86·5% was obtained in the present research when the entire peach trees infested with *S. amygdali* were sprayed with *B. bassiana* conidia formulated in invert emulsion. This indicates good efficacy of treatment with the formulated *B. bassiana* conidia in invert emulsion under field conditions. Such efficacy could be explained by the high viability of applied fungus conidia formulated in invert emulsion and the ability of the fungus mycelium to grow saprophytically in the galleries of *S. amygdali* adults then the ability of the conidia borne by this mycelium to infect the beetles inside the galleries. No studies have been reported on infecting host insects by the mycelium of *B. bassiana*, but infection usually occurs by the conidia. The presence of the fungus in the galleries of *S. amygdali* following its application on infested trees was shown in the present research by microscopical examination and successful isolation of the fungus on OMA + chloramphenicol plates (72·3% of the examined cases revealed a successful isolation of the fungus). A comparable observation and efficacy of *B. bassiana* against the coffee berry borer (*Hypothenemus hampei* Ferrari, Coleoptera: Scolytidae) was reported by Carrion and Bonet (2004). The investigators have confirmed the presence of the fungus in the fruit galleries of borer beetles following the treatment by spraying conidial suspension of the fungus on infested fruits under field conditions.

Another important issue for explaining the treatment efficacy of *S. amygdali* infesting peach trees by the formulated *B. bassiana* in invert emulsion is the secondary cycling of the fungus in the environment after application. For this aspect, we have observed that the dissection of the nearby peach trees' bark infested with *S. amygdali*, but untreated with formulated or unformulated *B. bassiana* has revealed the infection of *S. amygdali* adults with the fungus 1 month after the treatment of the nearby

trees existing in the experimental orchard (61·3% infection with *B. bassiana* among the nearby examined beetle adults). This indicates the possibility of *B. bassiana* secondary cycling in the environment either by the contact with infected insect cadavers or by the flight of infected but not killed adult insects from trees treated with the fungus following their emergence to incite a new infestation on untreated trees. The secondary cycling of the fungus in the environment and its horizontal transmission from infected to uninfected insects, in addition to the possibility of its overwintering in certain sites (e.g. galleries and holes of the tree bark) was reported by Dowd and Vega (2003); Carrion and Bonet (2004); Kreutz *et al.* (2004b). In addition, by molecular monitoring, Wang *et al*. (2004) have proved that the introduced *B. bassiana* strains into a local environment may persist for a long period following its application and can infect nontarget insect hosts.

It is noteworthy to mention that the main difference observed in the present research between the strains 149 and Medea of *B. bassiana* was the germination percentage of their conidia in the invert emulsion. However, the formulation used maintains viability of the conidia of both strains and improves storage stability when compared with unformulated (dry) samples. Other differences (but not statistically significant) were observed in the treatment efficacy of these strains against *S. amygdali* adults. These differences were demonstrated by the mortality percentage of treated insects. Overall, both strains of the fungus have demonstrated a high efficacy against the treated adults of *S. amygdali* as discussed earlier.

In conclusion, biocontrol of almond bark beetles with entomopathogenic fungi like *B. bassiana* is an interesting and important approach and the use of new fungus formulation, e.g. invert emulsion, is a new and interesting aspect. The tested strains of *B. bassiana* formulated in invert emulsion demonstrated a high efficacy in biocontrol of *S. amygdali* adults on peach trees. The formulated fungus conidia in invert emulsion could be sprayed directly on infested trees. The applied fungus conidia can penetrate, after germination, the bark of treated trees through the holes of *S. amygdali* and then can grow saprophytically in the insect galleries before producing conidia able to infect the insect and kill it. This research demonstrated benefits to efficacy and storage stability of *B. bassiana* provided by a simple formulation made from inexpensive ingredients prepared by a simple mixing procedure.

References

Avidov, Z. and Harpaz, I. (1969) *Plant Pests of Israel*. Jerusalem: Israel University Press.

Batta, Y. (2003a) Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metch.) Sorokin (Deuteromycotina: Hyphomycetes). *Crop Prot* **22**, 415–422.

Batta, Y. (2003b) Symptomatology of tobacco whitefly and red spidermite infection with the entomopathogenic fungus *Metarhizium anisopliae* (Metch.) Sorokin. *Dirasat: Agric Sci* **30**, 294–303.

Batta, Y. (2005) Control of the lesser grain borer (*Rhyzopertha dominica* F., Coleoptera: Bostrichidae) by treatments with residual formulations of *Metarhizium anisopliae* (Metch.) Sorokin (Deuteromycotina: Hyphomycetes). *J Stored Products Res* **41**, 221–229.

Behle, R.W. (2006) Importance of direct spray and spray residue contact for infection of *Trichoplusia ni* larvae by field application of *Beauveria bassiana*. *J Econ Entomol* **99**, 1120–1128.

Ben-Yehuda, S., Tolasch, T., Francke, W., Gries, R., Dunkelbum, E. and Mendel, Z. (2002) Aggregation pheromones of almond bark beetle *Scolytus amygdali* (Coleoptera: Scolytidae). *IOBC-WPRS Bull* **25**, 1–12.

Bruck, D.J. and Lewis, L.C. (2002) Whorl and pollen-shed stage applications of *Beauveria bassiana* for suppression of adult western corn rootworm. *Entomol Exper Appl* **103**, 161–169.

Carrion, G. and Bonet, A. (2004) Mycobiota association with the coffee berry borer (Coleoptera: Scolytidae) and its galleries in fruit. *Ann Entomol Soc America* **97**, 492–499.

Doberski, J.E. (1981) Comparative laboratory studies on three fungal pathogens of the elm bark beetles, *Scolytus scolytus*: effect of temperature and humidity on infection by *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus*. *J Invertebr Pathol* **37**, 195–200.

Dowd, P.F. and Vega, F.E. (2003) Autodissemination of *Beauveria bassiana* by sap beetles (Coleoptera: Nitidulidae) to overwintering sites. *Biocont Sci Technol* **13**, 65–75.

Elzinga, R.J. (1997) *Fundamentals of Entomology*, 4th edn, Upper Saddle River, New Jersey: Prentice Hall.

Hajek, A.E., Huang, B., Dubois, T., Smith, M.T. and Li, Z. (2006) Field studies of control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) using fiber bands containing the entomopathogenic fungi *Beauveria brongniartii*, *Metarhizium anisopliae*. *Biocont Sci Technol* **16**, 329–343.

Haraprasad, N., Niranjana, S.R., Prakash, H.S., Shetty, H.S. and Wahab, S. (2001) *Beauveria bassiana* – a potential mycopesticide for the efficient control of coffee berry borer, *Hypothenemus hampei* (Ferrari) in India. *Biocont Sci Technol* **11**, 251–260.

Kreutz, J., Vaupel, O. and Zimmermann, G. (2004a) Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the laboratory under various conditions. *J Appl Entomol* **128**, 384–389.

Kreutz, J., Vaupel, O. and Zimmermann, G. (2004b) Horizontal transmission of the entomopathogenic fungus *Beauveria*

bassiana among the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae) in the laboratory and under field conditions. *Biocont Sci Technol* **14**, 837–848.

Lecuona, R.E., Edelstein, J.D., Berratta, M.F., La Rossa, F.R. and Arcas, J.A. (2001) Evaluation of *Beauveria bassiana* (Hyphomycetes) strains as potential agents for control of *Triatoma infestans* (Hemiptera: Reduviidae). *J Med Entomol* **38**, 172–179.

Liu, H. and Bauer, L.S. (2006) Susceptibility of *Agrilus planipennis* (Coleoptera: Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. *J Econ Entomol* **99**, 1096–1103.

Luz, C. and Fargues, J. (1999) Dependance of the entomopathogenic fungus, *Beauveria bassiana*, on high humidity for infection of *Rhodnius prolixus*. *Mycopathologia* **146**, 33–41.

Luz, C., Tigano, M.S., Silva, I.G., Cordeiro, C.M. and Aljanabi, S.M. (1998) Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to control *Triatoma infestans*. *Mem Inst Oswaldo Cruz, Rio de Janeiro* **93**, 8.

Mahhou, A. and Dennis, F.G. (1992) The almond trees in Morocco. *HortTechnology* **2**, 488–492.

Mendel, Z., Ben-Yehuda, S., Marcus, R. and Nestel, D. (1997) Distribution and extent of damage by *Scolytus* spp. to stone and pome fruit orchards in Israel. *Insect Sci Appl* **17**, 175–181.

Shimazu, M. (2004) A novel technique to inoculate conidia of entomopathogenic fungi and its application for investigation of susceptibility of the Japanese pine sawyer, *Monochamus alternatus*, to *Beauveria bassiana*. *Appl Entomol Zool* **39**, 485–490.

Vidal, C., Fargues, J., Rougie, M. and Simths, N. (2003) Effect of air humidity on the infection potential of hyphomycetous fungi as mycoinsecticides for *Trialeurodes vaporariorum*. *Biocont Sci Technol* **13**, 183–198.

Wang, C., Fan, M., Li, Z. and Butt, T.M. (2004) Molecular monitoring and evaluation of the application of the insect-pathogenic fungus *Beauveria bassiana* in Southeast China. *J Appl Microbiol* **96**, 861–870.

Zada, A., Ben-Yehuda, S., Dunkelbum, E., Harel, M., Assael, F. and Mendel, Z. (2004) Synthesis and biological activity of four stereoisomers of 4-Methyl-3-Heptanol: main component of the aggregation pheromone of *Scolytus amygdali*. *J Chem Ecol* **30**, 631–641.