Beauveria bassiana (Ascomycota: Hypocreales) Wound Dressing for the Control of Euzophera pinguis (Lepidoptera: Pyralidae)

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ABSTRACT Injury to olive tree trunks and branches because of biotic and abiotic factors, such as pruning and mechanical harvesting, attracts the olive pyralid moth Euzophera pinguis Haworth (Lepidoptera: Pyralidae). This moth has become increasingly important in the Mediterranean region during recent years. The use of an entomopathogenic fungus for wound dressing for pest control is reported for the first time in this study. Beauveria bassiana (Ascomycota: Hypocreales) strain EABb 08/04-Ep was originally obtained from a diseased E. pinguis larva and has shown effective E. pinguis control in an olive crop in Jaén, Andalusia, Spain, under field conditions during the spring and fall of 2008 and 2009 and the spring of 2011. Experimental artificial 30 by 30-mm square wound cages were large enough to allow the E. pinguis females to oviposit. Approximately 80 and 40–60% of the control wounds contained live larvae in the experiments that occurred during the spring and fall, respectively. The B. bassiana wound dressing gave similar results as the chlorpyrifos wound dressing throughout the experiment, with efficacies reaching 80–85% in the spring and 90–95% in the autumn. The B. bassiana fungus was recovered from 60–90% of the wounds at the completion of the experiments and after 60 d of treatment. These data indicate that strain EABb 08/04-Ep applied to the pruning wounds can be an effective tool for the microbial control of E. pinguis in olive crops. Moreover, B. bassiana may be used within integrated pest management strategies to minimize chemicals, depending on the population density of the pyralid moth.

KEY WORDS branch protection zone, natural target pruning, olive pyralid moth, boring larva, pruning paint

The olive pyralid moth Euzophera pinguis Haworth (Lepidoptera: Pyralidae) is a serious bark and woodborer olive pest in most Mediterranean regions and has become increasingly important during recent years (Durán et al. 1998, IOOC 2012). E. pinguis has two overlapping generations each year, a spring-summer generation and a winter generation, with adult flight activity that may extend for 10 mo and with larvae present throughout the year (Navarro and Campos 2004, Tzanakakis 2006, Durán et al. 2010, Patanita 2010). The adult moths emerge in early spring and continue their first flight period until mid-June (De Andrés 1991). The female moths lay eggs in unprotected areas on the trunks of trees with injuries, such as tumors caused by the bacteria Pseudomonas syringae subsp. savastanoi (Smith) Janse and wounds caused by frost, wind, hail, or the fusion of branches. After hatching, the larvae feed endophytically into the main trunk and secondary branches, causing the interruption of sap flow, which leads to branch drying, the yellowing of leaves, the defoliation of branches, and even the loss of young olive trees (Grouard 2001, Navarro and Campos 2004). Similarly, female egg-laying activity is favored when olive tree trunks and branches are injured during pruning and mechanical harvesting, which occurs in the new intensive and super-intensive olive groves.

Because of the endophytic behavior of the larvae, E. pinguis control is extremely difficult through application of the organophosphate insecticide chlorpyrifos (48%), which is the only method used by farmers for the control of this moth (Ministerio de Agricultura, Alimentación, y Medio Ambiente [MAGRAMA] 2012). However, because of the ecological and health-related problems of insecticide application, alternative systems based on pheromone mating disruption have been recommended for the control of E. pinguis (Ortiz et al. 2004, 2007). Durán et al. (2010) reported that the efficacy of using of a synthetic pheromone for mating disruption of the olive pyralid moth is insufficient to prevent an increase in both the percentage of trees affected and the number of active galleries per tree. Biological control of E. pinguis is not widespread.
Spain (37.0% Tween 80 to a final concentration of 4.1 bioassay was adjusted by colony-forming unit assay (Goettel et al. 2011). Neuroptera and ants are less important predators of E. pinguis, and it has been reported that two braconids, Isenella myeloletonta (Wilkinson) and Phanerota ocellaris Kohl, have some impact on populations of closely related species of E. pinguis.

In a survey of the natural enemies of E. pinguis in Jaén, Andalusia in southern Spain, during 2007, a strain of the entomopathogenic, mitosporic, ascomycete Beauveria bassiana (Balsamo) Vuill. (Ascomycota: Hypocreales) was found infecting E. pinguis larvae within the branches of several olive trees (Quesada-Moraga 2011). The objective of this study was to evaluate wound dressing with this strain of B. bassiana for the microbial control of the olive pyralid moth under field conditions in an olive crop in Jaén, Andalusia, Spain.

Materials and Methods

Fungal Strain and Cultivation. The B. bassiana strain EABb 08/04-Ep (C.R.A.F., University of Cordoba Entomopathogenic Fungi Collection, Córdoba, Spain) was isolated from a dead E. pinguis larva from an olive crop in Jaén, Spain. This strain was deposited under the Budapest Treaty in the Spanish Type Culture Collection located at the University of Valencia under accession number CECT 20746 on 2 February 2009. Monosporic cultures of this strain were grown on malt agar slants at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C. To obtain a spore suspension, slant cultures of the isolates were subcultured in petri plates containing malt agar (BioCult B Laboratories, Madrid, Spain) for 15 d at 25°C in the dark and stored at 4°C.

Two methods were used to evaluate the presence of fungus in the wounds at the completion of each experiment (2 mo). Several pieces of bark were removed from the spring of 2008 and were stored in plastic bags until transportation to the laboratory. In addition, the surface of each wound was cleaned with a sterile cotton swab, which was also transported to the laboratory. A water suspension was prepared from the pieces of bark and the sterile cotton swabs. This suspension was then stirred with a rotary shaker (P-Selecta shakers, Barcelona, Spain) at 12 rpm for 60 min. The presence or absence of B. bassiana in the pieces of bark and cotton swabs was determined using Sabouraud chloramphenicol agar culture medium in the colony-forming unit assay.

Statistical Analysis. The data were analyzed using a one-way analysis of variance (Statistix 9.0, Analytical Software, Tallahassee, FL). Tukey’s honestly significant difference test was used to compare the means.

Results

E. pinguis Flight Activity in the Study Area. E. pinguis flight activity was monitored with pheromone traps. As shown in Fig. 1, there was similar flight behavior of the adult populations in the successive monitoring campaigns. There were two E. pinguis flights or population peaks per year, with a high level of activity during spring-summer and a low level of activity during autumn (Fig. 1).

Evaluation of the B. bassiana Wound Dressing Treatments for E. pinguis Control. In 2008, the spring B. bassiana wound dressing had a significant effect on...
the percentage of wounds without larval infestation (WWI) ($F_{2,8} = 117.4; P < 0.001$). The WWI was significantly reduced after wound dressing with *B. bassiana* and chlorpyrifos compared with the controls, whereas the WWI was significantly higher (93.3%) for chlorpyrifos than *B. bassiana* (48.3%) (Table 1). There was also a significant reduction in the percentage of wounds that contained live larvae (WLL) in the chlorpyrifos and *B. bassiana* wound dressing treatments ($F_{2,8} = 37.5; P < 0.001$). The percentage of wounds without *E. pinguis* larvae but with signs of larval boring as evidenced by the presence of frass accumulations (WLB) was only significant for the *B. bassiana* wound dressing ($F_{2,8} = 139.7; P < 0.001$) (Table 1).

During the autumn of 2008, the *B. bassiana* treatment had a significant effect on both the WWI ($F_{2,8} = 10.7; P = 0.01$) and WLL ($F_{2,8} = 223.2; P < 0.001$) (Table 1). Although the WWI in the control was higher in the autumn than spring, it was ≈50% higher in the chlorpyrifos and *B. bassiana* treatments (Table 1). No live larvae were observed in the chlorpyrifos and *B. bassiana* treatments, whereas 60% of the control wounds presented with live larvae (Table 1). The WLB were found only in wounds dressed with the fungus (8.3%) (Table 1).

During 2009, the spring *B. bassiana* wound dressing had a significant effect on the percentage of wounds without larval infestation (WWI) ($F_{2,8} = 81.5; P < 0.001$). The WWI was significantly reduced after wound dressing with chlorpyrifos (93.3%) and *B. bassiana* (20.0%) compared with the controls without WWI (Table 1). In this experiment, the chemical and microbial treatments again resulted in a significant ($F_{2,8} = 318.4; P < 0.001$) reduction in the presence live larvae in the wounds, which was zero in both treatments and 83.3% in the controls (Table 1). Similarly, the *B. bassiana* wound dressing was the only treatment with a significant percentage of wounds without *E. pinguis* larvae but with signs of larval boring ($F_{2,8} = 985.6; P < 0.001$).

During the autumn of 2009, the *B. bassiana* treatment had a significant effect on the percentage of wounds without larval infestation ($F_{2,8} = 52.19; P < 0.001$), which was significantly increased by the chlorpyrifos and *B. bassiana* wound treatments (Table 1). There was also a significant reduction in the percentage of WLL ($F_{2,8} = 585.7; P < 0.001$). There were no wounds with live larvae in the chemical and fungal treatments, whereas 21.1% of the control wounds presented with live larvae (Table 1). Significant differences were also detected between the treatments for wounds with no *E. pinguis* larvae but signs of larval boring ($F_{2,8} = 985.6; P < 0.001$), which were observed only in the controls and fungal treatment (Table 1).

During the spring of 2011, the treatment significantly impacted the percentage of wounds without larval infestation ($F_{2,8} = 84.9; P < 0.001$), whereas this number was significantly reduced only for the chemical treatment (Table 1). Notably, the wounds dressed with *B. bassiana* showed the highest (43.3%) percentage of wounds with no larvae but signs of larval boring (Table 1) during this spring. There were also significant differences between the treatments for the percentage of wounds that contained live larvae ($F_{2,8} = 7.6; P = 0.022$), which was reduced by both the chemical and microbial treatments (Table 1).

**Presence of *B. bassiana* in the Wounds at the Completion of the Experiment.** The presence of *B. bassiana* was detected at the time of wound opening. Based on the results, no *B. bassiana* was detected...
Table 1. Percentage of wounds attacked by the olive pyralid moth of olive bark with artificial wounds per treatment type

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<tbody>
<tr>
<td>WWI</td>
<td>83.3</td>
<td>3.3</td>
<td>16.6</td>
<td>21.1</td>
<td>52.6</td>
<td>20.0</td>
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<tr>
<td>WLL</td>
<td>1.6</td>
<td>1.6</td>
<td>4.4</td>
<td>2.0</td>
<td>4.4</td>
<td>0.0</td>
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<tr>
<td>WLB</td>
<td>100.0</td>
<td>100.0</td>
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The numbers in the columns followed by the same letter are not significantly different, as determined by the Tukey test (P = 0.05).

Discussion

Pheromone trap monitoring indicated that olive pyralid moth activity occurred in the study area throughout the entire three growing seasons. The annual flight curves of *E. pinguis* largely coincide with those published by Durán et al. (1998, 2010), who used light and pheromone traps in the province of Seville in Andalusia in southwestern Spain, and with those recorded by Olivero et al. (2005), who used pheromone traps in the Andalusia province of Malaga in South Spain. Consistent with Olivero et al. (2005), this similarity in flight curve shapes among the olive crops from different regions of Andalusia highly support a similar behavior of the pest in geographically distant olive regions, most likely conditioned by macro-ammon variable conditions. Although there were slight differences in the start dates of the adult flight in 2008, 2009, and 2011, in general, the adult activity of *E. pinguis* begins with the onset of spring, with the optimum temperature for emergence in the range of 20–25°C (Durán et al. 1998). The daily evolution of adult *E. pinguis* is highly affected by rainfall (i.e., several raining days negatively influence adult flight) and wind (Patanita 2010).

Our experimental artificial 30 by 30-mm square wound cages allowed the *E. pinguis* females to oviposit, as demonstrated by the high percentage of wounds showing live larvae in the controls, with ~80% in the spring experiments and 40–60% in the fall experiments. The results of this study indicate that the *B. bassiana* EABb 08/04-Ep strain provided effective *E. pinguis* control by reducing both the percentage of wounds with live larvae and the percentage of wounds with no live larvae but signs of larval boring. Notably, we did not observe dead larvae in the wounds with signs of larval boring, which may be because the majority of larval death occurred in the first-instar neonate larvae, which according to our previous study, are the most susceptible to EABb 07/06-Ep infection (Quesada–Moraga 2011).

The fungal treatment gave similar results as the chlorpyrifos treatment throughout the experiment, with efficacies reaching 80–85% during spring and 90–95% during autumn. However, wounds with signs of larval boring were not observed in the chemical treatment, indicating either an excellent ovicidal or repellent activity (Nazir et al. 2001), whereas these two activities were not detected in the *B. bassiana* dressed wounds. Therefore, the use of the fungus either alone or within an IPM strategy to replace or from the control treatment for all years, whereas *B. bassiana* was variably detected from the treated wounds according to the two evaluation methods used during the experiment. No significant differences were found for the presence of *B. bassiana* between the two methods for all years, except for the treatment during the spring of 2008, in which *B. bassiana* was detected in 93.3% of the bark pieces removed from the wounds and in 63.3% of the sterile cotton swabs pieces (Table 2).
minimize the use of chlorpyrifos, which has several nontarget effects and continues threat of removing from the list of authorized insecticides, seems to be justified (Rafalimanana et al. 2002, Prischmann et al. 2005, Delpuech et al. 2009).

To the best of our knowledge, no previous studies on the use of entomopathogenic fungi for wound dressing against insect pests have been reported. Wound dressing to protect olive crops against E. pinguis has been performed only with chemicals. Olivero et al. (2005) reported that wound dressing with chlorpyrifos (48%) and a mixture of fenitrothion and esfenvalerate provided effective control of E. pinguis larval boring. Connell et al. (2005) also used wound dressing with diazinon and copper oil for control of the American plum borer Eucalypitopsis fimbriata. They found that both chemical treatments significantly reduced the number of plum borer strikes compared with the untreated control. The studies by Camilli et al. (2007) supported the need to apply pruning paints to all wounds on susceptible trees in areas with oak wilt. Our experiments highlight the potential for preventive E. pinguis control using B. bassiana for olive tree wound dressing; however, we are also interested in elucidating the activity of the fungus after borer feeding activity is observed in the wounds.

The presence of fungus in the treated wounds after 60 d of treatment, which ranged between 36 and 63% for the bare pieces recovered from the wounds and between 33 and 93% for the sterile cotton swabs, revealed the potential of the fungus for long-lasting control of the olive pyralid moth. Additional studies are required to clearly define the number of fungal applications that are necessary for seasonal or annual control.

Our studies indicate that B. bassiana strain EABb 08/04-Ep applied to pruning wounds can be an effective tool for the microbial control of E. pinguis in olive trees. Moreover, it can be used within IPM strategies to minimize chemicals, depending on the population density of the pyralid moth.

Acknowledgments

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Table 2. Presence of B. bassiana in the wounds at the time of wound opening

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<td>1</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>2</td>
<td>63.3 ± 3.3b</td>
<td>36.7 ± 5.1a</td>
<td>60.0 ± 10.0a</td>
<td>40.0 ± 5.7a</td>
<td>60.0 ± 6.7a</td>
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<tr>
<td>3</td>
<td>93.3 ± 3.3a</td>
<td>40.0 ± 3.2a</td>
<td>53.3 ± 3.3a</td>
<td>33.3 ± 3.3a</td>
<td>63.3 ± 11.5a</td>
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* 1 = control, 2 = pieces of bark, 3 = sterile cotton swab.
* The means within the columns with the same letter are not significantly different (P ≤ 0.05) according to the Tukey (HSD) test (P ≤ 0.05).

References Cited


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