Biological Control of Hazelnut Weevil (*Curculio nucum* L., Coleoptera, Curculionidae) Using the Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) Vuill. (Deuteromycotina, Hyphomycetes)

B. Paparatti and S. Speranza
Dipartimento di Protezione delle Piante
Università degli Studi della Tuscia
Via S. Camillo de Lellis, 01100, Viterbo
Italy

Keywords: IPM, microbiological control, larval mortality

Abstract

The need to biologically control phytophagous insects has led researchers to consider using microorganisms to control insect pests in the field. On this basis, we decided to conduct field tests to determine the effectiveness of a commercial product made of *Beauveria bassiana* (Bals.) against the key hazelnut pest *Curculio nucum* L. Trials were carried out in a hazelnut orchard in the province of Viterbo (Italy). Three untreated control cages and three cages treated with the fungus entomopathogens were used during the tests. The cages were located under the hazelnut canopy and buried to half their height. 200 mature *C. nucum* larvae were placed in each cage and allowed to burrow naturally. After the larvae had buried themselves, a commercial product was applied to the three treated cages. Mortality was monitored during the following spring. The treated cages showed 99.5% *C. nucum* mortality as opposed to 63.5% for the untreated cages. These data show the effectiveness of the *B. bassiana* biological control on key insect pests for hazelnut trees in central Italy.

INTRODUCTION

Biological control of cultivated plants has become pivotal in phytophagous insect management. This kind of management makes it possible to reduce damage to agro-ecosystems, avoids insects developing resistance to a number of active ingredients (a.i.), and avoids using another product that would leave residual pesticides. The need to find alternative biological control systems against phytophagous insect pests has led researchers to investigate the possibility of using living organisms against noxious insects in the field, making use of this natural control. These biological controls were used in the present work to control the hazelnut weevil (*Curculio nucum* L., Coleoptera, Curculionidae) in the area of Monti Cimini (Viterbo, Italy).

The Latium region is second most important in Italy for hazelnut production (*Corylus avellana* L.), being responsible for 29.5% of national production. More than 95% of hazelnut production in the region comes from plantations in the Northern Latium area (Viterbo province) (Carbone et al., 2004) where the weevil is the key insect pest (Paparatti and Pucci, 1987).

The adult of the species has the typical *rostrum* of the weevil family. The female lays one egg in the lower third of the fruit once the fruit has reached a diameter of approximately 11-12 mm. The larva feeds on the seed and, after four instars, leaves the nut by piercing a hole in the pericarp and dropping to the ground. It then burrows down, buries itself, and builds an overwintering chamber in which to hibernate. At the end of spring, adult insects emerge.

Traditionally, control of this weevil in the Monti Cimini area has involved the application of two or three chemical treatments per regular period (generally using Lambda-cyhalotrin a.i.). However, chemical treatments have a major effect upon the hazelnut agro-ecosystem, causing a build up of pesticides in nearby Lake Vico.

Earlier research identified the biological cycle of the weevil and established a preliminary treatment threshold (Paparatti, 1990; Pucci, 1991, 1992). More recently the adoption of this threshold, as part of guided and integrated pest control (IPM) strategies, has made it possible to considerably reduce the number of insecticide treatments against weevils. Despite the success of this pest control strategy, it was decided to carry out a preliminary trial with a biological control against *C. nucum*, using the entomopathogenous fungus *Beauveria bassiana* (Balsamo) vuill. (Deuteromycotina, Hyphomycetes).

B. bassiana was discovered by Bassi in 1835, during the description of a disease that affected silkworms (Bassi, 1836). However, it was only in the 80's that it developed its toxic action due to various toxic agents produced by the developing fungus (Roberts, 1981). *B. bassiana* can be found on several substrata (ground, dead insects, bark, etc.) and by 1987, Li had already catalogued over 700 species of arthropod.

Several studies have examined the use of *B. bassiana* as a biological control agent in agriculture (Deseo and Rovesti, 1982; Hluchy and Samsinakova, 1989; Rodriguez and Prassitoli, 1990; Ferron et al., 1991; Nguya, 1993; Puterka et al., 1994; Hsu and Quarles, 1995; Kaaya and Munyinyi, 1995; Quintela and McCoy, 1997; Yasuda et al., 1997; Krueger and Roberts, 1997; Bellotti et al., 1997; Bischoff and Reichmuth, 1997; Moino et al., 1998). In previous research, this entomopathogenic fungus has produced positive results in the control of species from the same insect family as *C. nucum* (cfr. Adane et al., 1996; Fernandez and Colmenares, 1997; Paparatti and Speranza, 1999; Meikle et al., 2001; Chikwenhere et al., 2001).

MATERIALS AND METHODS

The experiment was carried out in a biological hazelnut grove situated in the area of Caprarola (Viterbo, Italy) near Lake Vico (620 m above sea level). The hazelnut grove in question is typical of the hazelnut producing area of Northern Latium. The cultivars grown there are the 'Tonda Gentile Romana' and the 'Nocchione', used for pollination in a ratio of 10%. The plant layout was 5 x 5 m.

Sixteen sample plants were randomly chosen for our experiment. On 10th October 1998, the ground below the plant foliage was covered with nets. This made it possible to collect the last instars of the larvae of hazelnut weevil which had left the nuts to bury themselves and hibernate.

Ten cubic cages (60 x 60 x 60 cm) were constructed and covered with a thick nylon net (150 g/m^2, 160 warp-yarns and 60 weft-yarns) a few months before the beginning of the experiment. These cages were buried about 40 cm and then filled with the soil that had been dug out, thereby maintaining the original natural conditions

Larvae were collected by hand, twice a day: at around 8 a.m. and around 6 p.m. This frequent collection prevented the larvae stress that could be caused by their staying on the nets and trying to bury themselves. As soon as they had been collected, larvae were put on the soil inside the cages and allowed to bury themselves naturally.

Each cage contained 80 weevil larvae. On 10th October 2001, when all the larvae had been buried in the cages, the soil of five cages (treated cages) was treated with a larvicide product; *Beauveria bassiana* (Bals.)(strain JW-1, ATCC 74040, commercial name Naturalis® BioIntrachem Italia) containing 2.3 x 10^7 living spores per millilitre of product. The recommended dosage was used for the experiment: 12 cc of commercial formula, diluted in 1.5 L of water, evenly soaked around the inner soil layer of the five sample cages. The five sample cages were soaked with an equal quantity of water.

On 19th May 2002, the cages were collected and taken to the laboratory where larvae mortality was recorded together with the depth of hibernation. Data regarding larval mortality were collected and elaborated by INSTAT3 software (Graphpad, San Diego, CA, USA).

RESULTS

The emergence of larvae from the nuts, in the area of Lake Vico is summarised in Fig. 1, which shows its onset from the first ten days in September, with a peak in the second ten days of the same month. As can be seen, the emergence of larvae from nuts followed shortly after (a few days) rainfall at the end of summer. Indeed, more than 75% of larvae were collected in the two or three days following rainfall.

Recording the depth of larvae burial in the control cages allowed researchers to calculate percentiles for spatial distribution (Fig. 2). 25% of larvae were found in a layer between the surface and a depth of 10 cm; the remaining 75% were found between 10 and 20 cm from the surface.

Average natural mortality of *C. nucum* larvae in the control cages reached 63.5%. The average mortality observed in the treated cages (Fig. 3) reached 99.5%. This shows an average difference of 36% compared to the control cages. Statistical analysis of the relative larvae mortality risk allowed us to underline more than 45 times of mortality risk with comparison between the dead larvae of treated population and those dead of non-treated (control) population ($P \leq 0.0001$; 95% Confidence Interval: 11.385 to 179.23 using the Katz approximation).

DISCUSSION

This research permitted the identification of a correlation between rainfall in the month of September and the emergence of larvae from hazelnuts. This correlation will be further studied, but on first impressions it seems to have a bio-ethological basis. The larvae of last instar must carry out their burrowing and for this they need loose wet soil. If corroborated by future observations, the correlation may prove useful for indicating the best time at which to treat the soil. Depth of burying is strictly related to soil type and its humidity.

As observed in our research, the most important datum was the increase in mortality in hibernating larvae. Cages treated with *B. bassiana* showed a 35% increase in larvae mortality. This interesting datum confirmed a previous study and showed how the hypogeous period is critical for other weevils of the same family (Menu and Debouzie, 1995; Paparatti and Speranza, 1999).

Statistical analysis confirms the pure numerical data, showing a greater risk of mortality amongst treated larvae. These encouraging results encourage us to continue with our research with a view to soon formulating a strategy for achieving microbiological pest of the hazelnut weevil, which is a key pest in the hazelnut orchards of Northern Latium.

ACKNOWLEDGEMENTS

Research partially financed by Mi.P.A.F. Project "Studio e ottimizzazione della filiera coricola dell'area Cimino-Sabatina" D.M. 564/7303 10/11/2003.

Literature Cited

Adane, K., Moore, D. and Archer, S.A. 1996. Preliminary studies on the use of *Beauveria bassiana* to control of *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. J. Stored Product Res. 32:105-113.

Bassi, A. 1836. Del mal del segno, calcinaccio o moscardino, malattia che affligge i bachi da seta e sul modo di liberarne le bigattaie anche le più infestate. Orcesi, Lodi.

Bellotti, A.C., Cardona, C., Riis, L., Allsopp, P.G., Rogers, D.J. and Robertson, L.N. 1997. Burrowing bugs and whitegrubs, major soil pests of food crops in Colombia. Proc. 3rd Brisbane Workshop on Soil Invertebrates. p.130-133.

Bischoff, R. and Reichmuth, C. 1997. Virulence factors of entomopathogenic fungi of *Plodia interpunctella* (Hübner) and *Ephestia kuehniella* (Zeller) (Lep: Pyr.). Proc. Intl. Conf. on Pests on Agriculture, Le Corum, Montpellier, France, 6-8 January 1977, Vol. 3.

Carbone, A., Franco, S., Pancino, B. and Senni, S. 2004. Dinamiche territoriali e profili produttivi dell'agricoltura del Lazio. In: P.L. Cataldi (ed.), Quaderni di informazione socioeconomica della Regione Lazio, Italia.

Chikwenhere, G.P. and Vestergaard, S. 2001. Potential effects of *Beauveria bassiana* (Balsamo) Vuillemin on *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) a biological control agent of water hyacinth. Biological Control 21:105-110.

Fernandez, S.A. and Colmenares, X. 1997. Evaluacion de Beauveria spp. Para el control de *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) en el cultivo de la papa. Agron. Trop. Maracay 47: 249-257.

Ferron, P., Fargues, J. and Riba, G., 1991. Fungi as microbial insecticides against pests. p. 665-706. In: D.K. Arora, L. Ajello and K.G. Mukerji (eds.), Handbook of Applied Mycology: Humans, Animals and Insects, Vol. 2, Marcel Dekker, New York.

Kaaya, G.P. and Munyinyi, D.M., 1995. Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for tsetse flies (*Glossina* spp.) at developmental sites. J. Invertebrate Pathology 66:237-241.

Deseo Kovacs, K.V. and Rovesti, L. 1992. Lotta microbiologica contro i fitofagi. Edagricole, Bologna, Italia.

Hluchy, M. and Samsinakova, A. 1989. Comparative study of the cusceptibility of adult *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and larval *Galleria mellonella* (L.)(Lepidoptera: Pyralidae) to the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. J. Stored Products Research 25:61-64.

Hsu, C. and Quarles, W. 1995. Greenhouse IPM for western flower thrips. IPN Practitioner XVII, 4.

Krueger, S.R. and Roberts, D.W. 1997. Soil treatment with entomopathogenic fungi for corn rootworm (Diabrotica spp.) larval control. Biological Control: Theory and Applications in Pest Management 9: 67-74.

Leucona, R., Clement, J.L., Riba, G., Joulie, C. and Juarez, P. 1997. Spore germination and hyphal growth of Beauveria sp. on insect lipids. J. Econ. Entomol. 90:119-123.

Li, Z. 1987. A list of insect hosts of *Beauvera bassiana*. Proc. 1st. Nat. Symp. Entomogenous Fungi, Gongzhuling, Jilin (People's Rep. of China). p.1-10.

Meikle, W.G., Cherry, A.J., Holst, N., Hounna, B. and Markham, R.H. 2001. The effect of an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), *Sitophilus zeamais* Motschulsky (Col.: Curculionidae), and grain losses in stored maize in the Benin Republic. J. Invertebrate Pathol. 77:198-205.

Menu, F. and Debouzie, D. 1995. Larval development variation and adult emergence in the chestnut weevil *Curculio elephas* Gyllenhal (Col., Curculionidae). J. Appl. Entomol. 119:279-284.

Moino, Jr.A. Alves, S.B. and Periera, R.M. 1998. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored-grain pest. J. Appl. Entomol. 122:301-305.

Nguya, K. and Maniania, C. 1993. Effectiveness of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. For control of the sterm borer *Chilo partellus* (Swinhoe) in maize in Kenya. Crop Protection 12:601-604.

Paparatti, B. 1990. *Curculio nucum* L. Catture di adulti ed analisi dell'infestazione condotte nell'areale del lago di Vico (Viterbo) nel biennio 1989-90. Frustula Entomologica XIII(XXVI):93-112.

Paparatti, B. and Pucci, C. 1987. *Balaninus nucum* L. Coleottero Curculionide dannoso al nocciolo (*Corylus avellanae* L.) nell'areale Viterbese. Proc. Le problematiche del nocciolo, Capranica, Italia, 13 settembre. p.81-85.

Paparatti, B. and Speranza, S., 1999. Biological control of chestnut weevil (*Curculio elephas* Gyll.; Coleoptera, Curculionidae) with the entomopathogens fungus *Beauveria bassiana* (Balsamo) Vuill. (Deuteromycotina, Hyphomycetes). Acta Hort. 494:459-464.

Pucci, C. 1991. Il controllo dei fitofagi infeudati all'agroecosistema noccioleto nel viterbese. Proc. Lotta biologica ed integrata in agricoltura, Formia, Italia, 14-16 settembre. Biologia Oggi 5:13-18.

Pucci, C. 1992. Studies on population dynamics of *Balaninus nucum* L. (Col., Curculionidae) noxious to the hazel (*Corylus avellana* L.) in Northern Latium (Central Italy). J. Appl. Entomol. 114:5-16.

Puterka, G.J., Humber, R.A. and Poprawski, T.J. 1994. Virulence of fungal pathogens (imperfect fungi: Hyphomycetes) to pear psylla (Homoptera: Psyllidae). Environ. Entomol. 23:514-520.

Quintela, E.D. and McCoy, C.W. 1997. Pathogenicity anhancement of *Metarhizium* *anisopliae* and *Beauveria bassiana* to first instar of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with subletal doses of imidacloprid. Environ. Entomol. 26:1173-1182.

Roberts, D.W. 1981. Toxins of entomopathogenic fungi. p.441-465. In: H.D. Burges (ed.).

Rodriguez, C. and Prassitoli, D. 1990. Pathogenicity of *Beauveria brogniartii* (Sacc.) Petch and *Metarhizium anisopliae* (Mots.) Sorok. And its effect on the corn weevil and the bean beetle. Anais de Sociedade Entomologica di Brasil 19:302-306.

Yasuda, K., Yakayesu, K. and Uehara, K. 1997. Effects of temperature, humidity and conidial density on infection by *Beauveria bassiana* of adult sweer potato weevil, *Cylas formicarius* (Fabricius)(Coleoptera: Curculionidae). Japanese J. Appl. Entomol. Zool. 41:55-58.

Figures

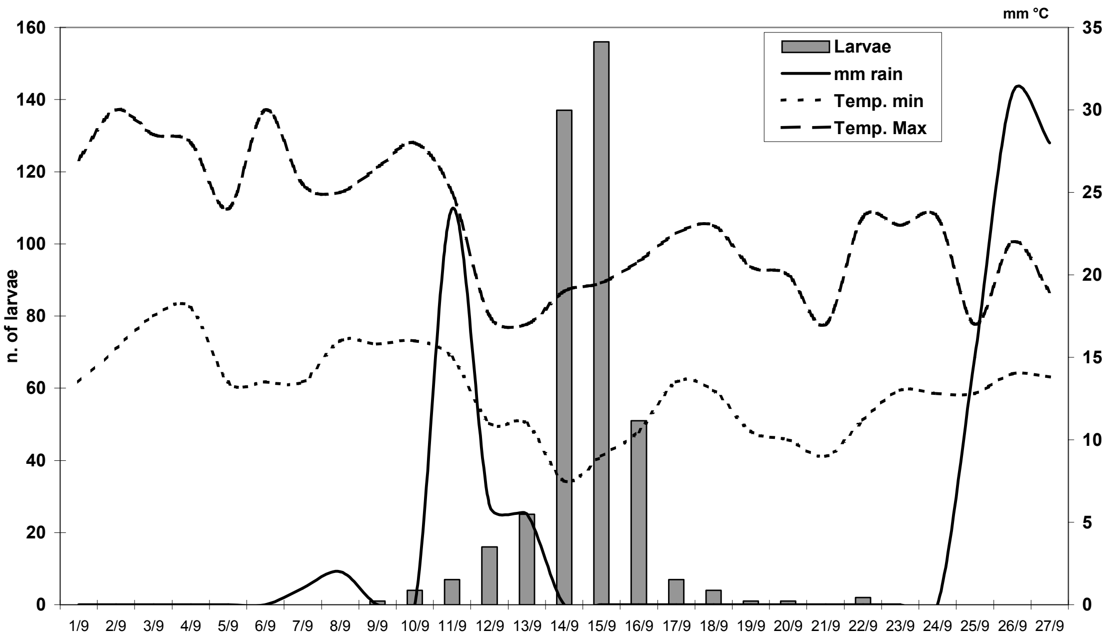

Fig. 1. Correlation between the emission of larvae from nuts and rainfall. Maximum and minimum temperatures are also shown.

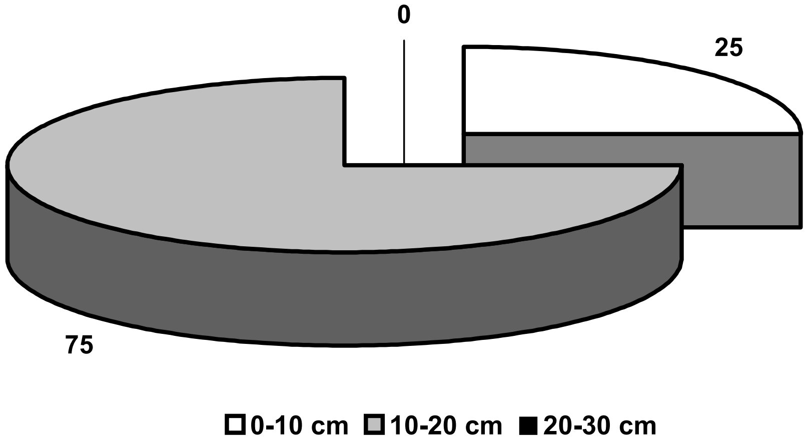

Fig. 2. Average depth of burial of *B. nucum* larvae in the cage control (percentage).

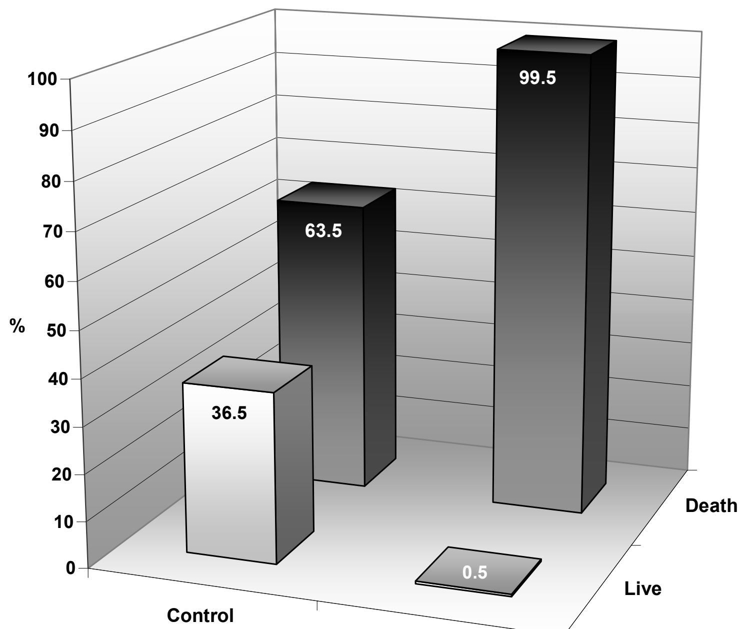

Fig. 3. Levels of mortality and average survival between the control cage and the cage treated with *B. bassiana*.