BIOLOGICAL CONTROL OF CHESTNUT WEEVIL (*Curculio elephas* Gyll.; Coleoptera, Curculionidae) WITH THE ENTOMOPATHOGEN FUNGUS *BEAUVERIA BASSIANA* (Balsamo) VUILL. (Deuteromycotina, Hyphomycetes)[1]

B. Paparatti and S. Speranza
Tuscia University,
Plant Protection Department
Via S. Camillo de Lellis
01100 Viterbo
Italy

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Abstract

This paper reports on results obtained in biological control of the chestnut weevil (*Curculio elephas* Gyll.) by means of the entomopathogen fungus *Beauveria bassiana* (Balsamo) Vuill. Experiments were carried out in a chestnut tree orchard of the Mountain Community of Monti Cimini (Viterbo, Italy). Cages measuring 60 cm in height, half-buried in the ground, were utilized. 100 larvae of chestnut weevils were placed in each cage, where they buried themselves spontaneously. A total of three trials was performed, in 1993-94, 1994-95 and 1997-98. A commercial strain of *B. bassiana* (Naturalis-L, Fermone Corporation, Phoenix, AZ) was utilized for the first two trials; while a strain provided by C.E.R. (Caffaro, Galliera, Bologna, Italy) was utilized for the third trial. Untreated control cages and cages treated with the entomopathogen fungus were used during the tests. The results, even though promising, presented high variability, probably due to the rainfall recorded during the test periods. Good results were obtained for the first and the second period (1993-94, 1994-95), while for the third period (1997-98) no significant differences between treated and untreated cages were observed.

1. Introduction

In the chestnut-growing area of the Monti Cimini (Viterbo, Italy) the key insect for table chestnut production is the chestnut weevil *Curculio elephas* (Gyll.) (Coleoptera, Curculionidae). In some areas and in certain years, infestation can affect as much as 90% of the production (Paparatti and Speranza, 1998).

Control of these insect populations presents a number of difficulties, for the following reasons:

- The chestnut ecosystem of the Monti Cimini area presents a considerable complexity, as shown in recent studies (Vitagliano *et al.*, 1993), which would be inadvisable to simplify;
- Since chestnut trees grow to a considerable height, with trees up to 25 m tall, spraying equipment (cannons) capable of directing the active ingredient up to the top of the canopy would be required, and this would lead to notable risk of drifting;
- In Italy the active ingredients annually registered for use on table chestnut are either extremely toxic, thus involving severe risks for public health and for the ecosystem in general, or else they are hazardous because of their mite-stimulating properties (cf. also Cinti *et al.*, 1995).

These problems prompted the authors to study biological and integrated methods for control of Curculionid populations. Trials were conducted with the entomopathogenic

[1] The authors equally contributed to the present research paper.

fungus *Beauveria bassiana* (Balsamo) Vuill., as this fungus had already been found on overwintering *C. elephas* larvae in the vicinity of the experimental field.

2. Biology of *Curculio elephas* (Gyll.)

In Central Italy adult curculionids appear between the end of August and mid-September (Cinti *et al.*, 1993; Speranza, 1998). Females lay their eggs by piercing the husk with their rostrum and inserting an egg into the hole. The newborn larva penetrates into the fruit within the husk and feeds on the amilaceous substratum of the kernel. As many as 19 larvae per chestnut fruit have been recorded, although generally each fruit hosts no more than 2 or 3 larvae (Cinti *et al.*, 1993; Desouhant, 1996). At the end of the larval stage the larva exits from the fruit by piercing a hole in the pericarp and dropping to the ground, where it buries itself at a depth ranging between 5 and 15 cm. It then builds a small overwintering chamber in the soil. (Cinti *et al.*, 1993). The pupae appear between the end of June and the month of July. The newly emerged adult remains in the overwintering chamber for three to four weeks (Fig. 1) and then exits from the soil for transfers to the canopy of the host trees. Thus the insect completes only one generation per year, although some larvae may remain in the ground in the larval state for several years (Menu, 1993; Paparatti and Speranza 1998).

3. Materials and methods

Investigations were carried out in a chestnut-growing area in the municipality of Vallerano (Viterbo, Italy), which lies at an altitude of 413 m, and is characterized by level ground. The most widespread chestnut variety in the experimental area (96%) is "Castagna" (or Maschio or Velletrana) (Bignami, 1990).

Experiments were repeated for three years, with the following methodologies:

1993-94: On 11, November 1993 four containers were buried, one of which had been treated, and the other three as controls. The containers measured 60x50x50 cm, and were buried up to half of their height. After one week, 100 mature *C. elephas* larvae were placed in each container (Fig. 2) and allowed to bury themselves naturally. Treatment was applied on 03 December 1993 using *Beauveria bassiana*-based NATURALIS-L insecticide (2,3 x 10^7 conidia per milliliter of product) manufactured by the Fermone Corporation Inc. The insecticide was supplied by Intrachem Italia s.r.l. of Grassobbio (Bergamo, Italy) and applied at the dose of 750 ml/300 l. water, as recommended in the manufacturer's instructions.

1994-1995: On 07 October 1994 four containers were buried, two of which had been treated and the other two used as controls. Treatment was applied on 13 June 1995. Number of larvae, active ingredient, doses and mode of treatment were the same as in the previous year.

1997-1998: On 13 October 1997 six containers were buried, 3 of which had been treated and the other three used as controls. In contrast to the two previous years, larvae were introduced one week after soil treatment. For this treatment *Beauveria bassiana* was supplied by the C.E.R. (Caffaro) (9x107 C.F.U./G). A dose of 4 g. of active ingredient per liter was used.

In all three trial years container soil tests were performed in the months of July and August of the following year. Tests were performed by transporting the soil to the laboratory in appropriate containers, where manual inspection for presence of insects (dead and alive) was carried out.

4. Results

As can be seen from the graphic (Fig. 3), natural mortality in the untreated containers was found to be elevated, reaching percentages of 71.33, 74 and 89% respectively in the three trial years.

In the first two years, mortality in the *B. bassiana*-treated containers was 100 and 99% respectively; in the third year, mortality reached no more than 90.76%, which was only slighly different from the natural mortality rate in the untreated containers. The increase in mortality observed in the first two years between treated and untreated containers was 28.67% and 25% respectively. Statistical analysis of the data shows that the comparison between treated and untreated containers gives a highly significant difference ($p \geq 0.01$) for 1994/95, significant ($p \geq 0.05$) for 1993/94 and non-significant for 1997/98.

Analysis of meteorological data (Fig. 4) shows that rainfall over the three trial years was 906.4, 453.4, and 1065.21 mm respectively during the experimental period (early October - end of August). These marked differences, particularly noteworthy in the third year (1997/98), may have affected the efficacy of the entomopathogen. In particular, it can be noted that in 1997, in the four weeks following treatment, substantial rainfall was recorded (a total of 383.2 mm), which may have washed away or affected the efficacy of the active ingredient.

Given the promising but so far dishomogeneous results of the trials, the authors intend to repeat the experiments with these bioinsecticides in the open field, over more extensive areas.

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Figure 1. Adult in overwintering chamber

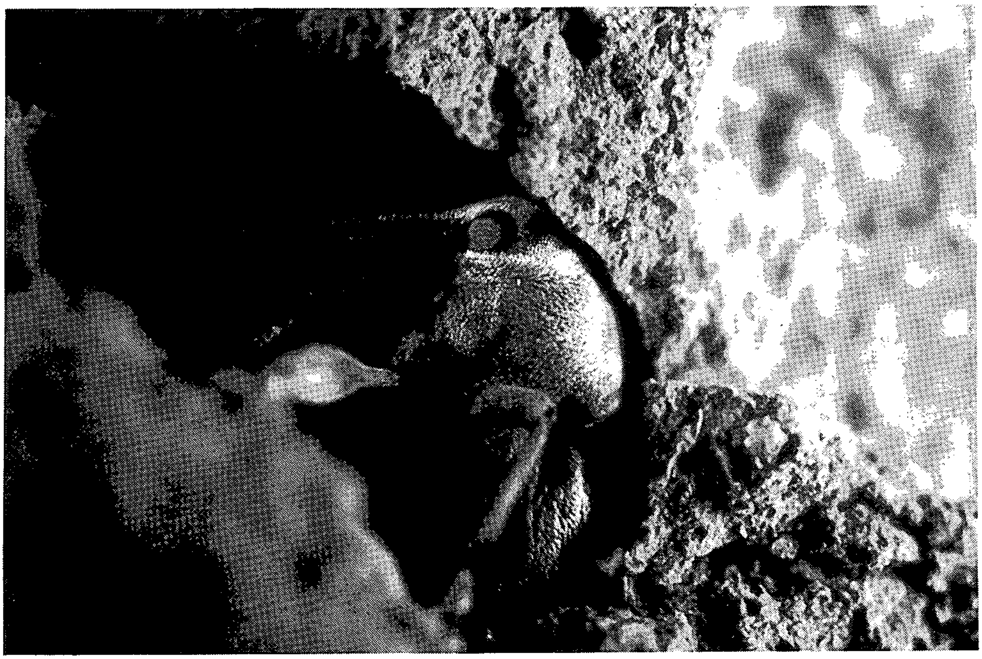

Figure 2. Experimental area with containers

Figure 3. Mortality of *C. elephas*

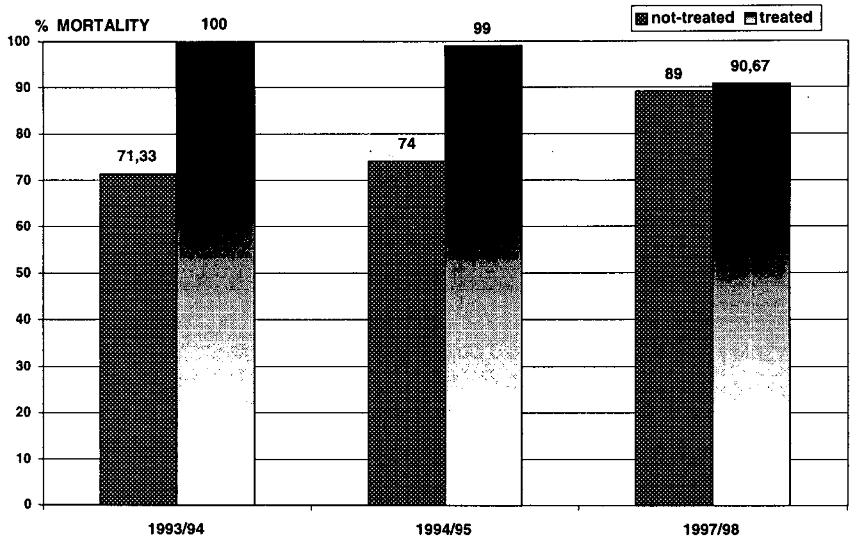

Figure 4. Meteorological data