

Biological notes on *Parahypopta caestrum* and first microbiological control assays

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Abstract

We provide notes about the biology and the ethology of *Parahypopta caestrum* (Hubner) (Lepidoptera Cossidae), based on field observations conducted in asparagus plantations in Apulia region, Italy. Furthermore, the effect of 6 entomopathogenic nematode (EPN) strains (Steinernematidae and Heterorhabditidae) and 3 entomopathogenic fungal (EPF) isolates (*Beauveria bassiana*) was evaluated in laboratory assays against III instar larvae of the asparagus moth. The results showed that all the nematodes and fungal strains affected the asparagus moth survival, except the *Steinernema affine* and *Heterorhabditis bacteriophora* strains. *Steinernema feltiae* and *B. bassiana* showed the best performances, killing on average 90% of the *P. caestrum* larvae. Considering the lack of effective chemical control means, the microbiological control of the asparagus moth by EPNs and EPFs reveals promising perspectives and needs further investigations.

Key words: asparagus moth, *Parahypopta caestrum*, Cossidae, Southern Italy, *Steinernema feltiae*, *Beauveria bassiana*.

Introduction

Asparagus spp. is a high-value, labour-intensive specialty crop. In Italy the most dedicated areas are located in Veneto region, immediately followed by Emilia-Romagna and Apulia. In Apulia, the asparagus cultivations are concentrated in the North of the region, in the province of Foggia, which contributes with its 20.6% to the Italian asparagus production (ISTAT, 2012). *Parahypopta caestrum* (Hubner) (Lepidoptera Cossidae) is a highly-destructive pest of *Asparagus* spp. in Europe. The soil-borne larvae bore galleries into the roots and the shoots, causing the total destruction of plantations after 2-3 years (Pollini, 1989). Due to its high destructiveness and the lack of effective control options available, *P. caestrum* can be considered the key pest of *Asparagus* spp. in the Apulia Region, where its presence has been recorded since the 1992. The lack of expert knowledge regarding the biology of *P. caestrum* and the limited range of available pesticides have contributed to the increase and the spread of the pest populations in the last 15 years (Tarasco, 2002).

To date, no effective control means are available against this pest. Nevertheless, *P. caestrum* has received little attention from the research institutions, probably because asparagus has been considered a "minor crop". Few data are available about the biology and the ecology of this pest, while control options such as microbial control have never been studied so far.

Entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) could be promising alternatives to chemical pesticides and have emerged as excellent biological control agents for soil-inhabiting pests (Copping and Menn, 2000; Butt *et al.*, 2001; Choo *et al.*, 2002; Flores *et al.*, 2002; Vestergaard *et al.*, 2002; Ansari *et al.*, 2003; 2004; 2008; Milner *et al.*, 2003; Grewal *et al.*, 2005; Georgis *et al.*, 2006; Lonzano Tovar *et al.*, 2013; Campos Herrera *et al.*, 2015). However, to our knowledge, the potential of fungi as biocontrol agents of

P. caestrum has not been examined so far, and only one experience about the effectiveness of EPNs against the asparagus moth is available in literature (Salpiggidis *et al.*, 2008).

Materials and methods

Biological and ethological notes on *P. caestrum*

Field observations were performed over a 3-year period (2011, 2012 and 2013) in several asparagus plantations in Apulia Region, through samples collection and data recording about the *P. caestrum* biology. The plantations were visited on May - June (to recover adults and eggs), July - August (larvae) and April (pupae).

Potential control of *P. caestrum* with EPN and EPF

Third instar larvae of *P. caestrum* were collected from infested asparagus plantation at Ortanova (Foggia province, Apulia) and reared in our laboratory on *Asparagus officinalis* L. plants.

Nematodes strains

Six isolates of EPNs, belonging to *Steinernema feltiae* Filipjev (2 strains: MF1 and G16), *Steinernema carpocapsae* (Weiser) Wouts Mracek Gerdin et Bedding (MR7 strain), *Steinernema arenarium* (Artyukhovsky) Wouts Mracek Gerdin et Bedding (C31 strain), *Steinernema affine* (Bovien) (CZ7 strain), *Heterorhabditis bacteriophora* Poinar (CE1 strain) were collected using the "Galleria baiting technique" (Bedding and Akhurst, 1975) during a soil survey in different habitats in Italy (Tarasco *et al.*, 2015a; 2015b). Nematodes were cultured in greater wax moth last-instar *Galleria mellonella* L. (Lepidoptera Pyralidae) larvae at 22 °C. To obtain fresh infective juveniles (IJs), 10 *G. mellonella* were placed on filter paper in a 100 × 10 mm Petri dish and treated with ca. 2,000 IJs suspended in 1.5 ml of tap wa-

ter. After a two-week treatment, *G. mellonella* larvae were put on modified White traps (White, 1927) for the recovery of new generations of IJs. Nematodes suspensions were prepared diluting 300,000 IJs of each strain in 100 ml of tap water.

Fungal strains

Three strains of *Beauveria bassiana* (Balsamo) Vuillemin (RF1, ALB55 and OF13) were used in our experiment. These strains are deposited in the collection of Department of Soil, Plant and Food Sciences (Bari, Italy), stored at 4 °C in glass tubes provided with a cotton cap and containing potato-sucrose-agar. They were previously selected upon their *in vitro* thermal regime and virulence against several insects such as *G. mellonella*, *Tenebrio molitor* L., *Trialeurodes vaporariorum* (Westwood) and *Ceratitis capitata* (Wiedemann) (Oreste *et al.*, 2012; 2015; 2016).

Fungal inoculum was prepared by growing the strains in Petri dishes (90 mm diameter) containing 2% malt extract-agar and incubated at 25 °C in the dark for 15 days. Then, conidia were harvested by pouring sterile distilled water with the addition of 0.002% Tween 80 (Sigma Aldrich) in the Petri dish. The concentration of conidia was estimated using a Malassez chamber and adjusted to 2×10^8 conidia ml⁻¹.

Pathogenicity assay

The experiment was carried out in pots (16 cm diameter) containing field soil collected from asparagus plantations. In each pot, 8 III instar larvae of *P. caestrum* were introduced. After 24 hours (all the larvae had deepened in the soil), each pot was inoculated with 100 ml of nematodes or fungal suspension, while the control pots were treated with 100 ml of sterile water. The pots were then incubated at 25 °C for 7 days, irrigated daily with water in order to avoid the soil drying. A complete randomized block design was used, with four replicates, each including 1 pot. After 7 days, the larvae were removed from the pots and the following data were recorded: alive larvae, mycosed larvae, larvae infected by nematodes, dead larvae due to other causes (natural mortality). Mortality caused by the entomopathogenic nematodes and fungal strains was confirmed by re-isolation of nematodes and fungi. Data were submitted to the logistic regression, considering the independent variables “Microorganism” (EPNs/EPFs) and “Strain (Microorganism)” (strain nested within the microorganism factor). Means were then compared with the least-squares means statistics ($P < 0.05$).

Table 1. Logistic regression statistics for the pathogenicity of different microorganisms (EPNs/EPFs) and strains against *P. caestrum* larvae.

Source of variation	Degree of freedom	χ^2	$P > \chi^2$
Microorganism	2	106.68	<0.0001
Strain (Microorganism) ^a	7	126.59	<0.0001

^a The “Strain” variable is nested within the “Microorganism” variable.

Results

Biological and ethological notes on *P. caestrum*

The asparagus moth adults may be observed in the field from May until June. Their life span is extremely brief (from 3 to 8 days); they show nocturnal activity only, without feeding. After mating, at the end of May, the females lay 100-200 eggs in clusters of about 40 on the stem collar of asparagus, just beneath the surface of the soil; several females can lay on the same stem. The embryonic development lasts on average 15 days. At the hatching (end of June), the larvae are grouped in a silken shelter, they then move down into the ground in groups and penetrate, either the underground part of the stem at a depth of between 5 to 10 cm, or the bud. They first feed on sub surface stem and root buds and then move on the fleshy asparagus roots. In autumn, the mature larvae crawl deeper into the soil (40-50 cm of depth) and they overwinter in diapause for 5 months in silken cocoons. The overwintering larvae return near to the surface from the end of April to the beginning of June; then they spin their cocoons vertically orientated in the soil, where they pupate. The pupal development lasts from 3 to 5 weeks. The flight period of new-emerged moths ranged from May to June in field, while in tunnel plantations it occurs at the end of April. If rainy or cold conditions occur, particularly during the flight period, the biological cycle of *P. caestrum* could delay of 10-15 days.

The entire life cycle of *P. caestrum* occurs on *Asparagus* spp. The damages are caused by the trophic activity of the larvae, which attack the root system, feeding on the buds and hollowing out the roots. The larvae prefer the root system, but they can enter the plants through the roots and invade the shoots (20-40 larvae/shoot). The first symptoms are yellowing and drying of plants, which completely dry up during the summer: the above-ground stems wilt and healthy root buds are destroyed. Young plantations are particularly vulnerable. The infestations are almost always first observed at the edges of the plantation and may spread across an entire plot in a 2-3 years' time.

Potential microbial control of *P. caestrum* with EPNs and EPFs

Results of logistic regression revealed that both the selected variables “Microorganism” and “Strain (Microorganism)” influenced the larval mortality of *P. caestrum* (table 1). Considering the type of microorganism, EPFs resulted more effective than EPNs, although results could be influenced by the unbalanced number of strains tested. All the nematodes and fungal strains were pathogenic against *P. caestrum* larvae, except the two nematodes strains of *S. affine* CZ7 and *H. bacteriophora* CE1 (figure 1). *S. feltiae* MF1 and *B. bassiana* OF13 showed the best performances (killing the 96.87% and 93.75% of *P. caestrum* larvae respectively), followed by *B. bassiana* RF1 and *S. feltiae* GF16 (90.62%) and *B. bassiana* ALB55 (87.5%). The nematodes strains *S. carpocapsae* MR7 and *S. arenarium* C31 induced respectively the 53.12% and 40.62% of cumulative mortality. The natural mortality was the 3.12% in the control while in the other experiments it ranged from the 0 to the 6.25%.

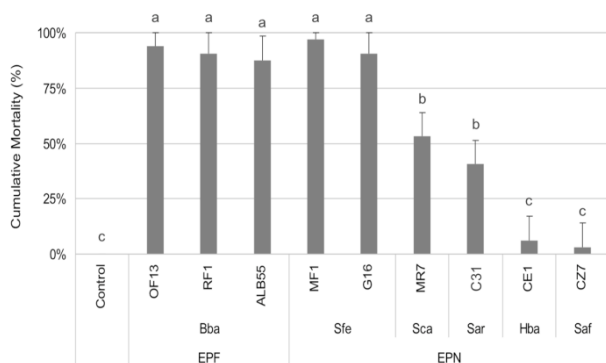


Figure 1. Pathogenicity of EPNs and EPFs strains on *P. caestrum* III instar larvae. Bba: *B. bassiana* strains (OF13, RF1, ALB55); Sfe: *S. feltiae* (MF1, G16); Sca: *S. carpocapsae* (MR7); Sar: *S. arenarium* (C31); Hba: *H. bacteriophora* (CE1); Saf: *S. affine* (CZ7). Within each variable, means with different letters are significantly different according to the least squares means statistics ($P < 0.05$). Vertical bars indicate the 95% upper confidence intervals.

Discussion and conclusions

Data from field and laboratory observations about the *P. caestrum* morphology and biology represent a contribution to the knowledge of this pest. Considering the morphological and biological features of the asparagus moth, we found differences with the literature. We recorded that mature larvae of *P. caestrum* are 55-70 mm in length while Grandi (1930) and Pollini (1989) reported lower sizes (40-50 mm). Furthermore, we found that the flight period ranged from May to the beginning of June in field (with 10-15 days delayed in raining periods) and from April to May in tunnel plantations, while other authors reported a wide flight period with peak in July (Grandi, 1930; Pollini, 1989).

Due to its biology and ethology, *P. caestrum* may be included in the category of pests residing in cryptic habitats, as insects which bore into the plant tissue (wood-boring insects) or under the bark (bark beetles) or in the soil (wireworms). These pests are very difficult to control because chemical pesticides are not able to penetrate into cryptic habitats and reach the target (Gumus *et al.*, 2015). Usually, the only option to reduce infestations is the removal and destruction of infested or injured plants (Langstrom *et al.*, 2004; Gumus *et al.*, 2015).

The asparagus moths cannot be effectively controlled by chemical insecticides. Repeated applications of insect growth regulators (IGRs) and other insecticides, such as Chlorpyrifos-ethyl, can reduce the damages but not under an economic damage threshold (Salpiggidis *et al.*, 2008). Removing cocoons before the adult emergence and postponing the harvest until June (cutting the stems 3-4 cm under the soil surface in order to remove the eggs) may limit the population increase, though these practices are highly labour-intensive and expensive.

Nematodes and fungi are able to penetrate the cryptic habitats because they are living organisms and may be horizontally transmitted by infected hosts. The potential

of EPNs and EPFs as alternatives to chemical insecticides against pests into cryptic habitats was revealed by several authors (Kreutz *et al.*, 2004; Marannino *et al.*, 2007; Martinez de Altube *et al.*, 2008; Demibilio *et al.*, 2010; Ashtari *et al.*, 2011; Gumus *et al.*, 2015).

Our results showed that EPNs and EPFs were effective against *P. caestrum* larvae in laboratory assays, although their effectiveness varied according to the species. *S. feltiae* and *B. bassiana* showed the best performances, by killing on average 90% of the *P. caestrum* larvae in laboratory assays. Considering the efficacy of EPNs, our results partially agree with those of Salpiggidis *et al.* (2008), who found that both *S. feltiae* and *H. bacteriophora* provided high larval mortality of *P. caestrum* (70-90%) in laboratory assays, although *S. feltiae* induced a more rapid effect. Furthermore, they recorded that diapausing larvae were less susceptible than the 1st and 2nd instar larvae. No data are available about the pathogenicity of EPFs against *P. caestrum*, thus our results represent the first report. Nevertheless, the effectiveness of the EPFs and EPNs as bio-agents against *P. caestrum* in asparagus needs to be confirmed in the field. The best period for the application of EPFs and EPNs in the field could be just after the egg hatching (on average 25-30 days after the first captures of adults, recorded with light traps), in order to reach the most susceptible stages (young larvae) during their migration to the roots. On the other hand, the soil moisture represents the main factor limiting the effectiveness of microbial control agents in asparagus field, particularly after the harvest, when usually the irrigation is suspended. To assure the optimal humidity level of soil for EPFs and EPNs survival and activity it would be necessary to provide supplementary irrigations after the microbial agents application in field (2-3 times/week or more in relation to the climatic conditions). Considering the high destructiveness of the asparagus moth, it is advisable a major attention from the research institutions and a territorial approach to the *P. caestrum* control, including the microbial control as a valid alternative to chemical pesticides.

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