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Potential of entomopathogenic fungi and nematodes against the two cryptic species *Parahypopta caestrum* and *Cossus cossus* in laboratory assays

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Abstract: Preliminary assays were performed in laboratory conditions in order to evaluate the infectivity of several entomopathogenic fungi (EPFs) and entomopathogenic nematodes (EPNs) autochthonous strains against *P. caestrum* and *C. cossus* larvae. Results revealed the efficacy of these microbial control agents in killing the larvae, although a wide inter- and intra-specific variability in virulence was detected among different microbial strains. Considering the lack of effective chemical control means, the microbial control of the Asparagus moth and the goat moth by EPNs and EPFs reveals promising perspectives and needs further investigations.

Key words: *Cossus cossus*, *Parahypopta caestrum*, microbial control

Introduction

Parahypopta caestrum (Hübner) and *Cossus cossus* (L.) (Lepidoptera, Cossidae) are highly-destructive cryptic pests in Europe. *P. caestrum* can be considered the key pest of *Asparagus* spp. in Italy, due to its high destructiveness and the lack of effective control options available. The soil-borne larvae bore mines into the roots and the shoots, causing the total destruction of plantations after 2-3 years. The goat moth *C. cossus* (L.) is a wood-boring pest whose larvae bore large galleries under the bark and even deeply into trunks and branches of fruit and forest trees, reducing plant growth and vigour, and causing limbs and branches to fall.

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) are very difficult to control because chemical pesticides are not able to reach the target (Gumus *et al*., 2015). Usually, the only option to reduce infestations is the removal and destruction of infested or injured plants (Gumus *at al*., 2015). One potential alternative to chemical insecticides for the control of cryptic insects can be the use of microbial control agents, as entomopathogenic nematodes (EPNs) and fungi (EPFs), because they may be able to penetrate into cryptic habitats and to be horizontally transmitted within the pest populations (Kreutz *et al*., 2004; Marannino *et al*., 2007; Demibilio *et al*., 2010; Ashtari *et al*., 2011; Gumus *et al*., 2015).

Material and methods

III instar larvae of *P. caestrum* (collected from infested Asparagus plantation) and *C. cossus* (from infested cherry orchard) were treated with 5 nematodes (*Steinernema feltiae, S. carpocapsae, S. arenarium, S. affine, H. bacteriophora*) and 3 fungal (*B. bassiana*) entomopathogenic autochtonous strains. The experiment agaist *P. caestrum* was carried out in pots (16 cm diameter) containing field soil collected from asparagus plantations. In each pot, 5 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 (300,000 Ijs) or fungal (2×10^8 conidia/ml) 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. The assay agaist *C. cossus* was carried out in Petri dishes provided, with filter paper and cherry bark pieces. In each dish, 5 larvae were introduced. Larvae were treated with single nematodes (300 Ijs/larva) or fungal (immersion for 20 seconds in 2×10^8 conidia/ml suspension) strain. The Petri dishes were then incubated at 24 °C for 7 days, recording the daily mortality. The following data were recorded: alive larvae, larvae with signs of fungal outgrwoth, 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 and means were then compared with the least-squares means statistics ($P < 0.05$).

Results and discussion

As shown in Figure 1, all the nematodes and fungal strains affected the Asparagus moth and the Goat moth survival. *Steinernema feltiae, M. anisopliae* and *B. bassiana* showed the best performances against both the pests, killing more than the 90% of the treated larvae. Considering the lack of effective chemical control means, the microbial control of the cryptic species by EPNs and EPFs reveals promising perspectives and needs further investigations. Nematodes and fungi are able to penetrate the cryptic habitats because they are living organisms and may be horizontally transmitted by infected hosts; nevertheless, their effectiveness as bio-agents against *P. caestrum* and *C. cossus* needs to be confirmed in the field.

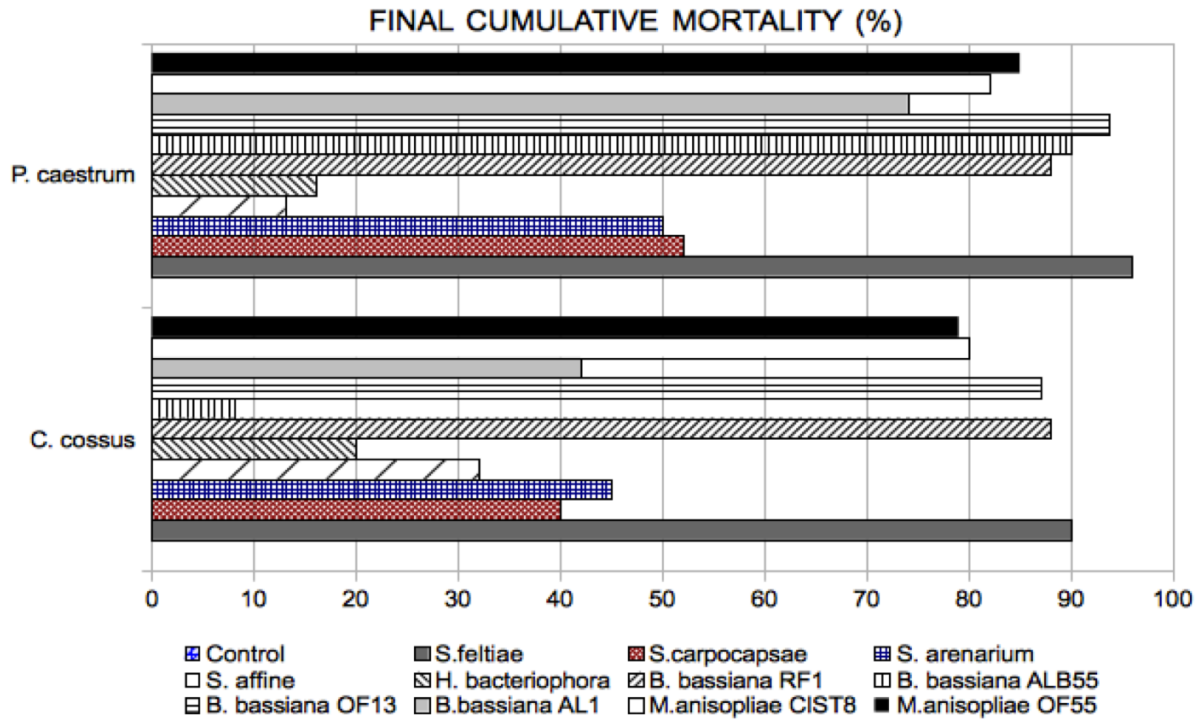

Figure 1. Effect of EPFs and EPNs on *P. caestrum* and *C. cossus* final cumulative mortality.

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