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Potential microbial control of xylophagous pests with entomopathogenic nematodes and fungi

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Abstract: The effects of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and fungi strains (*Beauveria bassiana*) were evaluated in laboratory assays against larvae of four xylophagous pests: the Asparagus moth *Parahypopta caestrum*, the European goat moth *Cossus cossus*, the pine longhorn *Arhopalus syriacus* and the black Buprestid *Capnodis tenebrionis*. Due to their biology and ethology, these insects may be included in the category of pests residing in cryptic habitats. The control of these species is very difficult, due to the inability of chemical pesticides to penetrate the cryptic habitats and reach the targets. The results showed that all the nematodes and fungal strains affected the insect survival. *Steinernema feltiae* and *Beauveria bassiana* showed the best performances. Considering the lack of effective chemical control means, the microbial control of the xylophagous pests by EPNs and EPFs reveals promising perspectives. Nematodes and fungi are able to penetrate the cryptic habitats because they are living organisms and may be horizontally transmitted by infected hosts. The distribution of EPF as preventive control method and the injection of EPNs suspensions to reach and infect the larvae inside the wood galleries can be a combined sustainable control system.

Introduction

Entomopathogenic fungi (EPFs) and nematodes (EPNs) could be promising alternatives to chemical pesticides and have emerged as excellent biological control agents for soil-inhabiting pests (Grewal et al., 2005; Lonzano Tovar et al., 2013; Campos Herrera et al., 2015). However, the potential of EPF and EPN as biocontrol agents against xylophagous insects has not been studied enough. Xylophagous or wood-boring insects reside in cryptic habitats in the xylem or under the bark of the plant which make them difficult to control by chemical insecticides. Among xylophagous insects in the Mediterranean, *Parahypopta caestrum* (Hübner) (Lepidoptera, Cossidae), *Cossus cossus* (Linnaeus) (Lepidoptera, Cossidae), *Capnodis tenebrionis* (Linnaeus) (Coleoptera, Buprestidae) and *Arhopalus syriacus* (Reitter) (Coleoptera, Cerambycidae) are highly destructive cryptic pests.

Firstly, *Parahypopta caestrum* can be considered the key pest of *Asparagus* spp. in Italy, as it is highly destructive and due to the lack of effective control options. The soil-borne larvae bore mines into the roots and the shoots, causing the total destruction of plantations in 2-3 year

(Pollini, 1989). The goat moth *C. cossus* 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 vigor, and causing limbs and branches to fall (Gumus et al., 2015). The Mediterranean flat-headed peachborer *C. tenebrionis* is a destructive pest of cultivated Rosaceae fruit trees. As referred by its name it is present in many Mediterranean countries, for example in southern Italy heavy attacks were reported on stone fruit trees. *Capnodis tenebrionis* usually attacks stressed or declining trees but also healthy plants. Eggs are laid during the summer on the trunk base and in the vicinity of it, either on superficial roots or freely in the soil (Marannino and de Lillo, 2007). Lastly, *Arhopalus syriacus* is native of the eastern Mediterranean region, but also found in Italy and Greece (Brown, 1968; Martelli, 1952). It develops in pine trees mainly. This wood borer has induced the mortality of numerous trees in Salento Italy and Lebanon in the middle East. The larvae feed first under the bark and they bore later into the sapwood of dying trees. Adults of this nocturnal species can be found on the host plants at night or in bark crevices and under loose bark (Webb and Eldridge, 1997).

Pests residing in cryptic habitats, as xylophagous insects that bore into the plant tissue (wood-boring insects) or under the bark (bark beetles) are very difficult to control because chemical pesticides are not able to reach them in their habitat (Gumus et al., 2015). Due to the absence of effective treatment methods, phytosanitary control measures of cutting and incineration of infested trees are considered (Gumus et 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). Therefore, the aim of the present work is to test the pathogenicity of native entomopathogenic fungi (EPFs) and nematodes (EPNs) against four different xylophagous insects and to evaluate their potential use against larval stages as substitute to the chemical insecticides being ineffective to control the xylophagous pests and also prohibited in forest habitats.

Material and methods

Nematodes and fungal isolates

Bioassays were carried out with isolates of EPNs belonging to *Steinernema feltiae* (Filipjev) (GR1, MF1, GF16, ALG18, OT15, GR1, LIB 132), *S. affine* (Bovien), *S. arenarium* (Artyukhovsky) (C31), *S. carpocapsae* (Weiser) (MR7), *S. apuliae* (CS3, LE13) and *Heterorhabditis bacteriophora* (Poinar) (LU1, D1). EPNs were collected using the "*Galleria* baiting technique" (Bedding, 1975) during a soil survey in Lebanon and Italy (Tarasco et al., 2015; Noujeim et al., 2016). To obtain fresh infective juveniles (IJs), nematodes were inoculated in last-instar *Galleria mellonella* (Lepidoptera, Pyralidae) larvae at a temperature of 22 °C on a 100 × 10 mm Petri dish with one 90 mm filter treated with ca. 2,000 IJs in 1.5 ml of tap water (Tarasco et al., 2015). Dead last-instar larvae were put on modified White traps (White, 1927); juveniles emerging from *Galleria* cadavers were collected and used in the bioassay. Isolates of *B. bassiana* (OF13- RF1-ALB55-CG2-OF13) obtained from soils of Italy, within previous studies (Tarasco and Triggiani, 1997; Tarasco and Triggiani, 2007) were used. *Beauveria bassiana* solutions and concentrations were prepared following its culture on Malt dextrose agar and counted using a Neubauer Hemacytometer.

Infectivity bioassays of Entomopathogenic nematodes and fungi against xylophagous larvae
The pathogenicity of *S. feltiae, H. bacteriophora and, B. bassiana* was tested under laboratory conditions against four xylophagous pests: the *Asparagus* moth *Parahypopta caestrum*, the European goat moth *Cossus cossus*, the pine longhorn *Arhopalus syriacus* and the black Buprestid *Capnodis tenebrionis.* Assays were conducted in Glass petri dishes (95 × 32 mm) filled with bark and frass from same environment of the larvae. For each xylophagous pest, five larval individuals were treated with a single fungal (2×10^5 conidia/ml) or nematodes strain (100 and 200 IJs/individual) and a single larva was considered a replicate. Sterile distilled water with 0.002% Tween 80 was used as control for EPF bioassays while only tap water was used as a control in EPN bioassays.

Another technique was adapted using EPN suspension of 100 and 200 IJs per larva. After the collection of the *Capnodis tenebrionis* and *Arhopalus syriacus* larvae from the wood trunks, they were introduced again into the galleries. Then, the two concentrations of EPNs (100 and 200 IJs/individual) diluted in 10 ml of tap water were injected into galleries using a micropipette. Then, the galleries holes were covered by a mesh fabric.

Larval mortality was recorded after 72-hrs of exposure to IJs. Afterwards, the dead larvae were removed from the boxes, rinsed in tap water and dissected after 48 hours to determine effective nematode infection.

Statistical analysis
Statistical analyses and plots were performed using the software IBM SPSS-Statistics 22. The results data were analysed using a general linear model procedure (ANOVA – analysis of variance) and significant differences among means were separated by HSD Tukey's test. The minimum level of significance was taken as $p < 0.05$.

Result and discussion

The bioassays showed that all the nematodes and fungal strains affected the survival rate of *P. caestrum, C. cossus, C. tenebrionis* and *A. syriacus.* Various strains of *Steinernema feltiae* and *B. bassiana* showed the best performances against all the pests, killing between 67% and 100% of the treated larvae. In the bioassays against *P. caestrum,* all *Beauveria bassiana* strains (OF13, RF1, ALB55) caused high mortality (87-98%), also three *S. feltiae* strains (GR1, MF1, GF16) induced between 87% and 100% mortality to *P. caestrum* larvae. However, low mortality rates were recorded by *S. affine, S. arenarium* and *H. bacteriophora.* In the experiments targeting *C. cossus,* three *S. feltiae* (GR1-ALG18-OT15) caused the highest mortality (80-100%). Nevertheless, . *bacteriophora* (LU1) and the remaining *S. feltiae* strains were the least effective. As for *B. bassiana,* the strains (OF13, RF1, CGD2) caused 90% of mortality. Concerning *C. tenebrionis, S. feltiae* in the strains OT15 and GR1 caused respectively 100% and 80% mortality rate. But no mortality we recorded by *H. bacteriophora.* As for the entomopathogenic fungi, two *B. bassiana* strains (CG2, OF13) recorded different results, respectively 23% and 76%. Lastly, the bioassays against only *S. feltiae* (LIB 132) succeeded to kill 67% of *Arhopalus syriacus* larvae.

Considering the lack of effective chemical control means, the microbial control of the cryptic species by EPNs and EPF reveals promising perspectives and needs further investigations. As living organisms, nematodes and fungi are able to penetrate the cryptic habitats and may be horizontally transmitted by infected hosts. The distribution of EPF as preventive control method and the injection of EPNs suspensions to reach and infect the larvae inside the wood galleries could be a combined sustainable control system. Nevertheless, their

effectiveness as bio-agents against *P. caestrum*, *C. cossus*, *C. tenebrionis* and *A. syriacus*, needs to be confirmed in the field.

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