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Susceptibility of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) pupae to entomopathogenic nematodes

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Abstract

The olive fruit fly *Bactrocera oleae* is one of the most serious and economically damaging insects worldwide, affecting the quality and quantity of both olive oil and table olives. Laboratory bioassays were conducted for the first time to evaluate the susceptibility of *B. oleae* pupae to two entomopathogenic nematodes (EPN) species, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. The nematodes tested caused pupal mortality of 62.5% and 40.6%, respectively. The most noteworthy result was obtained with *S. carpocapsae* which was able to infect 21.9% of the emerged adults. Since this tephritid fly spent several months in the soil as pupa, the use of EPNs could be a promising method to control this pest.

Key words: biological control, fruit flies, *Heterorhabditis*, olive, *Steinernema*, Tephritidae

Introduction

Olive cultivation constitutes a key element of the Mediterranean agricultural sector. Italy ranks second in the world (after Spain), producing approximately 2 mln t of olive fruit, harvested from 1,156,784 ha (FAOSTAT 2015). Like many other crops, olive trees are affected by a wide number of pests. Among them, the most serious and economically damaging insect worldwide is the olive fruit fly, *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae). Over the last 40 years, the principal method of managing this insect pest has been through the use of conventional pesticides, particularly organophosphates. Recently, even though most pest management strategies still rely on the use of synthetic pesticides, elevated awareness of negative impacts due to the use of such products on the environment and human health have resulted in efforts to reduce reliance on chemical controls (Skouras *et al.* 2007; Amvrazi and Albanis 2009).

In order to reduce the use of insecticides, alternative and environmentally friendly plant protection methods are constantly being developed (Daane and Johnson 2010). This may include the use of entomopathogenic organisms, such as entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis*, which are widely regarded as promising biological control agents for a broad range of insect pests with life stages in the soil and cryptic habitats (Grewal *et al.* 2005).

Third instar larvae (Yee and Lacey 2003; Toledo *et al.* 2005; Barbosa-Negrisoli *et al.* 2009; Malan and Manrakhan 2009; Rohde *et al.* 2012; Langford *et al.* 2014; Shaurub *et al.* 2015) and pupae (Barbosa-Negrisoli *et al.* 2009) of several tephritid flies were reported to be susceptible to EPNs. To date, only one laboratory study has been carried out by Sirjani *et al.* (2009) on the olive fruit fly. They evaluated the susceptibility of the third-instar (pre-pupal) stage of *B. oleae* to six commercially available EPNs.

Under laboratory conditions we determined for the first time the susceptibility of *B. oleae* pupae to two EPN species, the most frequently used *Steinernema carpocapsae* (Weiser, 1955) and *Heterorhabditis bacteriophora* Poinar, 1976.

Materials and Methods

Bactrocera oleae pupae used in the experiments were obtained from naturally infested fruit collected from trees in an organic olive grove in Impruneta (Florence, Tuscany, Central Italy) in September 2016. Harvested olives were stored on Petri dishes at 4°C before being used and pupae were extracted from olives with sterilized forcipes and used within one week from collection.

The susceptibility of *B. oleae* pupae was tested with two Italian EPNs: *Steinernema carpocapsae* ItS-CAO1 (collection of CREA-DC, Torrini *et al.* 2014) (SCC) and *Heterorhabditis bacteriophora* ItH-LU1 (collection of the Section of Entomology and Zoology – DISSPA, University of Bari, Tarasco *et al.* 2015) (HB). These nematode species were reared at 24°C in greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae and the infective juveniles (IJs) that emerged from cadavers were recovered using modified White traps (Kaya and Stock 1997). After storage at 12°C for 2 weeks, they were kept at 20°C for 24 h prior to testing.

The susceptibility assays with *B. oleae* pupae were performed in 24-well plates (COSTAR®, Corning, New York), filled with about 2 g of sterile sandy loam soil (pH 5.8–5.9).

A single pupa was inserted into the bottom of each well; 100 IJs/0.5 ml of distilled water were inoculated onto the soil surface (n = 32 for each strain). In the control (n = 32), only sterile water was added to each well. Wells were incubated in the dark at 20±2°C and 60–80% relative humidity (RH). Adult emergence and mortality were recorded daily for a period of 15 days. Dead pupae and adults were dissected at the end of the period to assess nematode infection. The mortality caused by EPNs was compared by means of contingency table analysis and the χ^2 test.

Results

The two EPNs caused higher cumulative mortality than the control, where no infection was observed (χ^2 = 28.537; df = 2; p = 0.0001). In particular, SCC induced 62.5% and HB 40.6% more mortality than the control (χ^2 = 29.091; df = 1; p = 0.0001 and χ^2 = 16.314; df = 1; p = 0.0001, respectively). However, there was no difference in pupal mortality between the two EPNs (χ^2 = 3.065; df = 1; p = 0.08).

Interestingly, while in HB the EPN caused the death of pupae in SCC we found a 21.87% mortality in emerged adults, probably infected during the emergence from the bottom to the top of each well.

Differences in pupae appearance were observed. The ones infected by *H. bacteriophora* developed a typical red colour due to the pigment produced by the enteric bacteria *Photorhabdus luminescens*, while the

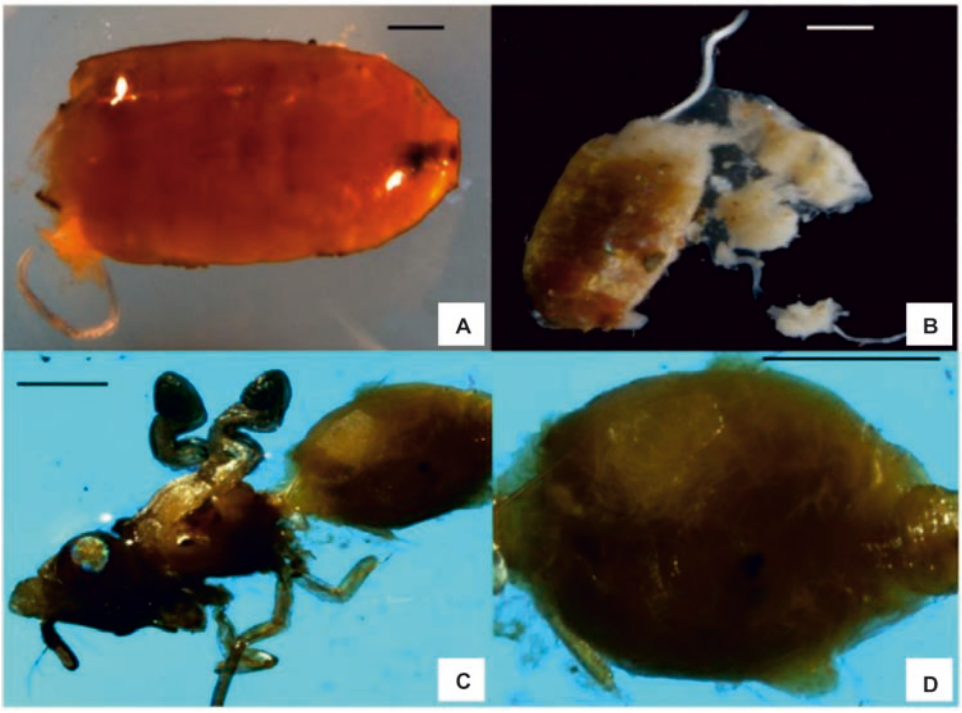

Fig. 1. Dead *Bactrocera oleae* pupa infected by: *Heterorhabditis bacteriophora* (A) and *Steinernema carpocapsae* (B). Dead *Bactrocera* oleae adult killed by *S. carpocapsae* (C) and a detail of entomopathogenic nematode strains (EPNs) infection inside the abdomen (D) (scale bars = 1 mm)

pupae with *S. carpocapsae* maintained a light-brown coloration (Forst and Nealson 1996; Singh *et al.* 2012) (Fig. 1A and B).

Steinernema carpocapsae also caused mortality of the *B. oleae* emerged adults. Dead adults showed wings that were not fully spread (Fig. 1C), and adult and juvenile nematodes in the entire body (e.g. abdomen; Fig. 1D).

Discussion

For the first time it was shown that *B. oleae* pupae are susceptible to the EPNs which were tested. Even though the olive fruit fly is economically important, only one laboratory study on the third-instar stage had been previously performed (Sirjani *et al.* 2009).

All of the studies conducted on tephritid flies have shown that larvae are the most susceptible stage to EPNs infection (i.e. Yee and Lacey 2003; Barbosa-Negrisoli *et al.* 2009; Langford *et al.* 2014; Shaurub *et al.* 2015). However, in the majority of olive groves in Italy, larvae drop onto and enter the soil at the end of autumn or in winter, when the temperatures are too low to use EPNs. The important results obtained in this study, regarding the capability of EPNs to cause mortality of *B. oleae* pupae and adults, give a new perspective in the integrated management of olive fruit flies. Indeed, EPNs could be applied beneath fruit tree canopies (Shaurub *et al.* 2015) at the beginning of spring, when adults emerge from the soil.

Further studies are necessary to identify the optimum climatic factors for *H. bacteriophora* and *S. carpocapsae* in order to obtain the highest infection rates of *B. oleae*. More research is required to assess the mechanism of nematode penetration into the pupae and the possibility of using these nematodes in the control of *B. oleae* under field conditions.

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