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Early and off-season biological control of medfly with entomopathogenic nematodes: From laboratory experiments to successful field trials

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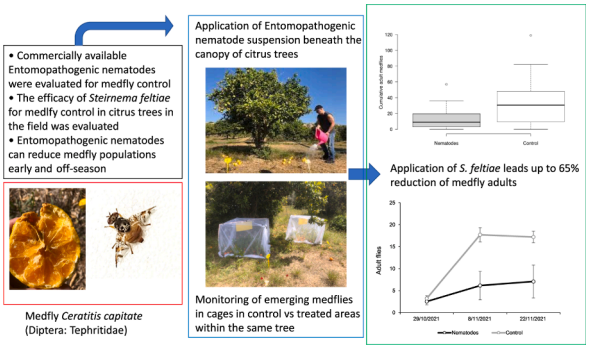
HIGHLIGHTS

- Commercially available Entomopathogenic nematodes were evaluated for medfly control.
- *Steinernema feltiae* provided the highest suppression of emerging adult medflies.
- The efficacy of nematodes for medlfy control in citrus trees was evaluated.
- Application of 2.5×10^6 *Steinernema feltiae*/m^2 leads to 65 % adult medfly suppression.
- Entomopathogenic nematodes can reduce medfly populations early and off-season.

GRAPHICAL ABSTRACT



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ABSTRACT

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is an important pest of citrus and other deciduous fruit trees. There is a need for sustainable pest management tools and the use of entomopathogenic nematodes have been explored for controlling the stages of medfly that occur in the soil. We have investigated further this approach by assessing the efficacy of commercially available entomopathogenic nematodes applied early season or off-season when the medfly populations are passing through their annual bottleneck period, aiming at reducing their population before the growing season. In laboratory experiments, the efficacy of commercial strains of *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora* and *H. downesi* at doses of 1.5×10^6 IJs/m^2 and 2.5×10^6 IJs/m^2, at 15C° and 25C° was assessed. *Steinernema feltiae* was found to result in up to 70 % reduction of adult medfly emergence at a dose of 2.5×10^6 IJs/m^2 and lower temperatures, confirming its superiority over other commercially available species. Field trials in citrus groves in Corinthos, Greece in Spring 2021 (early season) and Autumn 2021 (off-season) showed that a single application of *S. feltiae* at moderate dose regimes can provide about 62–65 % suppression of adult medlfies. Therefore, a single, moderate dose application of entomopathogenic nematodes early or off-season, which is more

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economically feasible can provide significant suppression of overwintering medflies and can be safely integrated with other tools for medfly management.

1. Introduction

The Mediterranean fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a polyphagous pest of significant importance, because it causes direct damage to a variety of fruit crops in many biogeographic regions in the world (Liquido et al., 1991; Morales et al., 2004). In addition, it is considered one of the most important quarantine pests with consequences for international fruit market due to quarantine restrictions (Hulme, 2009; Karsten et al., 2015; Malacrida et al., 2007; Rössler and Chen, 1994). Moreover, and because of climate change, the range of the medfly activity has been expanding over recent years in areas far beyond its traditional northermost distribution limit (Gilioli et al., 2022; Lux, 2018; Sultana et al., 2020; Zanoni et al., 2019). The control of medfly is based on a variety of tools ranging from pesticides (cover sprays and bait application), mass trapping, the Sterile Insect Technique (SIT) and the use of biological control, the latter involving predators, parasitoids and entomopathogens (Argov and Gazit, 2008; Bali et al., 2021; Beris et al., 2013; Konstantopoulou and Mazomenos, 2005; Mokrini et al., 2020; Wharton, 1989). The use of entomopathogenic nematodes (EPN) for control of medfly has been explored experimentally for several decades, mostly in laboratory studies (Gazit et al., 2000; Kapranas et al., 2021; Karagoz et al., 2009; Minas et al., 2016; Mokrini et al., 2020; Rohde et al., 2010). The free-living stage of EPN infective juveniles enter their insect hosts in the soil and through the release of symbiotic bacteria and toxins, they eventually kill them within 24–48 h (Kaya and Gaugler, 1993; Lu et al., 2017). Therefore, it has been proposed that the application of EPN in the soil beneath the tree canopy can kill a significant number of the soil dwelling stages of medflies.

The majority of the studies exploring the biological control potential of EPN for medfly suppression have two limitations. Most of the studies involve simple laboratory bioassays, whereas field trials are limited to a few published studies (Dolinski, 2016; Lindegren et al., 1990; Minas et al., 2016). Furthermore, both laboratory studies and limited field trials, although promising, concern species and/or strains of EPN that are not commercially produced (Gazit et al., 2000; James et al., 2018; Karagoz et al., 2009; Lindegren et al., 1990; Minas et al., 2016; Mokrini et al., 2020). The high efficacy of native strains of EPN in laboratory assays is positive in its own right, however, it has little impact on the adaptation of EPN as a biologically based and sustainable solution for medfly control, because these species/strains are not available to farmers (Kapranas et al., 2021). Nowadays, there are some EPN species which are commercially produced and used against a wide range of insects (Labaude and Griffin, 2018). Previous laboratory studies have indicated that some commercial EPN can exert significant suppression in number of emerging adults when applied in the soil substrate into which medfly larvae fall to pupate; *Steinernema feltiae* Filipjev reduced medfly emergence by up to 50 % because it had the highest immediate activity and a long residual activity over 4 weeks post application (Kapranas et al., 2021). Abiotic factors such as UV, soil type, moisture and temperature influence EPN efficacy (Stuart et al., 2015). For instance, low temperatures are associated with lower virulence rates; *Heterorhabditis bacteriophora* Poinar, *Steinernema glaseri* (Steiner, 1929), *Steinernema carpocapsae* Weiser and *S. feltiae* show increased infectivity in higher moistures in sandy loam soils (Grant and Villani, 2003). Temperature is also associated with infectivity and reproduction in EPNS (Grewal et al., 1994). Given that medfly infests fruit trees in temperate to warmer climates and in near arid conditions, it is important to consider thermal regimes of EPN species used as well as strategies for their application.

Low doses of commercial EPN can be used for medfly suppression off-season and early season in order to contain medfly populations before they build significantly as the season progresses (Kapranas et al., 2021). This approach might be both sustainable and economically feasible, because on-season multiple applications are required when medfly pest pressure is high and EPN efficacy might drop due to higher temperatures in the growing season, particularly in temperate Mediterranean agroecosystems. In this study, we further explore the efficacy of commercially available EPN for biological control of medfly. We approach this by firstly conducting laboratory assays in which we evaluate the efficacy of different commercially available nematodes at low and higher temperature regimes and at a lower and a higher dose. Then we present results from field trials on citrus groves wherein we test *S. feltiae* efficacy at early season and off-season in citrus.

2. Materials and methods

2.1. Laboratory experiments

2.1.1. Culture of nematodes and medflies

General procedures follow those of Kapranas et al. (2021). A laboratory culture of *C. capitata* was established with flies recovered from field-infested bitter oranges (*Citrus aurantium* L.), collected in the area of Attica-Greece during 2019. Adults were provided with water and a standard adult diet consisting of a mixture of yeast hydrolysate, sugar, and water at a 4:1:5 ratio, *ad libitum* in wire-screened wooden holding cages (35x35x50 cm). Eggs were collected from modified oviposition domes, provided with water and *Citrus aurantium* L. juice to stimulate oviposition. Larvae were reared on artificial diet prepared by mixing 200 g sugar, 200 g brewer's yeast, 100 g soybean flour, 4 g salt mixture, 16 g ascorbic acid, 16 g citric acid, 3 g sodium propionate, and 1 L water (Boller, 1985). Cultures of flies were kept under laboratory conditions at 25 ± 1 °C temperature, 55–65 % relative humidity and 16-h light–8-h dark photoperiod.

The nematodes used in the experiments *Heterorhabditis bacteriophora*, *Heterorhabditis downesi* Stock, Griffin and Burnell, *S. carpocapsae*, and *S. feltiae* were obtained from E-nema GmbH (Schwentinental, Germany). All nematodes were cultured at room temperature (23–24 °C) in *Galleria mellonella* (L.) larvae following methods by Kaya and Stock, (1997). Harvested infective juveniles (IJs) in tap water were stored in Nunc™ Cell Culture Treated Easyflasks 175 cm^2 (Thermo Scientific™) at 9–11 °C until use. The nematodes used in experiments were at most 3 weeks old.

2.1.2. Bioassays

Each experimental unit consisted of a laboratory tray (W × L × H: 25 × 25 × 8 cm) with soil substrate (7:3 sand:potting soil) of 5–6 cm depth that was placed in a wooden holding cage (30 × 30 × 30 cm). The sand and the potting soil were oven dried before starting the experiment and moisture content of the substrate was adjusted to 10 %. Nematode suspension was applied evenly to the substrate surface (400 cm^2) and then, 100 late instar larvae of medfly, at the time of leaving the artificial diet, were placed on the top of the soil substrate to burrow and pupate. Control units received only water. Humidity of the soil substrate was adjusted to 10–15 % by sprinkling it weekly with water. We used two doses of nematodes a lower (150IJ/cm^2 or 1.5 × 10^6 IJs/m^2) and a higher (250IJ/cm^2 or 2.5 × 10^6 IJs/m^2) one. The wooden cages were kept at either a temperature of 15 °C or at 25 °C, about 50 % relative humidity and permanent light. Adult medflies in 25 °C emerged after approx. 12 days. However, medflies, in the 15 °C assays were transferred in a room with 20 °C after one week to enhance medfly emergence which ensued in approx. 26 days. Because the susceptibility of the medflies to the nematodes is reduced considerably once they pupate, and pupation ensues soon after the mature larvae burrow into the soil, we suggest that

the infectivity of EPN is evaluated mostly at 15 °C rather at 20 °C. For each treatment combination (nematode, dose, temperature) we ran 20 replicates. For *H. downesi* no tests were done at the higher temperatures.

2.2. Field trials

Field trials took place in Koniario Intitute, Corinthos Greece, citrus groves (22.993895E, 37.890034 N). The groves had clay loam soil (approximately 37 % clay, 24 % silt and 39 % sand), and the ground was covered in 90 to 95 % of vegetation, 5 to 10 cm height, consisting mainly of *Oxalis pes-caprae* L. (>80 %) and *Avena sterilis* L. (10–15 %). Climatic condition data were obtained from AMONI, Corinthos station of the meteorological stations network of the National Observatory of Athens (http://meteosearch.meteo.gr) (see Suppl material S1). Before the nematode applications, fruit number per tree was estimated by counts in few trees, whereas their infestation was assessed in samples, about four days before. In each sample, about one or two fruits were collected both from the tree canopy and the soil beneath and then stored in plastic container with sterilized sand for four weeks at 25 °C in the laboratory. Containers were checked for pupae every-seven days by sieving the sand.

Nematode suspensions were prepared from commercial units and adjusted in dose at the day of application. Nematodes were applied in half of the soil surface beneath the canopy of each tree with a drencher and then both parts (treatment vs control) were labeled. Before the nematode application, the fallen fruits were manually distributed equally to the control and treatment side of each tree. The surface area of the canopy of citrus trees was on average 12 m^2 and about 0.5 L of water suspension per m^2 was applied in the treated side. A cage (W × L × H: 1.2 × 1.2 × 0.8 m) made of galvanized iron frames and insect cloth was erected approx. 10 days post application in each side of the tree at random position. In the top of each cage a two-sided yellow sticky trap (40 × 20 cm) was placed and medflies caught there were directly recorded.

2.2.1. Early season trials

The early season trials were run in Spring 2021 (March - May). Application of nematodes was on 24/3/2021 and 26/3/2021. Cages were erected on 9/4/2021 and medfly counts on sticky traps were recorded on 21/4, 06/05 and 27/5. In these trials two doses of nematodes were applied: 1.5 × 10^6 IJs/m^2 and 2.5 × 10^6IJs/m^2. Nematodes were applied in a mixture of Navel oranges *Citrus sinensis* (L.) Osbeck (11 and 10 trees with lower dose and higher dose, respectively) and sour oranges *Citrus aurantium* L. (13 and 15 trees with lower and higher dose, respectively). In total there were 49 replicate trees.

2.2.2. Off-season trials

The off-season trials were run in Autumn 2021 (October- November 2021). Application of nematodes was on 13/10/2021. Cages were erected on 20/10/2021 and medfly counts were recorded on yellow sticky traps on 29/10/2021, 08/11/2021 and 22/11/2021. A dose of 2.5 × 10^6IJs/m^2 nematodes was applied in 50 Valencia trees.

2.3. Statistical analysis

We directly compared adult fly emergence in different treatments including controls. Data were analyzed separately for lower and higher temperatures (15C° and 25C°) and doses of 1.5 and 2.5 × 10^6 IJs/m^2. One-way comparison of mean flies emerging in experimental units in different treatments was performed with a Welch's ANOVA followed by a Games–Howell multiple comparisons post-hoc test, which is a more robust test when the variance among groups is unequal. Comparisons of average fly numbers emerging between different dose and temperature treatments were performed with two tailed *t*-tests.

In the field trials, comparisons of medflies collected in traps set in cages in control vs nematode treated areas within each tree were performed with non-parametric paired tests, because in most cases the difference between control and treatment did not follow a normal distribution. Owing to the non-symmetrical distribution of these differences (skewed data) we used related samples signed tests (Sprent and Smeeton 2021).

3. Results

3.1. Laboratory experiments

All nematode treatments (different species and dose) led to significant reduction in emerging medflies compared to control, both at lower and higher temperatures (Welch ANOVA for 15 °C/1.5 × 10^6 IJs: F_4 = 50.163, P < 0.001; 25 °C/2.5 × 10^6 IJs: F_4 = 90.266, P < 0.001; 25 °C/1.5 × 10^6 IJs: F_3 = 241.194, P < 0.001; 25 °C/2.5 × 10^6 IJs: F_3 = 402.639, P < 0.001;Fig. 1). *Steinernema feltiae* had the highest efficacy leading to less emerging flies in all comparisons. Moreover, application of higher nematode dose led to significantly fewer emerging flies, except in the case of *H. downesi*, where the number of emerging medflies did not differ significantly between the different dose treatments (Fig. 1). In respect to temperature, significantly less medflies emerged at lower than at higher temperature, expect in the case of the 2.5 × 10^6 IJs /m^2 dose of *H. bacteriophora* (Table 1).

3.2. Field trials

Infestation of medfly was much higher in autumn than in spring, and was negligible in navel oranges (Table 2). In spring 2021, EPN treatments at both dose regimes resulted in lower median numbers of emerging adult medflies, irrespective of whether all trees were included in the analysis or only sour oranges with medfly infestation (Fig. 2). Application of 1.5 and 2.5 × 10^6 IJs /m^2 *S. feltiae* led to approx. 50 % and 65 % medfly suppression, respectively. In autumn 2021, application of 2.5 × 10^6 IJs /m^2 *S feltiae* led to lower emergence of medflies from the second sampling day onwards (Fig. 3a). Nematode treatments lead to a lower cumulative median of medfly emergence than in the control (Fig. 3b). Nematode treatment resulted in a 62 % suppression of medfly numbers in this case.

4. Discussion

Medfly larvae pupate within 3 cm of the soil surface (Jackson et al., 1998). Entomopathogenic nematodes are typically applied and are successful against pests in the soil and cryptic habitats (Shapiro-Ilan et al. 2012). Therefore, EPN application beneath the canopy of fruit trees can be used as a sustainable control measure that protects agroecosystem and consumer health (Bempelou et al., 2021). Entomopathogenic nematodes can show high efficacy against medfly in the laboratory (Gazit et al., 2000; Kapranas et al., 2021; Karagoz et al., 2009; James et al., 2018; Mokrini et al., 2020; Rohde et al., 2010; Yağcı et al., 2021). However, there are several obstacles that currently hinder the use of nematodes for medfly control in the field. Firstly, locally adapted species and strains that showed earlier promise are not commercially available (Kapranas et al., 2021; Lewis et al., 2006). Moreover, on-season applications of EPN are not economically viable because controlling increasing populations of medfly, associated with large numbers of larvae in the soil for a long period, would require repeated applications of EPN within a year at high doses. Lastly, EPN efficacy is reduced when temperatures increase > 30 °C (Stuart et al., 2015) which is typical in temperate fruit producing regions as the season progress. An alternative strategy is to utilize commercially available EPN for medfly control in a single application of 1.5–2.5mi IJs/m^2 off-season or early season. The lower threshold for medfly development and emergence is about 12.5 °C, whereas increased survival and significant emergence is observed from 15 °C (Duyck and Quilici, 2002; Grout and Stoltz, 2007; Quesada-Moraga et al., 2012; Ricalde et al.,

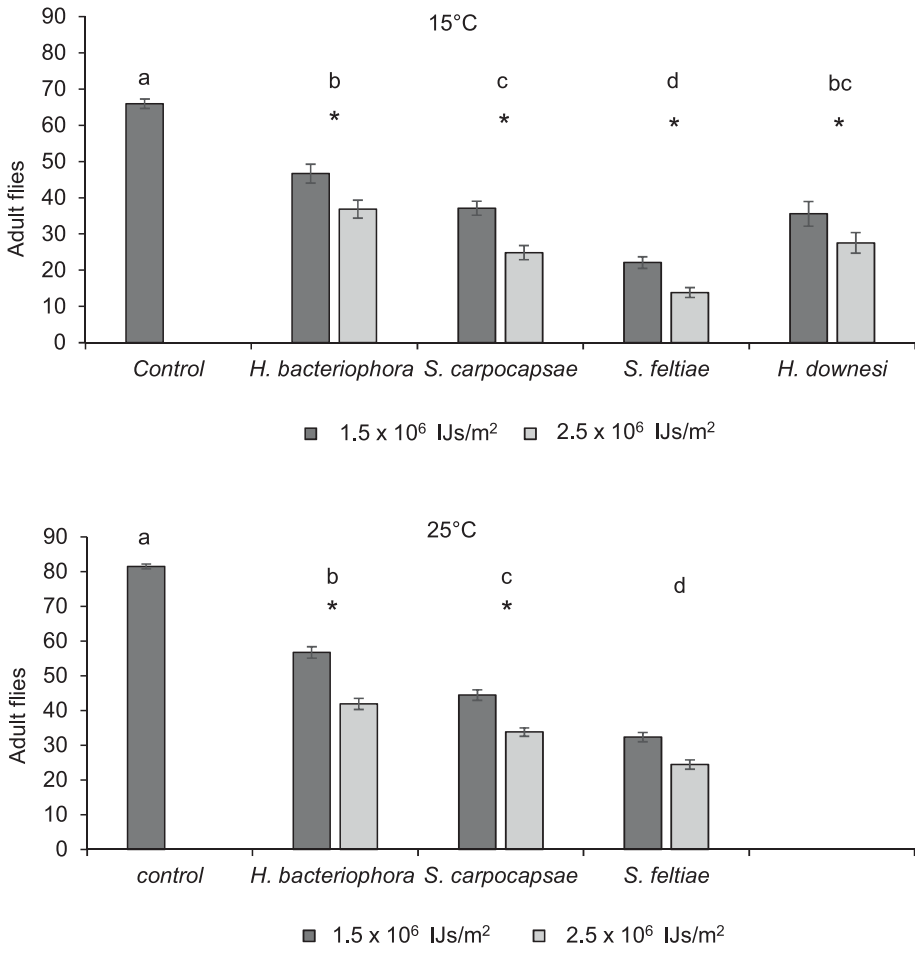

Fig. 1. Emerging adult medflies (means ± SE) from substrates to which different treatments were applied [*Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae* and *H. downesi* suspensions (at 150 IJs cm^2 and 250 IJs/cm^2) and water (control)]. 100 third-instar larvae of medflies were added immediately at 15 °C (a) and 25 °C (b). Bars for each dose and temperature with the same letter do not differ significantly at $P \leq 0.05$. Post –hoc tests for different doses indicated similar results and therefore, letters refer to both post post-hoc tests. Asterisks show significant differences between different doses.

Table 1
Comparison of medfly emergence (average ± S.E.) between 15 °C and 25 °C, for each treatment (two tailed t-tests).

	Dose	t	P	15C°	25C°
control		−10.534	<0.0001	65.95 ± 1.30	81.5 ± 0.68
H. bacteriophora	1.5×10^6 IJs	−3.272	0.002276	46.65 ± 2.61	56.75 ± 1.64
	2.5×10^6 IJs	−1.709	0.095526	36.85 ± 2.48	41.9 ± 1.60
S. carpocapsae	1.5×10^6 IJs	−2.988	0.004895	37.1 ± 1.92	44.45 ± 1.53
	2.5×10^6 IJs	−3.884	0.000398	24.85 ± 1.96	33.8 ± 1.20
S. feltiae	1.5×10^6 IJs	−4.897	0.000018	22.1 ± 1.61	32.35 ± 1.33
	2.5×10^6 IJs	−5.571	< 0.00001	13.85 ± 1.36	24.45 ± 1.32

2012; Vargas et al., 1996). Temperatures of 15–20 °C that are typical during autumn and early spring in temperate climates are broadly considered optimal for EPN performance given that minimum humidity requirements are met (Grant and Villani, 2003; Grewal et al., 1994; Stuart et al., 2015). The strategy of applying nematodes off-season, during autumn was also experimentally explored in the laboratory for the olive fruitfly *Bactrocera oleae* Rossi (Diptera: Tephritidae) which has similar biological characteristics, but without any validation in real field conditions (Sirjani et al., 2009).

In our laboratory assays it was confirmed that *S. feltiae* application even at the lower dose could significantly reduce adult medflies; at 15 °C a suppression by 66.5 % and 78.9 % in lower and higher dose, respectively and at 25 °C a suppression by 60 % and 70 % was observed, respectively. This efficacy can be explained by higher virulence of this species for dipteran larval pests and also by the long residual activity in moderate temperatures (~20 °C) which could provide control over a four weeks period (Kapranas et al. 2021). In addition, *S. feltiae* and to a lesser degree *S. carpocapsae* are better adapted to lower temperatures even if the latter has a stricter thermal range for reproduction which is 20–30 °C (Grewal et al., 1994; Hazir et al., 2001). *S. feltiae* is considered

Table 2
Sampling for medfly infestation in fruits before the entomopathogenic nematode application.

Trial	Tree	Estimated number of fruits/tree		Number of fruits sampled		Number of pupae/fruit	
		Canopy	Ground	Canopy	Ground	Canopy	Ground
Spring'Early season'	Orange Navel	250	90	50	50	0	0
	Sour Orange	350	100	50	32	0.62	1.59
Autumn 'Off-season'	Orange Valencia	120	180	49	33	4.75	5.78

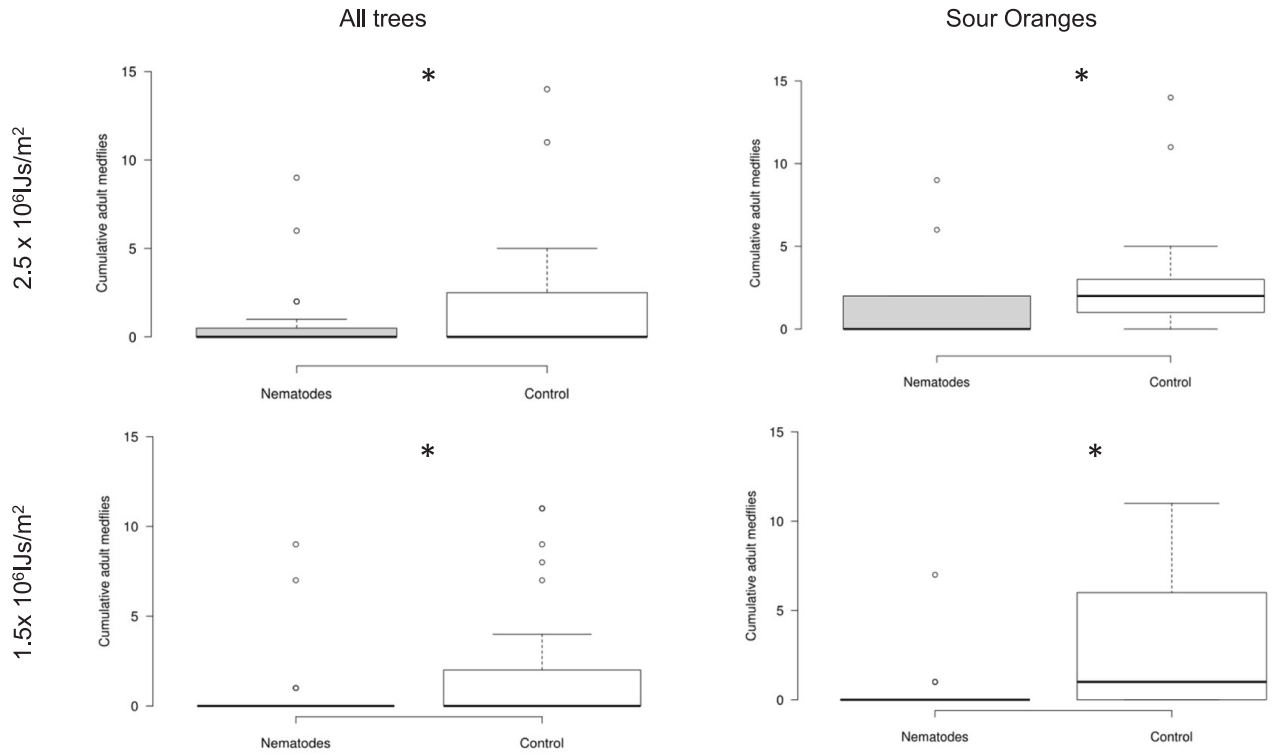

Fig. 2. Boxplots showing cumulative adult medfly numbers captured in traps in control versus *Steinernema feltiae* treated areas of citrus trees during the early season trial (9/4/2021 to 27/5/2021). Boxes indicate median (horizontal line within the box), 25–75 % quartiles (upper and lower box margins), and maximum/minimum range (whiskers), and outliers (dots > 1.5 above box height). Analysis was conducted separately for all trees (left panel) and for only sour orange trees (right panel). Asterisks indicate significant differences between control and treated areas (related samples signed tests, $P < 0.05$).

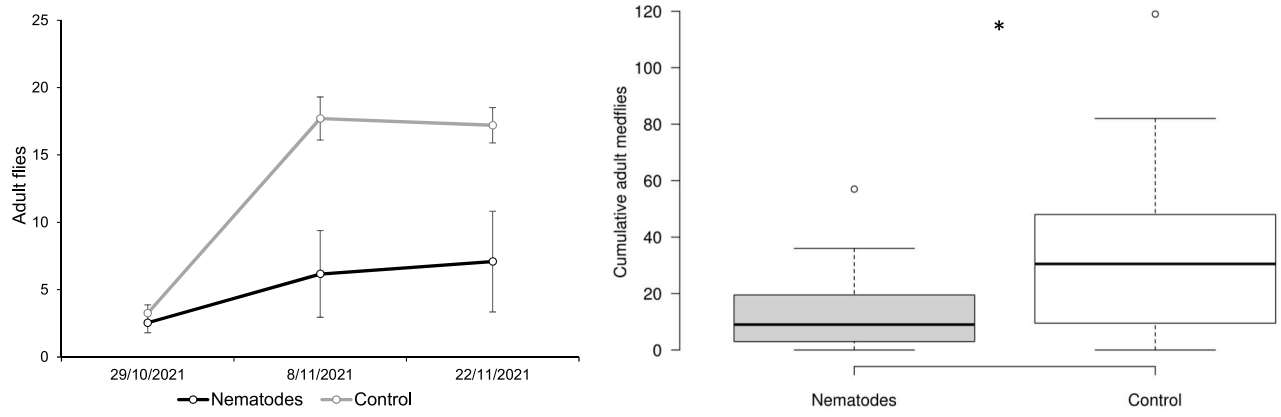

Fig. 3. Comparison of medflies captured in traps in control versus *Steinernema feltiae* treated areas of citrus trees during the off-season trial (20/10/2021 to 22/11/2021). a) average adult medfly numbers captured at different sampling dates. b) Boxplot showing cumulative adult medfly numbers. Boxes indicate median (horizontal line within the box), 25–75 % quartiles (upper and lower box margins), and maximum/minimum range (whiskers), and outliers (dots > 1.5 above box height). Asterisks indicate significant differences between control and treated areas (related samples signed tests, $P < 0.05$).

a cold adapted species that has shown good performance against larvae of the fruit fly *B. oleae* both in the soil and inside olives, because it could survive for long periods (95 weeks) and cause high infectivity at low temperatures even at 10 °C (Sirjani et al., 2009). The performance of heterorhabditids was not assessed as satisfactory. *Heterorhabditis downesi* is a species that is adapted to colder temperatures and has been used for biological control of coleopteran pests in cooler climates (Kapranas et al., 2017; Lola-Luz et al., 2005). However, it should be mentioned that *Heterorhabditis baujardi* LPP7 and *Heterorhabditis indica* IBCB n05 which are warmer climate adapted species led to significant high mortality > 87 % of medfly larvae in the field (Dolinski, 2016; Minas et al., 2016).

In the field trials, application of *S. feltiae* reduced medfly emergence by 50 % to 65 %. It is important to consider that during these two field trials there were important differences in experimental conditions, yet nematode applications led to similar outcomes. During spring 2021 medfly population was significantly lower than that of autumn 2021, while temperature and humidity conditions were better suited for nematode applications in autumn 2021 (see Suppl S1). Although nematode applications were performed on relatively cold days, temperatures increased during the course of the spring 2021 trial but decreased during

the trial in autumn 2021. These results suggest nematode efficacy is determined by both environmental conditions (spring temperatures) and pest pressure i.e., the number of larvae in the soil and inside fruits on the soil. EPN efficacy is affected by dose/pest density as shown in our study and other studies (Ebssa et al., 2012; Griffin, 2015; Minas et al., 2016; Kapranas et al., 2017). Nematode numbers significantly decline post application, especially in adverse environmental conditions (Griffin, 2015). In the spring trial, when there was a lower fruit fly infestation, increasing the dose by 67 % did not lead to an analogous increase in pest suppression. Examining the dose of nematodes needed for effective control is critical, and this is important in field conditions. The use of 25,000 IJs/m^2 of *Heterorhabditis baujardi* LPP7 in guava trees in Brazil led to significant mortality of medfly mature larvae > 87 % (Minas et al., 2016). Similarly, *Heterorhabditis indica* IBCB n5 strain applied in doses of 10,000 and 100,000 IJs/m^2 resulted to 66 and 93 % medfly larvae mortality, respectively in guava (Dolinski, 2016). Application of 5×10^6 IJs/m^2 of a Mexican strain of *S. feltiae* resulted in 86 % mortality of medflies in papaya trees in Hawaii (Lindegren et al., 1990). However, all the aforementioned studies concern experiments in semi-field conditions in warmer regions and not commercially available nematodes. In these trials, nematode suspensions were applied, a fixed number of medfly larvae were added and evaluation was assessed by measuring adult emerging adult medflies within a narrow period of time (approx. 2 weeks), whereas our experiments simulated a more realistic field scenario.

In both field trials there were also oranges lying on the soil of both treatments bearing significant numbers of medflies larvae susceptible to nematode infection. *Steinernema feltiae* can infect larvae of medlfies and other fruit flies as well as drosophilids inside oranges, apples, apricots, olives and blueberries (Hübner et al., 2017; Kapranas et al., 2021; Mokrini et al., 2020; Sirjani et al., 2009). The ability of EPN to infect medflies within fruits is important because in temperate climates, a large number of medflies overwinter in fruits (Martínez-Ferrer et al., 2006; Papadopoulos et al., 1996). In addition, these fruits likely contribute to recurring populations of medflies as evinced by the protracted period of adult medfly emergence within the cages for a period of almost two months in both trials. However, this recurring population within cages is likely low due to suboptimum temperature conditions for medfly development especially in the autumn trial. In Autumn 2021, application of nematodes did not have an immediate result (first sampling date) likely due to the fraction of medflies in the pupal stage, which is not susceptible to EPN (Karagoz et al., 2009; Langford et al., 2014; Yee and Lacey, 2003).

In conclusion, our laboratory experiments together with the field trials provide validation for an alternative approach of using EPN for early season and off-season biological control of medfly. A single application of commercially available *S. feltiae* at 1.5 to2.5 $\times 10^6$ IJs IJs/m^2 beneath the canopy of citrus trees, early season and/or off- season, can reduce the emerging medfly population by at least 50 %. This intervention is more economically viable than multiple applications of EPN in the season, since medfly develops multiple generations in temperate Mediterranean orchards. Relatively cooler autumn temperatures could improve the residual activity and therefore the efficacy of *S. feltiae* against medfly larvae in the soil and in fruits. The efficacy and feasibility of this approach can be further improved if adjuvants, such as wetting agents that enhance nematode efficacy in conditions of e.g., dry soils or when there is ground vegetation, are added in the EPN suspensions (McGraw and Schlossberg, 2017; Schroeder and Sieburth, 1997; Shapiro-Ilan et al., 2012, 2006). Lastly, the use of EPN would be meaningful if through a scouting program (trapping) their application targets hot spots within the orchard or even at wider scale.

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CRediT authorship contribution statement

Apostolos Kapranas: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Anna Chronopoulou:** Investigation. **Arne Peters:** Resources. **Spyros Antonatos:** Investigation. **Ioanna Lytra:** Investigation, Resources. **Panagiotis Milonas:** Conceptualization, Formal analysis, Writing – review & editing. **Dimitrios Papachristos:** Conceptualization, Investigation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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