

## Short communication. Susceptibility of *Phoenix theophrasti* (Palmae: Coryphoideae) to *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) and its control using *Steinernema carpocapsae* in a chitosan formulation

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### Abstract

The invasive red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) is the most destructive pest of palms in the world. It has been reported on 19 palm species belonging to 15 different genera. The host status of the Cretan Date Palm, *Phoenix theophrasti*, remains unclear. Therefore, the present study was carried out to ascertain the host status of this protected palm species. Additionally, the efficacy of entomopathogenic nematodes in a chitosan formulation to control this pest in *P. theophrasti* was assessed. Our results showed that healthy 4-yr-old *P. theophrasti* palms were not infested by adult females after 9 days exposure in a population density of 3 adults per plant. However, infestation was successful when neonate larvae were artificially introduced in palms. Therefore, natural populations of *P. theophrasti* could be at risk. Gummy secretion was observed in both naturally and forced infested palms indicating the existence of antibiosis in this species. Curative applications with the entomopathogenic nematode *Steinernema carpocapsae* in a chitosan formulation in early infested *P. theophrasti* palms managed to reduce insect's activity and could help the palms to recover.

**Additional key words:** antibiosis; antixenosis; entomopathogenic nematodes; *Phoenix canariensis*.

### Resumen

**Comunicación corta. Susceptibilidad de *Phoenix theophrasti* frente a *Rhynchophorus ferrugineus* y su control mediante el uso de *Steinernema carpocapsae* en una formulación con quitosano**

El picudo rojo de las palmeras, *Rhynchophorus ferrugineus* Oliv. (Coleoptera, Curculionidae) es la plaga de palmeras más destructiva del mundo. Se ha citado en 19 especies de palmeras pertenecientes a 15 géneros diferentes. El estatus de la palmera datilera de Creta, *Phoenix theophrasti* como huésped del picudo rojo no está claro. Este estudio se llevó a cabo para determinar la respuesta de la palmera datilera de Creta frente al ataque de este curculiónido. Además se evaluó la eficacia de una formulación del nematodo entomopatógeno *Steinernema carpocapsae* con quitosano para su control. Fue imposible infestar palmeras de 4 años de edad con hembras adultas tras 9 días de exposición con 3 hembras por planta. Sin embargo, la infestación fue exitosa cuando las larvas neonatas se introdujeron artificialmente en las palmeras. Por lo tanto, las poblaciones naturales de *P. theophrasti* podrían estar en riesgo. Se observó una secreción de goma, tanto en las palmeras infestadas de forma natural, como en las forzadas, que indica la existencia de antibiosis. Las aplicaciones curativas con los nematodos entomopatógenos en *P. theophrasti* redujeron la actividad de los insectos y podrían ayudar a esta especie de palmera en su recuperación.

**Palabras clave adicionales:** antibiosis; antixenosis; nematodos entomopatógenos; *Phoenix canariensis*.

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The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is the most destructive pest of palms worldwide (EPPO, 2008, 2009; Dembilio and Jacas, 2011). Adult *R. ferrugineus* are large reddish brown beetles about 3 cm long with a characteristic long curved rostrum. Females lay eggs at the base of the fronds in separate holes made with their rostrum. Neonate larvae bore into the palm core and upon completion of development move back to the base of the fronds to pupate. A new generation emerges and adults may remain within the same host and reproduce until the palm eventually dies. Subsequently, adults move to new hosts (Dembilio and Jacas, 2011).

*Rhynchophorus ferrugineus* has been reported on 19 palm species (EPPO, 2008; Dembilio et al., 2009) with different susceptibility to the pest (Barranco et al., 2000; Dembilio et al., 2009). The Canary Island date palm, *Phoenix canariensis* hort. ex Chabaud, is especially susceptible to *R. ferrugineus* attack (Dembilio et al., 2009). Antixenotic and antibiotic mechanisms of resistance against *R. ferrugineus* have been described in *Chamaerops humilis* L. and *Washingtonia filifera* (Lindl.), respectively (Dembilio et al., 2009).

The Cretan date palm, *Phoenix theophrasti* Greuter (Palmae: Coryphoideae), is a threatened palm species native to the eastern Mediterranean region with a very restricted distribution (Crete and some Aegean islands) (OJ, 1992; IUCN, 2010). Infestations of *P. canariensis* palms by *R. ferrugineus* have been recorded close to the main habitats of *P. theophrasti* in Vai Forest (Kontodimas, personal communication), a designated type of natural habitat for conservation (OJ, 1992). *Phoenix theophrasti* proved to be susceptible to *R. ferrugineus* in the laboratory (Kontodimas et al., 2005-2006). However, no quantitative data on its susceptibility vis-à-vis with the highly susceptible *P. canariensis* exist. Furthermore, should the native habitat of this palm be invaded by *R. ferrugineus*, there would be an urgent need for sustainable control methods. The entomopathogenic nematode (EPN) *Steinernema carpocapsae* (Weiser) proved highly effective in a chitosan formulation against *R. ferrugineus* in *P. canariensis* (Llácer et al., 2009; Dembilio et al., 2010). For the abovementioned reasons, the objectives of this study were: (1) to elucidate the susceptibility of *P. theophrasti* against *R. ferrugineus* and (2) to determine the efficacy of *S. carpocapsae* to protect *P. theophrasti* against the weevil.

**Semi field assays:** Assays were carried out in Benaki Phytopathological Institute (BPI), Kifissia, Greece

(latitude: 38° 04' 53" N; Longitude: 23° 48' 47" W; altitude: 67 m) during May-June 2010. Tests were conducted in a metallic mesh house consisting of eight independent screened cages (3 × 1.5 × 2.3 m) within a glasshouse under natural light and temperature conditions (mean: 22.3°C, max: 40.3°C, min: 12.9°C).

**Plant material:** Trials were performed on 4-yr-old potted *P. theophrasti* and *P. canariensis* palms. Their stipe was around 30 cm high and 20 cm wide. They were planted in 10 L containers and were watered every other day.

**Experimental insects:** Weevils were captured in the province of Valencia (East Spain) in traps baited with the weevil aggregation pheromone and kairomones and transferred to BPI. Subsequently, weevils were kept in a Perspex cage (25 × 25 × 30 cm), which bore on its upper side a hole (8 cm in diameter) covered by a mesh used for insect manipulation and its bottom side consisted of a 2 mm metal mesh. The cage was set on top of a tray containing thin apple slices within a folded piece of moistened filter paper used by females for oviposition and by both males and females as food. Less than 48-h old neonate larvae obtained from the apple slices were used in the infestation assays.

**Natural infestation no-choice test:** Five palms per palm species, enclosed together in separate cages, were used. Each palm was exposed to three presumably-mated females of *R. ferrugineus* for 9 days. Six weeks later palms were dissected and larvae were extracted.

**Forced infestation test:** Five palms per palm species enclosed together in separate cages were used. Each palm was artificially infested with ten *R. ferrugineus* larvae. Ten holes 30 mm deep and 4 mm in diameter were drilled along a ring 10 cm below the palm apex (2 holes per frond) and one neonate larvae was introduced into each hole. Previously removed plant material was used to seal the holes. Palms were processed as above.

**Entomopathogenic nematodes (EPN) test:** Twenty-four *P. theophrasti* palms were artificially infested with 6 neonate larvae per palm following the forced infestation protocol described above. Twelve palms constituted the control group and were enclosed in one cage. The remaining 12 palms were treated with the commercial product Biorend-R® palmeras (*S. carpocapsae* with a chitosan adjuvant) (IDEBIO S.L., SPAIN). The product was applied 14 days after the infestation at the recommended dose of 20 × 10<sup>6</sup> IJs + 200 mL chitosan per palm tree in 300 mL water. The solution was applied on the trunk and the bases of the fronds by thorough

**Table 1.** Percentage of successfully infested palms and mean number of *Rhynchophorus ferrugineus* larvae found in natural and forced infestation tests of *Phoenix theophrasti* and *P. canariensis*. Five palms (replicates) per species and type of infestation were considered. Three presumably-mated females per palm were used in the natural infestation test. Ten neonate larvae per palm were introduced in the forced infestation test

Palm species	Natural infestation test		Forced infestation test	
	Successful infestation (%)	Larvae per palm (#)	Successful infestation (%)	Larvae per palm (#)
<i>P. theophrasti</i>	0	0 <sup>b</sup>	40.0	0.4 ± 0.3 <sup>a</sup>
<i>P. canariensis</i>	40.0	7.8 ± 5.3 <sup>a</sup>	60.0	1.2 ± 0.8 <sup>a</sup>
ANOVA results ( <i>F</i> ; <i>df</i> ; <i>p</i> )	—	NA <sup>1</sup>	—	1.07; 1, 9; 0.3319

<sup>1</sup> NA: not applicable.

soaking using a watering can. Twenty days later, the palms were dissected as above. In all tests, the number of infested palms (with successfully developing larvae) was recorded for estimation of percentage successful infestation; the number of live larvae per palm was counted as well as the number of empty galleries and those with the remaining of dead larvae (the latter two will be called «galleries» hereafter). Additionally in the EPN treatment, the contents of galleries, *i.e.* gummy secretion, unidentified remains, etc. were examined under binocular stereoscope for presence of EPN.

When appropriate, results were subjected to either analysis of variance (ANOVA) or non-parametric Kruskal-Wallis test. The data were checked for normality and transformed with the angular transformation prior to analysis of variance.

In the natural infestation test, no infestation was observed in 4-year-old *P. theophrasti*. However, 40% *P. canariensis* were successfully infested (Table 1). In the forced infestation test, 60 and 40% *P. canariensis* and *P. theophrasti*, respectively, were successfully infested (Table 1). In both infestation treatments, small galleries, evidence of larval activity, were observed in *P. theophrasti*. The preimaginal mortality of *R. ferrugineus* in forced infested *P. theophrasti* was 96.0% and this value was not significantly different from that

registered in *P. canariensis*, 88.0% (ANOVA,  $F = 0.84$ ;  $df = 1, 9$ ;  $p = 0.3866$ ). Starting 3 days after infestation, a gummy secretion was observed oozing from the holes made with the drill in *P. theophrasti*. The same secretion was observed in the oviposition holes of the naturally infested *P. theophrasti* palms.

Successful infestation percentage of *P. theophrasti* in EPN test was 17 and 0% for control and EPN treatment, respectively. Infestation percentage based on the presence of galleries was higher (92% and 50% for the control and the EPN treatment, respectively) (Table 2). Thirty and seven galleries in total were found in control and EPN-treated palms, respectively. The mean number of galleries per palm was significantly higher in control than in EPN-treated palms (Table 2). When palms were dissected, 40 and 14% of the galleries in control and EPN treatments, respectively, bore the gummy secretion already observed in the susceptibility tests. EPN were recovered in the secretion from EPN-treated palms. The galleries found in control palms were longer (approximately 25 mm) than those in EPN-treated palms (less than 8 mm).

Results show that natural infestation of 4-yr-old palms of *P. theophrasti* by *R. ferrugineus* adult females is not successful under our experimental conditions. Kontodimas *et al.* (2005-6) successfully infested

**Table 2.** Percentage of successfully infested palms and mean number of *Rhynchophorus ferrugineus* larvae and galleries found per palm in forced infestation tests of *Phoenix theophrasti*. Twelve palms per treatment were considered. Palms were artificially infested with six neonate larvae each

Treatment	Successful infestation (%)	Larvae per palm (#)	Galleries per palm (#)	Efficacy (%)
Control	17	0.2 ± 0.1 <sup>a</sup>	2.5 ± 0.5 <sup>a</sup>	—
<i>S. carpocapsae</i> + chitosan	0	0 <sup>b</sup>	0.6 ± 0.2 <sup>b</sup>	100
Kruskal-Wallis test results	—	NA <sup>1</sup>	$K = 10.2017, p = 0.0014$	—

<sup>1</sup> NA: not applicable.

*P. theophrasti* palms exposed to a population density of 6 females per 2-yr-old seedlings for 100 days but both the infestation rate (33%) and the number of adults obtained were low and adult size was small.

Forced infestation of *P. theophrasti* was possible indicating that previously harmed palms (by other pests, the wind, etc) could be naturally infested. In this case, red palm weevil larvae in *P. theophrasti* suffered from high mortality rates, which were comparable to those in *P. canariensis*. Mortality of *R. ferrugineus* larvae in *P. canariensis* was higher than the one reported by Dembilio *et al.* (2009) (51.7%) perhaps due to low night temperatures during the experiment, which can affect young stages (Dembilio and Jacas, 2011).

Occurrence of gummy secretion in infested *P. theophrasti* palms where null or low infestation was recorded indicates the existence of antibiosis, as observed in *W. filifera* (Dembilio *et al.*, 2009). The extent that this mechanism can contribute to protect *P. theophrasti* should be further investigated, *e.g.* in relation to the age and nutritional-sanitary state of palms or population density of *R. ferrugineus*.

The results of the EPN application are comparable to those obtained in *P. canariensis*, where the same *S. carpocapsae* formulation achieved efficacies from 73 to 81% (Llácer *et al.*, 2009). In the present study, survival and activity of EPN in the palms was proved over a period of high temperatures (up to 40.3°C). Moreover, live EPN were recovered in the gummy secretion suggesting that EPN could cope with plant defenses without being hindered.

Only recently, natural infestation was recorded for the first time in three offshoots of *P. theophrasti* in the field in Heraklion Prefecture-Crete (Plant Protection Centre of Heraklion, personal communication), which urges the need for plant protection measures in protected areas where the natural populations of *P. theophrasti* exist. The efficacy observed when applying *S. carpocapsae* in a chitosan formulation as a curative treatment could help the recovering of early infested palms.

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