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Field efficacy of Imidacloprid and Steinernema carpocapsae in a chitosan formulation against the Red Palm Weevil Rhynchophorus ferrugineus (Coleoptera: Curculionidae) in Phoenix canariensis.

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Running title: Control of *Rhynchophorus ferrugineus* in *Phoenix canariensis*

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Abstract

BACKGROUND: the invasive red palm weevil, *Rhynchophorus ferrugineus*, has become the major pest of palms in the Mediterranean Basin. Chemical control against this species is difficult because of its cryptic habits and it is mainly based on the repeated application of large quantities of synthetic insecticides. The aim of this work has been to evaluate in the field the efficacy of imidacloprid (Confidor® 240 OD) and *Steinernema carpocapsae* with chitosan (Biorend R® Palmeras) as soil and stipe treatments, respectively, alone or in combination, against this pest.

RESULTS: all treatments significantly reduced the mean number of immature stages of *R. ferrugineus* per palm. However, there were no significant differences among the different treatments considered. Efficacies ranged from 83.8 to 99.7 % for the mean number of immature stages found in the palms and resulted in a significant increase in palm survival compared to the untreated control (75.0-90.0 % versus 16.5 %, respectively).

CONCLUSION: both imidacloprid and *S. carpocapsae* in a chitosan formulation proved highly effective against *R. ferrugineus* in the field and their efficacies did not significantly change when used in combination.

Keywords: Mortality; *Phoenix canariensis*.

1 INTRODUCTION

The invasive red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), has become the major pest of palms in the Mediterranean Basin, where it spread slowly during the mid 1990's and very quickly during the last five years. The pest is currently widely distributed in Oceania, Asia, Africa and Europe[1] and has been recently found in the Caribbean.[2] Females lay their 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 these adults may remain within the same host and reproduce until the palm eventually dies. Subsequently, adults will move and look for a new palm host. *Rhynchophorus ferrugineus* has been reported on 19 palm species belonging to 15 different genera.[1,3,4] Several control methods have been applied against this pest within an Integrated Pest Management strategy. Its main components are phyto-sanitation, which involves cutting down and burning infested palms, use of insecticides and use of pheromone traps for adult monitoring and mass trapping.

Chemical control against *R. ferrugineus* is mainly based on the repeated application of large quantities of synthetic insecticides, which are applied in a range of preventative and curative procedures designed to limit and contain the spread of infestation. These procedures have been developed and refined since commencing in India in the 1970s.[5] Methods range from general dusting of the leaf axils after pruning or spraying of the palm stipe, to localized direct injections of chemicals into the trunk.[6] Researchers have concluded that because of the cryptic habitat of the boring stages of this weevil, chemical insecticides have to be applied frequently and over a long period of time for effective management of established populations.[5, 7] However, there are deep concerns about the environmental pollution caused by these treatments, especially in public areas where ornamental palms are grown.[6] Furthermore, many of the currently used insecticides especially organophosphates and carbamates, are not effective enough.[8] Imidacloprid showed a good efficacy against different stages of *R. ferrugineus* in both laboratory and glasshouse assays[8] and it is one of the few chemicals recommended for field applications against this pest in palm nurseries in Spain.[9] Imidacloprid is a chloronicotinyl nitroguanidine insecticide that was first introduced to the United States in 1994. It is used as a crop and structural pest insecticide, a seed treatment, and a flea-control treatment. Imidacloprid works by disrupting the insect nervous system and kills by contact and ingestion.[10] It is used to control sucking insects and is effective against adult or larval stages of various species.[11, 12]

An interesting alternative to the chemical control of *R. ferrugineus* could be the use of entomopathogenic nematodes (EPNs).[13-17] EPNs are safe for non-target vertebrates and to the environment, and since they are mass-produced in liquid media, production costs have been significantly reduced in recent times.[18] The infective third juvenile stages (Dauer Juvenile, DJ) survive outside the insect and can actively search for hosts. DJs enter the insect host through any opening (mouth, anus, spiracles) and grow into the parasitic stage. The death of the insect due to nematode parasitism is caused by Gram-negative bacteria which are carried within the gut of the DJs.[19] *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae), which is mutualistically associated with the bacterium *Xenorhabdus nematophila* (Enterobacteraceae), is the most studied, available, and versatile of all EPNs. Although field experiments in date palms, *P. dactylifera* L., conducted several years ago, produced inconsistent results,[14] recent laboratory and semi-field assays using *S. carpocapsae* with chitosan showed efficacies around 80% in *Phoenix canariensis* Hort. ex Chabaud (Arecaceae).[17] The commercial product Biorend R® Palmeras contains *S. carpocapsae* and a chitosan adjuvant. Chitosan is a biodegradable organic product with the active ingredient N-acetyl-glucosamine, which can activate defense mechanisms in the plant,[20] increase lignification, and promote root development.[21] The use of nematodes with chitosan is patented[22] and nowadays a formulation of *S. carpocapsae* with chitosan is included in the list of authorized products against *R. ferrugineus* in Spain.[9]

The aim of this work has been to evaluate in the field the efficacy of imidacloprid and *S. carpocapsae* with chitosan as soil and stipe treatments, respectively, alone or in combination against *R. ferrugineus*.

2 EXPERIMENTAL METHODS

2.1 Location and set up. Field experiments were conducted from December 2007 to January 2009 in a *P. canariensis* nursery located in a *R. ferrugineus*-infested area near the town of Algemesí, Spain (Lat.: 39º 19' 36" N; Long.: 00º 43' 77" W; alt.: 18 m). *Phoenix canariensis* palms were 6-8 years old (palm stipe around 0.5 m in diameter and 1.7 m high). An area of 750 m^2 within the nursery containing 360 palms regularly planted forming a grid was selected. The grid was cross-divided into 4 rectangular sections of the same size containing 72 palms each by removing the two central rows and columns of palms. Palms exhibiting typical symptoms of infestation such as bitten fronds, fallen central shoot, small holes in the leaf, scars and oozing out of a reddish-brown fluid and extrusion of fibers from these holes,[23] were removed and only those that were presumed to be pest-free were further considered. Five different insecticide treatments (Table 1) plus a control were included in each block (4 to 6 palms per treatment and block and 8-10 palms for control and block). Palms on the borders of each block were left untreated.

Three white traps baited with the weevil aggregation pheromone (ferrugineol) and kairomones (ethyl acetate and pieces of palm fronds) located near the nursery were used for monitoring population dynamics of *R. ferrugineus* adults from September 2007 until October 2008. The traps consisted of a 10 l capacity white plastic bucket with four openings (2.5 × 6 cm^2) regularly distributed 4 cm below the upper rim of the bucket.

2.2 Pesticide application. The commercial products Confidor® 240 OD (Bayer Crop Science S.L., Alcàsser, Valencia) and Biorend R® Palmeras (Idebio S.L., Salamanca, Spain) were applied at the doses shown in Table 1 either alone or in combination. Imidacloprid was injected into the soil with a probe connected to a high pressure hydraulic sprayer to a depth of 10-15 cm around the trunk. Biorend R® Palmeras was directly sprayed onto the top of the palm stipe with a Mauricio® 18 l Manual Knapsack Sprayer (Pulverizadores Mauricio S.A., Valencia). Pesticide applications started in December 2007 for both products (Table 1).

2.3 Data collection. The nursery was inspected fortnightly. At each inspection, palms showing symptoms of infestation were removed and taken to laboratory for dissection. All specimens of *R. ferrugineus*, dead or alive, were extracted and checked for presence of nematodes.[17]

2.4 Statistical analysis. Results were subjected to a two-way-analysis of variance (ANOVA, the two factors being treatment and block). The mean numbers of immature stages found alive were further separated using Duncan's test whereas palm mortality results were separated using Dunnett's test. The efficacies of the different treatments based on mean numbers of immature stages found alive were calculated according to Abbott.[24]

3 RESULTS

Trap captures of *R. ferrugineus* adults were maximal in October 2007, two months before the beginning of the assay. They dropped during winter, slowly recovered during spring 2008 and peaked again in summer (Figure 1) in correspondence with the sudden increase of dead palms found in control plots (Figure 2).

Newly infested palms were not detected in the nursery until the month of March (Figure 2). First dead palms were observed in control and Confidor®+Biorend R® (I)-treated blocks. From that month onwards, dead palms were progressively detected in all treatments except in the Confidor®+ Biorend R® (II)-treated palms, where first detection occurred in August. During this month mortality suddenly increased in the control and significant differences in the percentage of surviving palms between control and the rest of treatments appeared. These differences did not disappear until the end of the experiment in January 2009, when efficacies were finally calculated (Table 2).

Blocks A and B had a significantly lower incidence of *R. ferrugineus* than blocks C and D (6.26 ± 1.76 versus 22.87 ± 4.26 individuals per palm, respectively). However, interaction between block and treatment was not significant (Table 2). All treatments significantly reduced the mean number of immature stages of *R. ferrugineus* per palm and resulted in increased palm survival compared to the untreated control (Table 2). There were no significant differences among the different treatments considered. Efficacies ranged from 83.3 to 99.7 % for the mean number of immature stages found per palm and from 68.8 to 88.0 % for palm survival, which ranged from 73.8 to 90.0 %. Most of the grubs found dead in *S. carpocapsae*-treated palms proved positive in the laboratory for the presence of nematodes. In many cases palms treated with imidacloprid showed, internal darkened areas which were attributed to initial galleries where young larval stages died.

4 DISCUSSION

Adult weevil captures in pheromone traps are in agreement with seasonal incidence of *R. ferrugineus* in the region of Valencia.[25] Maximal captures are recorded from late-summer to early fall and reach a minimum in winter.

Although all blocks looked similar at the beginning of the assay and selected palms showed no signs of infestation at that time, incidence of *R. ferrugineus* in the nursery resulted heterogeneous and palms in blocks C and D resulted more heavily infested than those in blocks A and B. The south orientation of blocks C and D and their shorter distance to already known infested foci than blocks A and B may partially explain these results.

Both imidacloprid and the nematode formulation with chitosan proved highly effective against *R. ferrugineus* in the field. The efficacy of imidacloprid was independent of when it was applied (December-April-May or December-May-July) and did not significantly change when used in combination with nematodes. The half-life of imidacloprid is 48-190 days depending on groundcover.[26] It breaks down faster in soils with plant groundcover as opposed to fallow soils. According to Tattar et al.,[27] when soil applied, imidacloprid takes from 4-8 weeks for smaller trees and 8-12 weeks for larger ones to move, via the vascular system of the plant, to the foliage. In a preliminary study (same authors, unpublished results), soil injections of Confidor ® 240 OD (10 ml per palm) in 30-yr old *P. canariensis* palms in spring took around 6 weeks to become detectable in the foliage and could be detected for up to 4 additional months. We therefore hypothesize that healthy palms treated with imidacloprid in December 2007 were protected against the new generation of *R. ferrugineus* when females emerged in spring 2008. The additional spring applications of imidacloprid probably kept palms protected during the rest of the year. Adult females actually laid eggs in these palms, but neonate larvae died soon after eclosion as inferred from the darkened internal areas observed when dissecting imidacloprid-treated palms at the end of the assay in January 2009. Kaakeh[8] applied imidacloprid as a soil drench around the stipe of date palms. Three weeks later percent larval mortality reached 61.9 % and all larvae collected alive from these palms died within 48 h.

In semi-field trials with Biorend R®, Llácer et al.[17] obtained efficacies for the mean number of immature stages from 80 % to 98 % in curative and preventive assays, respectively. These results are in agreement with 99.7 % efficacy obtained in our assays based on the number of immature stages found in the palm. The efficacies obtained are very high, especially when compared to chemical pesticides used against this pest.[8, 28-30] Our results contrast with the inconsistent results obtained by Abbas et al. [14] when using entomopathogenic nematodes in date palms. One important difference between Abbas et al.[14] experiments and those reported here is the use of chitosan as an adjuvant. Chitosan is presumed to protect nematodes from environmental conditions and therefore increase and stabilize efficacy as compared to formulations where nematodes are applied without it. Our results confirm that *S. carpocapsae* does not stay on the outside of the palm waiting for its host, but, rather, penetrates in the palm crown actively looking for and infecting *R. ferrugineus* larvae.

These results differ from the general consensus that this species is a classic ambusher,[31, 32] but are in agreement with results obtained by Llácer et al. [17] These authors reported that *S. carpocapsae* with chitosan can survive in the palm for at least two weeks without losing its efficacy. Dillon et al.[33] found that the percentage of *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps parasitized by *S. carpocapsae* increased between two and four weeks and they attributed this fact to both the time taken by the nematodes to find the insects and that taken by the insects to die after EPN infection. In our assays, a monthly application of this product resulted in efficacies statistically equal to those obtained with imidacloprid alone or in combination with the nematode. However, when compared to imidacloprid, the nematode formulation resulted more laborious to apply. To solve the problem of having to reach the top of the palm when treating old taller palms with nematodes or other pesticides, the use of a fixed 4-mm line holding 2 to 4 micro-sprinklers on the top of the stipe has been proposed. In cities like Valencia, Spain, most palms in public gardens have such a line fixed on the top of the stipe down to a height of 2.5 m. When needed, this line is directly connected to a pump on a carrying platform and the pesticide is applied from it with no need to actually get to the top of the palm stipe.

Efficacies obtained from the combined treatments of imidacloprid and *S. carpocapsae* with chitosan were not significantly different from those obtained with the same products when applied alone. The rationale when designing the combined treatments was to protect the palms almost immediately with the nematodes[17] while imidacloprid progressively accumulated in the plant tissues. Our results demonstrate that such tandem effect did not occur and both the entomopathogenic nematodes and imidacloprid effectively protected the palms in a short time. However, the palms used in this assay were about 1.5 m high and the tandem effect could actually happen and become crucial in older palms several meters high, where imidaclorpid translocation to the palm crown could take longer.

Management options to reduce *R. ferrugineus* populations in palms in the Mediterranean basin are limited both because of the cryptic nature of the pest and the limited number of active ingredients available.[9] Both entomopathogenic nematodes and imidacloprid offer an efficient alternative for its control.[9]

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Table 1. Products, doses and application dates of the 5 different treatments applied against *R. ferrugineus* on 6-8 year old *P. canariensis* palms.

Treatment / Product	a.i.	Dose	Application	Application dates
Confidor® 240OD (I)	Imidacloprid	10 ml in 2 l. water (0,042-0,062 %)[1]	Soil injection	December 2007, March and May 2008
Confidor® 240OD (II)	Imidacloprid	10 ml in 2 l. water (0,042-0,062 %)[1]	Soil injection	December 2007 May and July 2008
Biorend R®	*Steinernema carpocapsae* Chitosan	5 x 10^6 DJs (50 ml)[1]	Stipe crown spray	Monthly from December 2008 to December 2009
Confidor®+Biorend R® (I)	Imidacloprid *Steinernema carpocapsae* chitosan	10 ml 5 x 10^6 DJs (50 ml)[1]	Soil injection Stipe crown spray	Confidor in December 2007, March and May 2008. Biorend R® in March and September 2009
Confidor®.+Biorend R® (II)	Imidacloprid *Steinernema carpocapsae* chitosan	10 ml 5 x 10^6 DJs (50 ml)[1]	Soil injection Stipe crown spray	Confidor in December 2007, May and July 2008. Biorend R® in May and September 2009

[1]Authorized doses in Spain (MARM, 2009)

Table 2. Mean number of immature stages of *R. ferrugineus* found in *P. canariensis* palms and percentage palm survival of the 5 different treatments applied against *R. ferrugineus* on 6-8 year old *P. canariensis* and efficacies (%) based on both parameters. Confidor®-treated palms received three treatments in December, March and May (I) or in December, May and July (II). Biorend R® was applied monthly when alone and twice, in coincidence with the second Confidor treatment and in September, when combined with Confidor.

Treatment	Number of immature stages alive			Percentage palm survival		
	n	Mean ± SE [1, 2]	Efficacy	n	Mean ± SE [3]	Efficacy
Control	40	36.60 ± 3.96a		4	16.5 ± 5.8b	
Confidor® 240OD (I)	20	5.50 ± 4.60b	91.2 ± 4.0	4	90.0 ± 6.7a	88.0 ± 8.0
Confidor® 240OD 2 (II)	20	9.50 ± 4.60b	88.8 ± 3.9	4	75.0 ± 11.1a	70.1 ± 13.2
Biorend R®	18	1.29 ± 5.55b	99.7 ± 0.2	4	73.8 ± 10.9a	68.6 ± 13.0
Confidor® 240OD + Biorend R® (I)	19	12.5 ± 5.01b	97.8 ± 2.2	4	85.0 ± 5.8a	82.1 ± 6.9
Confidor® 240OD + Biorend R® (II)	20	10.24 ± 4.74b	83.3 ± 12.7	4	85.0 ± 11.1a	82.0 ± 13.2
Statistical Analyses	$F_{treatment}$= 8.54; *df* = 5, 117; $P < 0.0001$ F_{block}= 4.98; *df* = 3, 117; $P = 0.0030$ (Block A = B > C = D) $F_{interaction}$= 1.64; *df* = 15, 117; $P = 0.0785$			Means compared to control using Dunnett's test. All comparisons were significant.		

[1]Means followed by different letters are significantly different (ANOVA, $P = 0.005$; Duncan's test).

[2]Data subjected to the logarithmic transformation prior to analysis.

[3]Data subjected to the angular transformation prior to analysis.

Figure 1. Dynamics of *R.ferrugineus* adults captured in traps baited with the weevil aggregation pheromone (ferrugineol) and kairomones (ethyl acetate and pieces of palm fronds) located near the palm nursery used in this assay.

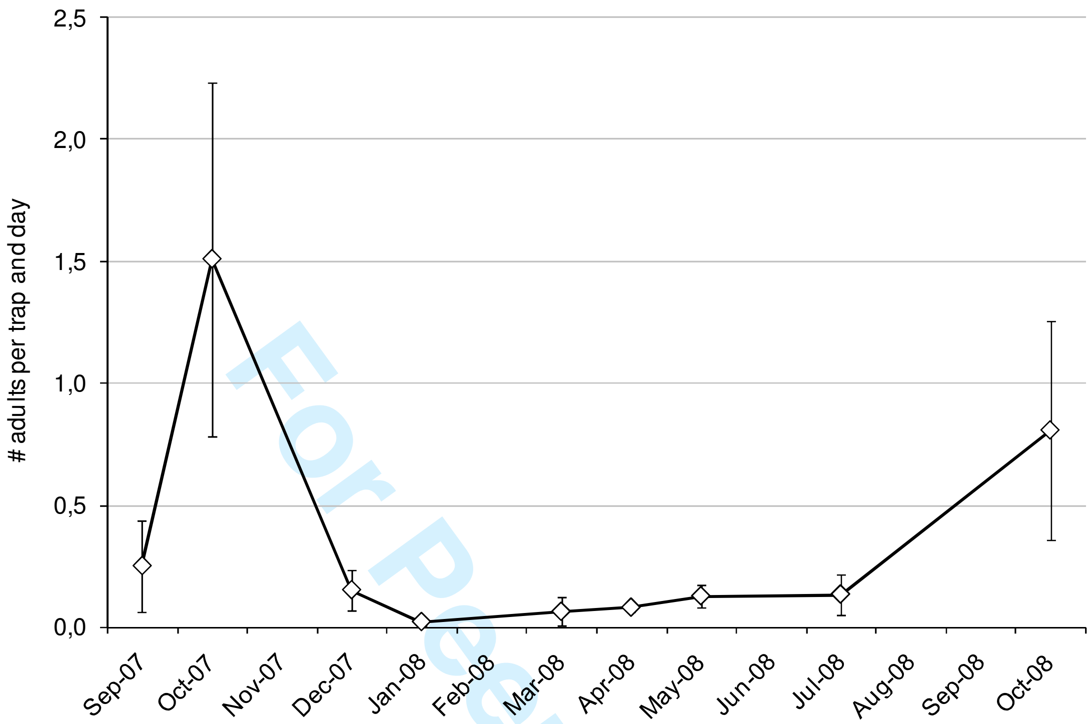

Figure 2. *Phoenix canariensis* survival (%) under different pesticide treatments. Confidor®-treated palms received three treatments in December, March and May (I) or in December, May and July (II). Biorend R® was applied monthly when alone and twice, in coincidence with the second Confidor treatment and in September, when combined with Confidor.