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

8

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

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11

12 Óscar Dembilio<sup>a1</sup>, Elena Llácer<sup>a1</sup>, María del Mar Martínez de Altube<sup>3</sup>, Josep A. Jacas<sup>a2</sup>

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14 <sup>a</sup>Unitat Associada d'Entomologia Agrícola Universitat Jaume I (UJI) -Institut Valencià  
15 d'Investigacions Agràries (IVIA)

16 <sup>1</sup>IVIA; Departament de Protecció Vegetal i Biotecnologia; Ctra Montcada-Nàquera km  
17 4.5; E-46113-Montcada (Spain).

18 <sup>2</sup>UJI; Departament de Ciències Agràries i del Medi Natural, Campus del Riu Sec; E-  
19 12071-Castelló de la Plana (Spain).

20 <sup>3</sup>Idebio S. L., Polígono Industrial el Montalvo, Calle Bell 3, 37008 Salamanca, Spain

21

22 Corresponding author: Josep A. Jacas

23 Universitat Jaume I, Departament de Ciències Agràries i del Medi Natural,  
24 Campus del Riu Sec; E-12071-Castelló de la Plana (Spain).

25 Tel: +34 964729401

26 Email: jacas@camn.uji.es

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29 **Abstract**

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

37  
38 RESULTS: all treatments significantly reduced the mean number of immature stages of  
39 *R. ferrugineus* per palm. However, there were no significant differences among the  
40 different treatments considered. Efficacies ranged from 83.8 to 99.7 % for the mean  
41 number of immature stages found in the palms and resulted in a significant increase in  
42 palm survival compared to the untreated control (75.0-90.0 % versus 16.5 %, respectively).

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

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53 Keywords: Mortality; *Phoenix canariensis*.

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## 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<sup>1</sup> and has been recently found in the Caribbean.<sup>2</sup> 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.<sup>1,3,4</sup> Several control methods have been applied against this pest within an Integrated Pest Management strategy. Its main components are phytosanitation, 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.<sup>5</sup> 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.<sup>6</sup> 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.<sup>5,7</sup> However, there are deep concerns about the environmental pollution caused by these treatments, especially in public areas where ornamental palms are grown.<sup>6</sup> Furthermore, many of the currently used insecticides especially organophosphates and carbamates, are not effective enough.<sup>8</sup> Imidacloprid showed a good efficacy against different stages of *R. ferrugineus* in both

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3 83 laboratory and glasshouse assays<sup>8</sup> and it is one of the few chemicals recommended for  
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5 84 field applications against this pest in palm nurseries in Spain.<sup>9</sup> Imidacloprid is a  
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7 85 chloronicotinyl nitroguanidine insecticide that was first introduced to the United States  
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9  
10 86 in 1994. It is used as a crop and structural pest insecticide, a seed treatment, and a  
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12 87 flea-control treatment. Imidacloprid works by disrupting the insect nervous system and  
13  
14 88 kills by contact and ingestion.<sup>10</sup> It is used to control sucking insects and is effective  
15  
16 89 against adult or larval stages of various species.<sup>11, 12</sup>  
17  
18 90 An interesting alternative to the chemical control of *R. ferrugineus* could be the use of  
19  
20 91 entomopathogenic nematodes (EPNs).<sup>13-17</sup> EPNs are safe for non-target vertebrates  
21  
22 92 and to the environment, and since they are mass-produced in liquid media, production  
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24 93 costs have been significantly reduced in recent times.<sup>18</sup> The infective third juvenile  
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26 94 stages (Dauer Juvenile, DJ) survive outside the insect and can actively search for  
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28 95 hosts. DJs enter the insect host through any opening (mouth, anus, spiracles) and  
29  
30 96 grow into the parasitic stage. The death of the insect due to nematode parasitism is  
31  
32 97 caused by Gram-negative bacteria which are carried within the gut of the DJs.<sup>19</sup>  
33  
34 98 *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae), which is  
35  
36 99 mutualistically associated with the bacterium *Xenorhabdus nematophila*  
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38 100 (Enterobacteraceae), is the most studied, available, and versatile of all EPNs. Although  
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40 101 field experiments in date palms, *P. dactylifera* L., conducted several years ago,  
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42 102 produced inconsistent results,<sup>14</sup> recent laboratory and semi-field assays using *S.*  
43  
44 103 *carpocapsae* with chitosan showed efficacies around 80% in *Phoenix canariensis* Hort.  
45  
46 104 ex Chabaud (Arecaceae).<sup>17</sup> The commercial product Biorend R<sup>®</sup> Palmeras contains *S.*  
47  
48 105 *carpocapsae* and a chitosan adjuvant. Chitosan is a biodegradable organic product  
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50 106 with the active ingredient N-acetyl-glucosamine, which can activate defense  
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52 107 mechanisms in the plant,<sup>20</sup> increase lignification, and promote root development.<sup>21</sup> The  
53  
54 108 use of nematodes with chitosan is patented<sup>22</sup> and nowadays a formulation of *S.*  
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56 109 *carpocapsae* with chitosan is included in the list of authorized products against *R.*  
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58 110 *ferrugineus* in Spain.<sup>9</sup>

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3 111 The aim of this work has been to evaluate in the field the efficacy of imidacloprid and *S.*  
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5 112 *carpocapsae* with chitosan as soil and stipe treatments, respectively, alone or in  
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7 113 combination against *R. ferrugineus*.  
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## 11 115 **2 EXPERIMENTAL METHODS**

12 116 **2.1 Location and set up.** Field experiments were conducted from December 2007 to  
13  
14 117 January 2009 in a *P. canariensis* nursery located in a *R. ferrugineus*-infested area near  
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16 118 the town of Algemés, Spain (Lat.: 39° 19' 36" N; Long.: 00° 43' 77" W; alt.: 18 m).  
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18 119 *Phoenix canariensis* palms were 6-8 years old (palm stipe around 0.5 m in diameter  
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20 120 and 1.7 m high). An area of 750 m<sup>2</sup> within the nursery containing 360 palms regularly  
21  
22 121 planted forming a grid was selected. The grid was cross-divided into 4 rectangular  
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24 122 sections of the same size containing 72 palms each by removing the two central rows  
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26 123 and columns of palms. Palms exhibiting typical symptoms of infestation such as bitten  
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28 124 fronds, fallen central shoot, small holes in the leaf, scars and oozing out of a reddish-  
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30 125 brown fluid and extrusion of fibers from these holes,<sup>23</sup> were removed and only those  
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32 126 that were presumed to be pest-free were further considered. Five different insecticide  
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34 127 treatments (Table 1) plus a control were included in each block (4 to 6 palms per  
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36 128 treatment and block and 8-10 palms for control and block). Palms on the borders of  
37  
38 129 each block were left untreated.

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40 130 Three white traps baited with the weevil aggregation pheromone (ferrugineol) and  
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42 131 kairomones (ethyl acetate and pieces of palm fronds) located near the nursery were  
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44 132 used for monitoring population dynamics of *R. ferrugineus* adults from September 2007  
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46 133 until October 2008. The traps consisted of a 10 l capacity white plastic bucket with four  
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48 134 openings (2.5 × 6 cm<sup>2</sup>) regularly distributed 4 cm below the upper rim of the bucket.

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50 135 **2.2 Pesticide application.** The commercial products Confidor® 240 OD (Bayer Crop  
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52 136 Science S.L., Alcàsser, Valencia) and Biorend R® Palmeras (Idebio S.L., Salamanca,  
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54 137 Spain) were applied at the doses shown in Table 1 either alone or in combination.  
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56 138 Imidacloprid was injected into the soil with a probe connected to a high pressure

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3 139 hydraulic sprayer to a depth of 10-15 cm around the trunk. Biorend R<sup>®</sup> Palmeras was  
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5 140 directly sprayed onto the top of the palm stipe with a Mauricio<sup>®</sup> 18 l Manual Knapsack  
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7 141 Sprayer (Pulverizadores Mauricio S.A., Valencia). Pesticide applications started in  
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9 142 December 2007 for both products (Table 1).

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11 143 **2.3 Data collection.** The nursery was inspected fortnightly. At each inspection, palms  
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13 144 showing symptoms of infestation were removed and taken to laboratory for dissection.  
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15 145 All specimens of *R. ferrugineus*, dead or alive, were extracted and checked for  
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17 146 presence of nematodes.<sup>17</sup>

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19 147 **2.4 Statistical analysis.** Results were subjected to a two-way-analysis of variance  
20  
21 148 (ANOVA, the two factors being treatment and block). The mean numbers of immature  
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23 149 stages found alive were further separated using Duncan's test whereas palm mortality  
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25 150 results were separated using Dunnett's test. The efficacies of the different treatments  
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27 151 based on mean numbers of immature stages found alive were calculated according to  
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29 152 Abbott.<sup>24</sup>

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### 34 154 **3 RESULTS**

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36 155 Trap captures of *R. ferrugineus* adults were maximal in October 2007, two months  
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38 156 before the beginning of the assay. They dropped during winter, slowly recovered during  
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40 157 spring 2008 and peaked again in summer (Figure 1) in correspondence with the  
41  
42 158 sudden increase of dead palms found in control plots (Figure 2).

43  
44 159 Newly infested palms were not detected in the nursery until the month of March (Figure  
45  
46 160 2). First dead palms were observed in control and Confidor<sup>®</sup>+Biorend R<sup>®</sup> (I)-treated  
47  
48 161 blocks. From that month onwards, dead palms were progressively detected in all  
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50 162 treatments except in the Confidor<sup>®</sup>+ Biorend R<sup>®</sup> (II)-treated palms, where first  
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52 163 detection occurred in August. During this month mortality suddenly increased in the  
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54 164 control and significant differences in the percentage of surviving palms between control  
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56 165 and the rest of treatments appeared. These differences did not disappear until the end  
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58 166 of the experiment in January 2009, when efficacies were finally calculated (Table 2).

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3 167 Blocks A and B had a significantly lower incidence of *R. ferrugineus* than blocks C and  
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5 168 D ( $6.26 \pm 1.76$  versus  $22.87 \pm 4.26$  individuals per palm, respectively). However,  
6  
7 169 interaction between block and treatment was not significant (Table 2). All treatments  
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9 170 significantly reduced the mean number of immature stages of *R. ferrugineus* per palm  
10  
11 171 and resulted in increased palm survival compared to the untreated control (Table 2).  
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13 172 There were no significant differences among the different treatments considered.  
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15 173 Efficacies ranged from 83.3 to 99.7 % for the mean number of immature stages found  
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17 174 per palm and from 68.8 to 88.0 % for palm survival, which ranged from 73.8 to 90.0 %.  
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19 175 Most of the grubs found dead in *S. carpocapsae*-treated palms proved positive in the  
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21 176 laboratory for the presence of nematodes. In many cases palms treated with  
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23 177 imidacloprid showed, internal darkened areas which were attributed to initial galleries  
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25 178 where young larval stages died.  
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#### 31 180 4 DISCUSSION

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33 181 Adult weevil captures in pheromone traps are in agreement with seasonal incidence of  
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35 182 *R. ferrugineus* in the region of Valencia.<sup>25</sup> Maximal captures are recorded from late-  
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37 183 summer to early fall and reach a minimum in winter.  
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39 184 Although all blocks looked similar at the beginning of the assay and selected palms  
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41 185 showed no signs of infestation at that time, incidence of *R. ferrugineus* in the nursery  
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43 186 resulted heterogeneous and palms in blocks C and D resulted more heavily infested  
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45 187 than those in blocks A and B. The south orientation of blocks C and D and their shorter  
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47 188 distance to already known infested foci than blocks A and B may partially explain these  
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49 189 results.  
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52 190 Both imidacloprid and the nematode formulation with chitosan proved highly effective  
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54 191 against *R. ferrugineus* in the field. The efficacy of imidacloprid was independent of  
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56 192 when it was applied (December-April-May or December-May-July) and did not  
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58 193 significantly change when used in combination with nematodes. The half-life of  
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60 194 imidacloprid is 48-190 days depending on groundcover.<sup>26</sup> It breaks down faster in soils



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3 195 with plant groundcover as opposed to fallow soils. According to Tattar et al.,<sup>27</sup> when soil  
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5 196 applied, imidacloprid takes from 4-8 weeks for smaller trees and 8-12 weeks for larger  
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7 197 ones to move, via the vascular system of the plant, to the foliage. In a preliminary study  
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10 198 (same authors, unpublished results), soil injections of Confidor<sup>®</sup> 240 OD (10 ml per  
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12 199 palm) in 30-yr old *P. canariensis* palms in spring took around 6 weeks to become  
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14 200 detectable in the foliage and could be detected for up to 4 additional months. We  
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16 201 therefore hypothesize that healthy palms treated with imidacloprid in December 2007  
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18 202 were protected against the new generation of *R. ferrugineus* when females emerged in  
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20 203 spring 2008. The additional spring applications of imidacloprid probably kept palms  
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22 204 protected during the rest of the year. Adult females actually laid eggs in these palms,  
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24 205 but neonate larvae died soon after eclosion as inferred from the darkened internal  
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26 206 areas observed when dissecting imidacloprid-treated palms at the end of the assay in  
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28 207 January 2009. Kaakeh<sup>8</sup> applied imidacloprid as a soil drench around the stipe of date  
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30 208 palms. Three weeks later percent larval mortality reached 61.9 % and all larvae  
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32 209 collected alive from these palms died within 48 h.  
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34 210 In semi-field trials with Biorend R®, Llácer et al.<sup>17</sup> obtained efficacies for the mean  
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36 211 number of immature stages from 80 % to 98 % in curative and preventive assays,  
37  
38 212 respectively. These results are in agreement with 99.7 % efficacy obtained in our  
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40 213 assays based on the number of immature stages found in the palm. The efficacies  
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42 214 obtained are very high, especially when compared to chemical pesticides used against  
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44 215 this pest.<sup>8, 28-30</sup> Our results contrast with the inconsistent results obtained by Abbas et  
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46 216 al.<sup>14</sup> when using entomopathogenic nematodes in date palms. One important  
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48 217 difference between Abbas et al.<sup>14</sup> experiments and those reported here is the use of  
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50 218 chitosan as an adjuvant. Chitosan is presumed to protect nematodes from  
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52 219 environmental conditions and therefore increase and stabilize efficacy as compared to  
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54 220 formulations where nematodes are applied without it. Our results confirm that *S.*  
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56 221 *carpocapsae* does not stay on the outside of the palm waiting for its host, but, rather,  
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58 222 penetrates in the palm crown actively looking for and infecting *R. ferrugineus* larvae.  
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3 223 These results differ from the general consensus that this species is a classic  
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5 224 ambusher,<sup>31, 32</sup> but are in agreement with results obtained by Llácer et al.<sup>17</sup> These  
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7 225 authors reported that *S. carpocapsae* with chitosan can survive in the palm for at least  
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9 226 two weeks without losing its efficacy. Dillon et al.<sup>33</sup> found that the percentage of  
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11 227 *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps parasitized by *S.*  
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13 228 *carpocapsae* increased between two and four weeks and they attributed this fact to  
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15 229 both the time taken by the nematodes to find the insects and that taken by the insects  
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17 230 to die after EPN infection. In our assays, a monthly application of this product resulted  
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19 231 in efficacies statistically equal to those obtained with imidacloprid alone or in  
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21 232 combination with the nematode. However, when compared to imidacloprid, the  
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23 233 nematode formulation resulted more laborious to apply. To solve the problem of having  
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25 234 to reach the top of the palm when treating old taller palms with nematodes or other  
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27 235 pesticides, the use of a fixed 4-mm line holding 2 to 4 micro-sprinklers on the top of the  
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29 236 stipe has been proposed. In cities like Valencia, Spain, most palms in public gardens  
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31 237 have such a line fixed on the top of the stipe down to a height of 2.5 m. When needed,  
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33 238 this line is directly connected to a pump on a carrying platform and the pesticide is  
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35 239 applied from it with no need to actually get to the top of the palm stipe.  
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37 240 Efficacies obtained from the combined treatments of imidacloprid and *S. carpocapsae*  
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39 241 with chitosan were not significantly different from those obtained with the same  
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41 242 products when applied alone. The rationale when designing the combined treatments  
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43 243 was to protect the palms almost immediately with the nematodes<sup>17</sup> while imidacloprid  
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45 244 progressively accumulated in the plant tissues. Our results demonstrate that such  
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47 245 tandem effect did not occur and both the entomopathogenic nematodes and  
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49 246 imidacloprid effectively protected the palms in a short time. However, the palms used in  
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51 247 this assay were about 1.5 m high and the tandem effect could actually happen and  
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53 248 become crucial in older palms several meters high, where imidacloprid translocation to  
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55 249 the palm crown could take longer.  
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3 250 Management options to reduce *R. ferrugineus* populations in palms in the  
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5 251 Mediterranean basin are limited both because of the cryptic nature of the pest and the  
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7 252 limited number of active ingredients available.<sup>9</sup> Both entomopathogenic nematodes and  
8  
9 253 imidacloprid offer an efficient alternative for its control.<sup>9</sup>  
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378 **Table 1.** Products, doses and application dates of the 5 different treatments applied against *R. ferrugineus* on 6-8 year old *P. canariensis*  
 379 palms.

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

380 <sup>1</sup>Authorized doses in Spain (MARM, 2009)



381 **Table 2.** Mean number of immature stages of *R. ferrugineus* found in *P. canariensis* palms and percentage palm survival of the 5 different  
 382 treatments applied against *R. ferrugineus* on 6-8 year old *P. canariensis* and efficacies (%) based on both parameters. Confidor®-treated palms  
 383 received three treatments in December, March and May (I) or in December, May and July (II). Biorend R® was applied monthly when alone and  
 384 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 <sup>1,2</sup>	Efficacy	n	Mean ± SE <sup>3</sup>	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	$F_{\text{treatment}} = 8.54; df = 5, 117; P < 0.0001$			Means compared to control using Dunnett's test. All		
	$F_{\text{block}} = 4.98; df = 3, 117; P = 0.0030$ (Block A = B > C = D)			comparisons were significant.		
	$F_{\text{interaction}} = 1.64; df = 15, 117; P = 0.0785$					

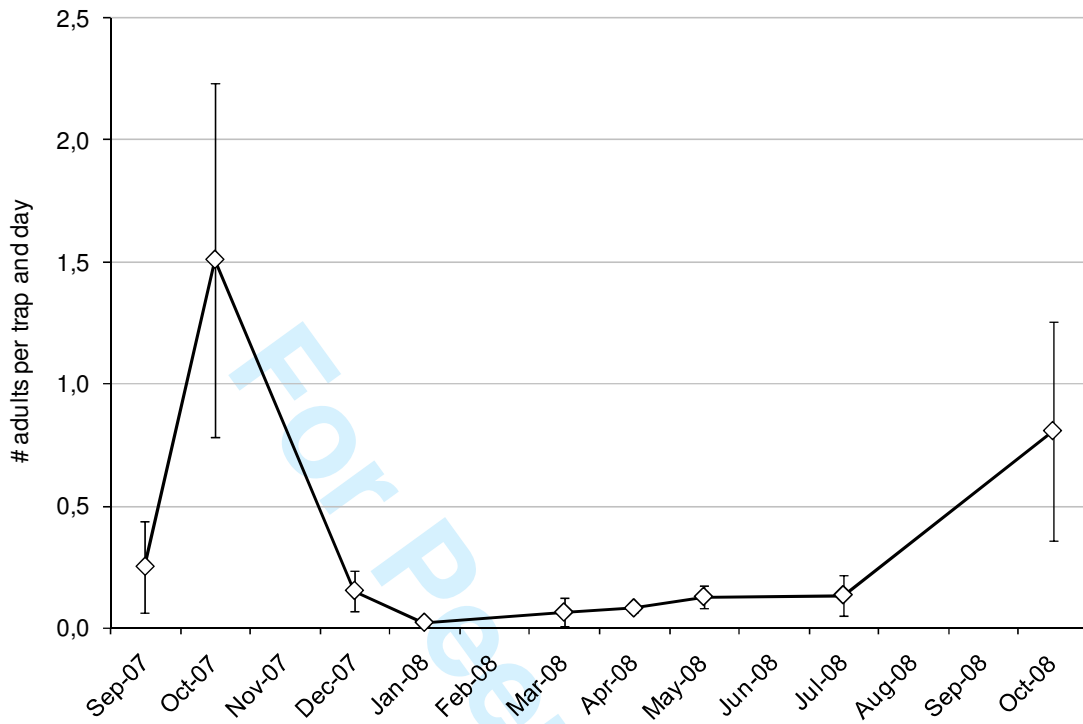
385 <sup>1</sup>Means followed by different letters are significantly different (ANOVA,  $P = 0.005$ ; Duncan's test).

386 <sup>2</sup>Data subjected to the logarithmic transformation prior to analysis.

387 <sup>3</sup>Data subjected to the angular transformation prior to analysis.

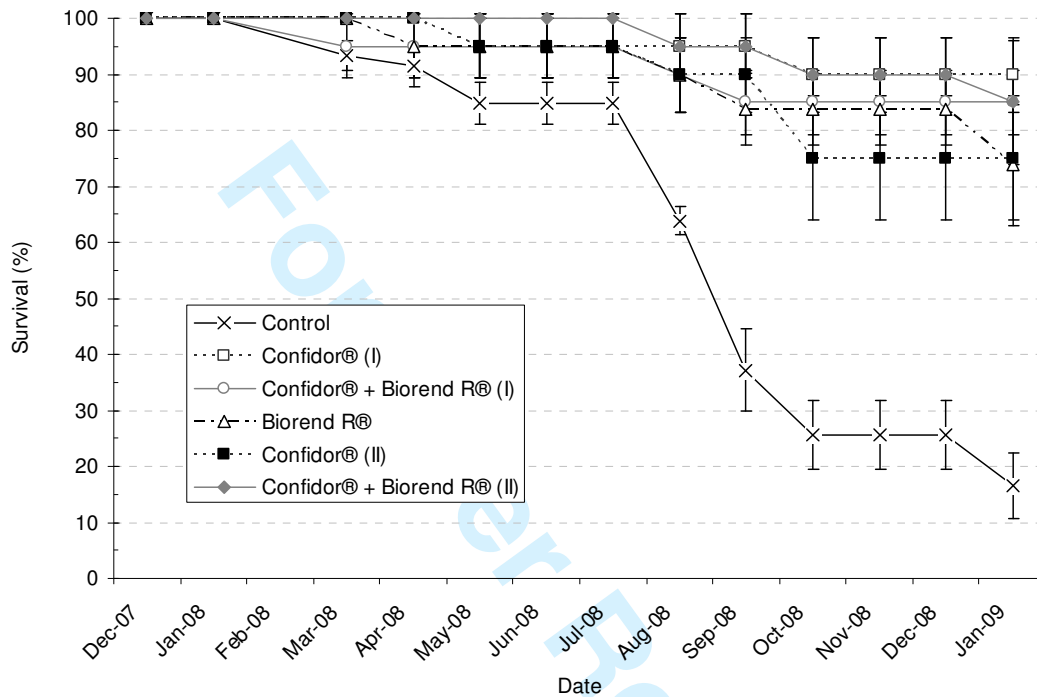
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388 **Figure 1.** Dynamics of *R.ferrugineus* adults captured in traps baited with the weevil  
389 aggregation pheromone (ferrugineol) and kairomones (ethyl acetate and pieces of palm  
390 fronds) located near the palm nursery used in this assay.



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



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