Research Article

Received: 17 May 2014 Revised: 30 July 2014 Accepted article published: 25 August 2014 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.3889

Mobility and efficacy of abamectin and imidacloprid against *Rhynchophorus ferrugineus* in *Phoenix canariensis* by different application methods

Óscar Dembilio,[a] Josep M Riba,[b] Miguel Gamón[c] and Josep A Jacas[a*]

Abstract

BACKGROUND: *Rhynchophorus ferrugineus* is the most destructive pest of palms. As detection of early infestation stages is difficult, preventive measures, mostly chemical control, are crucial. Stipe injection of insecticides has developed rapidly as a suitable technique. However, pesticide movement within palms and palm reaction to wounding remain controversial. We used abamectin and imidacloprid applied by crown spray, stipe and frond injections to disentangle how these pesticides move within *P. canariensis* and how tissues wounded by injection heal. Furthermore, we established their lethal doses to larvae of *R. ferrugineus*.

RESULTS: Maximum residues of imidacloprid (0.1 mg kg^{-1}) were detected in crown and frond samples for up to 2 months after stipe injection, whereas maximum residues of abamectin were found in frond tip samples (0.5 mg active substance kg^{-1}) 5 months after stipe injection. Based on the lethal concentrations calculated, these doses could satisfactorily protect palms for up to 3 months after treatment. No significant wound damage was observed 2 years after injection.

CONCLUSION: Stipe injection, irrespective of the active substance considered, resulted in better distribution and higher persistence compared with frond injection and, especially, crown spray. As a consequence, our results point to stipe injection as a good alternative to control *R. ferrugineus*.

Keywords: stipe injection; pesticides; lethal doses; mortality

1 INTRODUCTION

The invasive red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), has become the most destructive pest of palms in the world.[1] This species is native to south-east Asia, from India to the Philippines, and has enormously increased its geographical range to the Middle East, the Mediterranean Basin, the Macaronesia and the Caribbean as a result of multiple accidental anthropogenic introductions in recent decades.[2–4] This weevil has been reported on 26 palm species belonging to 16 different genera.[5] In the Mediterranean Basin, *R. ferrugineus* is the major pest of palms, mainly *Phoenix canariensis* hort. ex Chabaud, an endemic palm to the Canary Islands, commonly used as an ornamental in the northern half of the Basin, and *Phoenix dactylifera* L., mostly cultivated on the southern shores of the same region.

A serious problem coupled with *R. ferrugineus* is the difficulty of detecting the early stages of infestation on account of its cryptic habits. As a consequence, emphasis is generally focused on preventive measures, mostly relying on chemical control based on the repeated application of pesticides. These treatments can be combined with curative procedures, including sanitation by surgery, designed to contain the infestation. In Spain, a minimum of eight preventive treatments, including five pesticide applications, per season (from March to November) are recommended by official plant protection organisations.[6] However, only six active substances (ASs) are nowadays (May 2014) authorised against *R. ferrugineus* in palms in public areas in Spain. These pesticides are abamectin, chlorpyrifos, emamectin, imidacloprid, phosmet and thiamethoxam.[7] These pesticides are regularly applied as spray on the stipe and crown, or as a drench in the soil (in palm nurseries), with a consequent impact on the environment, including human health problems, as these products may be used in gardens and public areas where palms are often located. For these reasons, stipe (=stem) injection of systemic insecticides is an interesting alternative to protect palms against *R. ferrugineus*.[8] This method

* *Correspondence to: Josep A Jacas, Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I (UJI), Unitat Associada d'Entomologia Agrícola UJI-IVIA (Institut Valencià d'Investigacions Agràries), Campus del Riu Sec, E-12071, Castelló de la Plana, Spain. E-mail: jacas@uji.es*

a *Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I (UJI), Unitat Associada d'Entomologia Agrícola UJI-IVIA (Institut Valencià d'Investigacions Agràries), Castelló de la Plana, Spain*

b *ENDOterapia Vegetal (EV), Castelló d'Empúries, Girona, Spain*

c *Laboratorio Agroalimentario de la Generalitat Valenciana, EURL – Pesticide Residues in Fruit and Vegetables, Burjassot, València, Spain*

directly releases the product into the vascular system of the palm in an efficient and environmentally friendly way compared with other methods. At present, half a dozen systemic insecticides have been authorised in different EU countries against this pest. These substances belong to the neonicotinoid (clothianidin, imidacloprid and thiamethoxam) and the avermectin (abamectin and emamectin) pesticide groups established by IRAC according to their mode of action.[9] The efficacy of these products against *R. ferrugineus* has been proven.[8,10,11] Experiments carried out by El Sebay and Abbas[12] revealed that low-pressure injection was effective and safer for palms than high-pressure injection. However, there is an intense debate about the pros and cons of the application of this technology to palms.[13] One of the main objections is related to the injury caused by the drilling of the stipe prior to injection, which may provide opportunities for insect and pathogen invasion of the stipe. According to Tomlinson,[14] palms cannot repair injuries in their stems because, as monocotyledoneous plants, they have no vascular cambium and are essentially devoid of secondary growth. However, Thomas and de Franceschi[15] pointed out that damaged areas in palms are quickly isolated by paravascular parenchyma cells, which secrete tylose, phenolic compounds and an acid gel containing pectin. Because of this open debate, much effort is being devoted to the development of new injection devices to minimise this injury. Further, it would be interesting to find alternative injection zones in the palm where injuries would not be as important (the fronds, for instance). An added problem to the injection of systemic products in palms is associated with the stability of the suspension used. On the one hand, internal conditions of the palm stipe promote precipitation of the AS injected. On the other, a fast precipitation can occur even before injection if the insecticide is not adequately formulated for microinfusion (this is the case with imidacloprid).[16] Furthermore, proof of the negative impact of neonicotinoids on the behaviour of pollinators is questioning the use of this type of pesticide to control palm pests on a large scale.[17] Finally, little is known about the distribution of these products when injected into palms.

Neonicotinoids act as agonists on the postsynaptic nicotinic acetylcholine receptors of insects, causing an overstimulation of their nervous system which finally kills them. These insecticides are transported mainly in the xylem in both mono- and dicotyledonous plant species.[18] In particular, the systemic properties of imidacloprid allow it to become uniformly distributed in the young growing plant. After application, most of the residue of imidacloprid on the leaf surface consists of the unchanged parent compound, whereas when applied as a soil drench or seed treatment it is completely metabolised.[19] It has been demonstrated recently that some of these metabolites exhibit insecticidal potency against different species and may influence the residual insecticidal activity in imidacloprid-treated plants,[20,21] in spite of its relatively low concentration.[19,22] Imidacloprid is more effective when ingested by the target insect than by contact.[23] The other group of systemic insecticides authorised against *R. ferrugineus*, the avermectins, are natural fermentation products of the soil bacterium *Streptomyces avermitilis*.[24] These pesticides are generally used to control mites and insects on a wide variety of crops and also ornamentals. They interfere with the neural and neuromuscular transmissions by disrupting a specific type of synapsis that uses gamma-aminobutyric acid (GABA) as a transmitter. In insects, GABAergic synapsis occurs throughout the nervous system, making insects susceptible to these ASs at low doses. In particular, abamectin is highly unstable to light and has been shown to photodegrade rapidly on plant and soil surfaces and in water following agricultural applications. Abamectin is also readily degraded by soil microorganisms. In addition, abamectin does not persist or accumulate in the environment. This instability, as well as its low water solubility and tight binding to soil, limit abamectin bioavailability for non-target organisms and furthermore prevent it from leaching into groundwater or entering the aquatic environment.[25] Like imidacloprid, abamectin is most effective when ingested by the target organism.

For all the reasons above, field and laboratory experiments were carried out with the systemic pesticides abamectin and imidacloprid. These assays were aimed at (1) disentangling how these pesticides are translocated within *P. canariensis* and how tissues wounded by stipe injection heal, and (2) establishing lethal doses of these pesticides for larvae of *R ferrugineus*. The combination of the results of these assays should provide clues to increase the efficacy of chemical control of this pest.

2 EXPERIMENTAL METHODS

2.1 Laboratory experiments

2.1.1 Stock colonies

Stock colonies were maintained using standard procedures.[26] These colonies were started with adult weevils collected in the province of Valencia in traps baited with ferrugineol (the *R. ferrugineus* aggregation pheromone) and plant kairomones (ethyl acetate and pieces of palm fronds). These colonies were established in 2007 and have been periodically supplemented with the introduction of additional wild specimens. Adult weevils were reared in a controlled environment cabinet at 25 ± 1 °C, 75 ± 5% RH and a 16 h light photoperiod in perspex cages ($30 \times 30 \times 45$ cm depth) with a density of 100–120 weevils per cage. These cages had a round hole, 8 cm in diameter, on the upper side, covered by a mesh and used for manipulation of the specimens, and their bottom side consisted of a 2 mm metal mesh used by females for oviposition. Cages were set on top of a tray containing a folded piece of moistened filter paper containing thin apple slices used by female weevils as oviposition substrate and by both males and females as food. Apple slices were replaced 3 times per week.[26] Eggs were further kept on apple slices until hatching and then reared up to adulthood individually in 125 mL vials containing 45–50 g of the weevil's artificial diet.[27] Larvae were moved to a new vial fortnightly until ready for pupation (45-day-old larvae). At that moment, larvae were moved to another vial containing strands of dry esparto grass (*Stipa tenacissima* L.) used by the larvae to build a cocoon. About 1 month later, laboratory-reared adults emerged. These adults were eventually introduced into the rearing cages.

2.1.2 Experimental insects

Young larvae (L III to V; mean weight 441 ± 5 mg) and old larvae (L VII to IX; mean weight 2730 ± 20 mg) obtained from the stock colonies were exposed to different doses of imidacloprid (10, 1, 0.1 and 0.01 mg AS kg^{-1}) and abamectin (15, 1.5, 0.15 and 0.015 mg AS kg^{-1}). For each larval instar, three replicates of ten larvae per dose were considered. For each replicate, 500 g of diet was prepared, and the corresponding pesticide amount was added during cooling of the mixture, when the temperature dropped to 40 °C. This mixture was distributed among ten vials as above, where larvae were individually exposed to the pesticide. These containers were examined daily to check larval mortality for up to 30 days. Larvae were weighed at the beginning of the assay (wt_0)

and at the end (wt_f), either when they died or when the assay was terminated at day 30. The weight gain ($wt_f - wt_0$) was divided by the days elapsed since the beginning of the assay, and the growth rate was obtained for each pesticide and larval age. As avermectins are decomposed rapidly in sunlight, vials were wrapped in aluminium foil to avoid any interference of the light in our results.

2.2 Field experiments

2.2.1 Location and set-up

Experiments were performed in a *P. canariensis* nursery located in an *R. ferrugineus*-free marshland area near the town of Castelló d'Empúries, Spain (latitude: 42° 15′ 48″ N; longitude: 3° 10′ 30″ E; altitude: 8 m asl). Palms were 17 years old and were regularly planted in an area of 1300 m^2 within the nursery, containing 238 specimens. Within this area, 126 palms were selected in May 2012. These palms had never received any pesticide treatment. The nursery was rarely watered, as annual average rainfall in Castelló d'Empúries is 711.8 mm,[28] which makes irrigation usually unnecessary. In 2012, a total of 160 and 77.7 mm of rain was recorded there in spring and May respectively.[29] As a consequence, when the treatments took place, the nursery had not been previously irrigated, as the soil was at its field capacity.

2.2.2 Pesticide treatments

Abamectin and imidacloprid were selected. The commercial products Confidor® 20 LS (AS imidacloprid; Bayer Crop Science S.L., Valencia, Spain) and Vertimec® (AS abamectin; Syngenta S.L., Madrid, Spain) were either injected or sprayed at different concentrations according to the maximum authorised doses in Spain (Table 1). Twenty-one palms per treatment (spray, stipe and frond injections) and control were treated on 2 May 2012. Injections were performed using the low-pressure microinfusion system developed by ENDOterapia Vegetal S.L. (Castelló d'Empúries, Girona, Spain). This system uses one single injection point, unlike other systems that use several injection points distributed every 30–40 cm along the perimeter of the stipe. Spray treatments were applied using a Honda® portable power sprayer. When pesticides were injected, they were mixed with Endomix Palm® developed by ENDOterapia Vegetal at 1:1 ratio to stabilise the mixture, avoid precipitation and improve the movement of the AS inside the palm.

2.2.3 Sample collection

Three palms per treatment and control were sampled 24 h after the treatment and then every month until November 2012. At each sampling date (seven in total) the selected palms were cut down and dissected using a chainsaw. Five different samples per palm were collected. Crown and lower stipe discs (around 25 cm in diameter, taken from the central core) were obtained. Further, three fronds per palm were selected, and base, middle and tip samples were obtained by pooling into one single sample the bases, the middle and the tip sections of the three fronds respectively. These samples were kept refrigerated until they reached the laboratory, where they were frozen at −18 °C until further processing for analyses. At that moment, samples were cut into smaller pieces with hand pruners and then ground before analysis. Imidacloprid and abamectin residues were determined by the QuEChERS method with the use of European variation EN 15 662.[30,31] The homogeneous sample was extracted with acetonitrile, followed by the salting out of water from the sample using anhydrous magnesium sulphate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid–liquid partitioning.

2.2.4 Mechanical injury caused by injection

Six additional palms were injected into the stipe with Confidor®, as previously described, and dissected to assess the injury caused by the injector 12 and 24 months after treatment. These samples were obtained with a chainsaw and checked by the naked eye.

2.3 Statistical analyses

In the laboratory tests, for each larval age and pesticide concentration tested, the average survival time (AST) values were calculated using Kaplan–Meier survival analysis.[32] These values were further compared using the non-parametric Kruskal–Wallis test. When significant differences were found, medians were separated using a box and whisker plot. Final weight (wt_f) values for each larval age and pesticide were compared by ANOVA and means were separated according to Tukey's HSD test. When significant differences were obtained, growth rates ($wt_f - wt_0$/longevity) were compared using the non-parametric Kruskall–Wallis test as above. Furthermore, regression analysis was used to check the relationship between these rates and pesticide dose. Finally, for times of 5

Table 1. Products, doses and method of application of the five treatments applied to 17-year-old *Phoenix canariensis* palms

Pesticide	Active substance	Authorised use/s and dose/s in Spain[a]	Application technique and volume per palm	Dose (g AS $palm^{-1}$)
Confidor® 20 LS	Imidacloprid (200 g L^{-1})	**Injection**: 4–10 mL per treatment (1.5–2 m below crown; at 45–55 day intervals between March and November) **Spray**: 0.05–0.075 L hL^{-1} **Drench**: 8–10 L ha^{-1} (max. two treatments 30–40 days apart)	Stipe injection (0.02 L)	2.00
Confidor® 20 LS	Imidacloprid (200 g L^{-1})	**Injection**: 20–80 mL $palm^{-1}$ (max. two applications per year 15–45 days apart between March and November)	Frond injection (0.02 L)	2.00
Confidor® 20 LS	Imidacloprid (200 g L^{-1})		Crown spray (30 L)	4.50
Vertimec® 1.8 EC	Abamectin (18 g L^{-1})		Stipe injection (0.16 L)	1.44
Vertimec® 1.8 EC	Abamectin (18 g L^{-1})		Frond injection (0.16 L)	1.44

[a] Authorised doses in Spain (MAGRAMA, 2014).[7]

Table 2. Average survival time (AST, days after treatment), final weight and growth rate of *R. ferrugineus* young and old instars treated with imidacloprid in the laboratory. Initial larval weight was 0.441 and 2.726 g for young and old larvae respectively

	AST[a] (days)		Final weight (g)		Growth rate (mg day^{-1})	
Dose (mg kg^{-1})	Young larvae	Old larvae	Young larvae	Old larvae	Young larvae	Old larvae
0.010	7.67 ± 1.53 a	20.33 ± 4.16 a	0.355 ± 0.013 b	2.283 ± 0.096 b	−13.09 ± 1.79 b	−67.81 ± 18.06 b
0.100	4.67 ± 0.58 b	6.67 ± 1.15 b	0.347 ± 0.015 b	2.169 ± 0.047 b	−20.46 ± 1.32 c	−90.94 ± 9.88 c
1.000	2.06 ± 0.59 c	2.22 ± 0.38 c	0.387 ± 0.018 b	2.323 ± 0.060 b	−26.51 ± 0.71 d	−153.80 ± 3.07 d
10.000	1.39 ± 0.35 d	2.06 ± 0.59 c	0.366 ± 0.016 b	2.493 ± 0.050 b	−29.13 ± 3.10 d	−171.29 ± 16.15 d
Control	–[b]	–[b]	1.358 ± 0.059 a	4.127 ± 0.053 a	44.83 ± 0.59 a	39.48 ± 5.41 a
ANOVA	$\chi^2 = 9.88$; df = 3; $P = 0.020$	$\chi^2 = 9.60$; df = 3; $P = 0.022$	$F = 222.97$; df = 4, 149; $P < 0.001$	$F = 165.81$; df = 4, 149; $P < 0.001$	$\chi^2 = 13.00$; df = 4; $P = 0.011$	$\chi^2 = 12.43$; df = 4; $P = 0.014$

[a] AST values limited to the duration of the assay.
[b] AST values could not be calculated because the observed mortality was below 50%.

and 10 days after treatment, percentage mortality was corrected[33] and transformed into probits, and the corresponding probit lines were fitted.[34] A chi-square test was used to prove the goodness of fit. In addition, plots of standardised residuals were checked for their location within a horizontal band of ± 2 units about zero.[35] In the field test, for each pesticide–treatment combination, correlation between pesticide concentrations during the assay in the different samples was studied.

3 RESULTS

3.1 Mortality and growth rates of *R. ferrugineus* larvae

Both for abamectin and imidacloprid, the higher the dose and the younger the instar, the shorter were the AST values recorded (Tables 2 and 3). However, except for young larvae exposed to imidacloprid, no significant differences were observed for the two highest doses of each pesticide tested. Growth rates in control larvae were always positive (Tables 2 and 3). Interestingly, except for old larvae exposed to the lowest dose of abamectin, which increased weight at the same rate as the control, all treatments resulted in a weight loss during the assay, which for each pesticide was independent of the dose (Tables 2 and 3). When growth rates were plotted against pesticide dose, significant dose–response relationships were observed in all cases ($P < 0.005$; adjusted $R^2 > 0.7978$). Moreover, when mortality was plotted against pesticide dose and then subjected to probit analysis, young larvae yielded lower lethal concentration (LC) values than old larvae (Table 4).

3.2 Pesticide residues

As expected, control palms proved to be negative for abamectin residues. However, low imidacloprid residues (<0.007 ± 0.003 mg kg^{-1}) were detected in six control palms out of the 21 included in the study. These results were attributed to contamination from the sampling tools, basically the chainsaw, which is quite difficult to clean properly in the field. For each pesticide–treatment combination, pesticide concentrations in the different samples during the assay could not be satisfactorily correlated ($P > 0.05$).

3.2.1 *Abamectin injection into the stipe*

Residues between 0.150 and 0.550 mg kg^{-1}, which according to the laboratory results (Table 4) could cause mortality rates above 90% in larvae at day 10, were detected in the middle and tip samples of the frond between 2 and 5 months after the treatment (Fig. 1). Crown residues were much lower (0.100 mg kg^{-1}) and peaked 1 month after the treatment. According to the laboratory

Table 3. Average survival time (AST, days after treatment), final weight and growth rate of *R. ferrugineus* young and old instars treated with abamectin in the laboratory. Initial larval weight was 0.441 and 2.726 g for young and old larvae respectively

	AST[a] (days)		Final weight (g)		Growth rate (mg day^{-1})	
Dose (mg kg^{-1})	Young larvae	Old larvae	Young larvae	Old larvae	Young larvae	Old larvae
0.015	12.33 ± 1.53 a	–[b]	0.361 ± 0.014 b	3.904 ± 0.091 b	−9.21 ± 1.15 b	48.13 ± 3.05 a
0.150	5.00 ± 1.00 b	8.33 ± 3.21 a	0.399 ± 0.014 b	2.280 ± 0.060 c	−19.42 ± 1.82 c	−63.75 ± 5.39 b
1.500	1.89 ± 0.84 c	2.83 ± 0.29 b	0.378 ± 0.016 b	2.415 ± 0.041 c	−29.12 ± 4.70 d	−132.24 ± 5.64 c
15.000	1.28 ± 0.25 c	2.50 ± 0.50 b	0.368 ± 0.013 b	2.311 ± 0.049 c	−29.64 ± 8.50 d	−145.07 ± 8.91 c
Control	–[b]	–[b]	1.489 ± 0.060 a	4.159 ± 0.043 a	−56.34 ± 9.44 a	41.82 ± 3.01 a
ANOVA	$\chi^2 = 9.58$; df = 3; $P = 0.020$	$\chi^2 = 6.01$; df = 2; $P = 0.050$	$F = 280.27$; df = 4, 149; $P < 0.001$	$F = 245.56$; df = 4, 149; $P < 0.001$	$\chi^2 = 12.90$; df = 4; $P = 0.012$	$\chi^2 = 12.97$; df = 4; $P = 0.011$

[a] AST values limited to the duration of the assay.
[b] AST values could not be calculated because the observed mortality was below 50%.

Table 4. Probit lines adjusted to mortality data of immature *R. ferrugineus* at two time points (5 and 10 days) when fed on either imidacloprid- or abamectin-supplemented diet in the laboratory. Control mortality was below 5.6 ± 1.2% and 9.3 ± 2.3% for old and young larvae respectively

Active substance	Target	Time (days)	*n*	Slope ± SE	χ^2	*P*-value	LC_{50}; 95%FL (mg kg^{-1} diet)	LC_{90}; 95% (mg kg^{-1} diet)
Imidacloprid	Old larvae	5	120	1.148 ± 0.173	1.596	0.450	0.139; 0.075–0.253	1.813; 0.838–6.304
		10	120	1.232 ± 0.211	0.847	0.655	0.047; 0.024–0.084	0.513; 0.247–1.804
	Young larvae	5	120	1.840 ± 0.489	0.004	0.998	0.100; 0.042–0.165	0.498; 0.285–2.014
		10	120	1.036 ± 0.310	0.443	0.801	0.004; 0.001–0.010	0.077; 0.038–0.302
Abamectin	Old larvae	5	120	1.751 ± 0.291	0.719	0.698	0.305; 0.186–0.494	1.644; 0.923–4.331
		10	120	2.171 ± 0.415	0.585	0.746	0.126; 0.081–0.198	0.490; 0.289–1.307
	Young larvae	5	120	1.922 ± 0.491	0.003	0.999	0.166; 0.079–0.268	0.770; 0.440–2.895
		10	120	1.560 ± 0.340	0.139	0.933	0.028; 0.013–0.049	0.184; 0.097–0.664

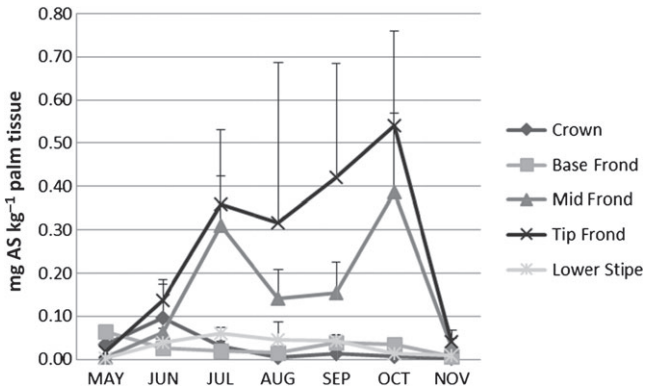

Figure 1. Residues of abamectin when injected into the stipe of 17-year-old palms. Only upper error bars are presented.

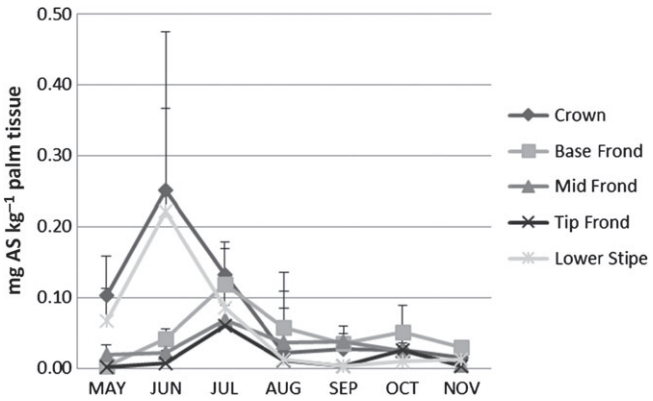

Figure 2. Residues of imidacloprid when injected into the stipe of 17-year-old palms. Only upper error bars are presented.

results, this concentration would cause between 50 and 90% mortality in young instars at day 10 and less than 50% mortality for older instars (Table 4). For the remaining dates and palm samples, residues ranged from nil to less than 0.050 mg kg^{-1}, and these concentrations would cause less than 50% mortality in all stages but young larvae (Table 4).

3.2.2 *Abamectin injection into the frond petiole*

Residues were lower than in the previous case. A peak of around 0.150 mg kg^{-1}, which according to laboratory results would always result in larval mortality rates below 90% (Table 4), was detected 1 month after the treatment in all frond samples. From 3 months after the treatment until the end of the assay, no residues of abamectin were detected.

3.2.3 *Imidacloprid injection into the stipe*

At 24 h after the treatment, residues of around 0.100 mg kg^{-1} of imidacloprid were detected in lower stipe and crown disc samples. This concentration is expected to cause more than 90% mortality in young larvae 10 days after the treatment (Table 3), and between 50 and 90% in older stages (Table 4). No imidacloprid was detected in the fronds until 1 month later (0.041 mg kg^{-1} in the base frond sample). At that time, imidacloprid residues peaked in the stipe (values of 0.200 mg kg^{-1} in the lower stipe and crown) and 1 month later in the frond base (0.120 mg kg^{-1}). From month 3 to month 6, residues were around 0.015 mg kg^{-1}, irrespective of the palm tissue considered (Fig. 2), and this concentration is expected to cause less than 50% mortality of young larvae at day 10. To sum up, imidacloprid concentrations resulting in more than 90% mortality in young larvae at day 10 (Table 4) were observed in the crown for 2–3 months after the treatment, and the same concentrations are expected to produce more than 50% mortality in older larvae (Table 4).

3.2.4 *Imidacloprid injection into the frond petiole*

The performance of imidacloprid in this case was completely different from that recorded when the pesticide was injected into the stipe. A low concentration was observed during the whole assay in all palm samples (lower than 0.030 mg kg^{-1}), with the exception of a peak of around 0.250 mg kg^{-1} in the frond base 3 months after injection. These concentrations could cause mortality rates between 50 and 90% in young larvae 10 days after the injection, and mortality rates below 50% in older stages (Table 4).

3.2.5 *Imidacloprid spray onto the crown*

The highest amount of imidacloprid was detected in the lower stipe disc sample 24 h after the application (around 0.500 mg kg^{-1}). In all other tissues and sampling dates, residues were lower than 0.030 mg kg^{-1} (Fig. 3). As in the case of the petiole injection, these concentrations are expected to cause mortality rates between 50 and 90% in young larvae 10 days after the treatment, and below 50% in older stages (Table 4).

3.3 Mechanical injury caused by injection

The injection area could be easily identified by the naked eye by a darker colour than the rest of the disc. However, it looked compact

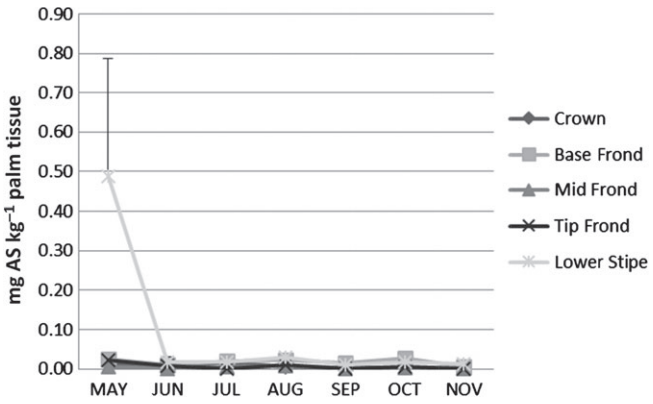

Figure 3. Residues of imidacloprid when sprayed onto the crown of 17-year-old palms. Only upper error bars are presented.

and healthy. No symptoms of fungal growth were observed 12 and 24 months after injection.

4 DISCUSSION

Abo-El-Saad *et al.*[11] studied the mortality of different stages of *R. ferrugineus*, including neonate and 25-day-old larvae, when exposed to abamectin. Residual toxicity was studied in transparent plastic cups sprayed under a Potter tower with an unspecified volume of different abamectin concentrations ranging from 200 to 1000 mg abamectin L^{-1}. LC_{50} values of 98.7 and 739.9 mg L^{-1} were obtained for neonate and 25-day-old larvae 24 and 48 h after the treatment respectively. Although the method used precludes direct comparison of these results with ours, these values are much higher than those obtained in our assays (Table 4). Several reasons could explain these enormous differences. On the one hand, we estimated LC_{50} later than these authors (Table 4). On the other hand, in our assays abamectin was mixed with the diet and protected from the light. As a consequence, larvae were exposed to the chemical both orally and by contact. Furthermore, photodegradation was prevented by wrapping the vials containing the diet with aluminium foil. Avermectins are mostly active by ingestion, and it is known that surface residues are decomposed rapidly;[36–38] their residual activity is mostly attributed to their translaminar activity, which provides them with a relatively prolonged residual activity.[36] Therefore, the method used by Abo-El-Saad *et al.*[11] may have underestimated abamectin toxicity to *R. ferrugineus*.

A few years earlier, Cabello *et al.*[39] studied the mortality of seven-day-old and 30-day-old larvae of *R. ferrugineus* when exposed to a dose of 100 mg imidacloprid L^{-1} diet. This dose, which is about 10 times higher than the highest dose used in our assays (10 mg kg^{-1}), resulted in 100% mortality 5 and 8 days after the treatment for older and young larvae respectively. As far as we know, this is the only case reported of older larvae of *R. ferrugineus* exhibiting higher sensitivity to a pesticide than younger ones. Indeed, our results show the opposite both for imidacloprid and abamectin (Tables 2 and 3). Furthermore, the highest dose used in our assays reached 100% mortality much earlier than that reported by these authors. In contrast, our results are in agreement with those obtained by Kaakeh[40] who exposed larvae of undetermined age to sugar cane stem pieces dipped in a solution of 6 mg imidacloprid L^{-1}, which resulted in 100% mortality 18 h after the treatment.

For each pesticide–treatment combination, concentrations found in the different palm tissues could not be satisfactorily related to each other. Because the tissues of *P. canariensis* that should be targeted to protect them against the weevil are basically the frond base, where oviposition and pupation take place, and the crown, where the weevils feed and develop,[41,42] this result precludes the use of easily accessible palm frond samples to estimate pesticide concentrations in the stipe. Furthermore, efficacy trials performed in *P. canariensis* detached frond petioles used as a proxy of the palm crown[43] should be taken with caution. In the case of abamectin, which moves and accumulates in the fronds, such an assay could overestimate efficacy (Fig. 1), whereas in the case of imidacloprid, which remains mostly in the stipe, the result would have been the opposite (Fig. 2). Whether the different distribution observed for these two insecticides could be taken as representative of avermectins and neonicotinoids, respectively, needs further research.

Stipe injection, irrespective of the AS considered, resulted in a better distribution within the palm and higher persistence compared with frond injection and, especially, crown spray. Once injected, abamectin moved and accumulated in the fronds and presented concentrations expected to cause 50–90% mortality in young instars and below 50% in older ones in the crown for only 1 month after the treatment. By contrast, upon injection, imidacloprid remained in the stipe, where concentrations expected to result in more than 90% mortality in young instars and 50–90% in older ones were observed for 2–3 months after the treatment (Figs 1 to 3, Tables 2 to 4). As a consequence, our results point to a better performance of imidacloprid relative to abamectin against *R. ferrugineus* under field conditions. The concentrations of imidacloprid observed in our assay over time are in good agreement with those obtained by Llácer *et al.*[44] when applying this insecticide as a soil drench in young palms (2 years old, 7.8 cm diameter). Imidacloprid concentration in the stipe increased for 45 days from application (reaching a peak of around 0.30 and 0.14 mg AI kg^{-1} when 0.36 and 0.72 g AI $palm^{-1}$ were applied respectively) and decreased afterwards following similar dynamics to that observed in the crown (Fig. 2). The same authors estimated the efficacy of the highest dose (0.72 g AI $palm^{-1}$), which was 92–96% when imidacloprid was applied as a curative treatment (palms infested before treatment) and 100% when used preventively (palms infested after the treatment). Interestingly, when persistence of the preventive treatment was studied (0.60 g AI $palm^{-1}$), 100% efficacy was recorded 45 days after treatment, which dropped to 86% after 2 months; these results are in agreement with what we found when imidacloprid was injected into the stipe.

The dose of imidacloprid used for the spray treatment and the total volume applied in palms were 2.25 and 1500 times higher than when used in trunk injections respectively. However, very low residues of this pesticide were found in sprayed palms during the whole assay. Neonicotinoid insecticides have excellent systemic uptake and translocation in plants.[45,46] However, this was not the case in our assays. Hence, good efficacies reported in grey literature for spray applications of imidacloprid against *R. ferrugineus* should probably be attributed to direct contact and ingestion by neonate larvae and adults in the periphery of palms. Because these sprays may result in run-off and accumulate in the soil, where residues can remain for up to 190 days,[47] sprays, if not properly applied, could result in undesired exposure to non-target organisms. However, if applied to small nursery plants, this accumulation in the substrate could result (soil properties not

leading to imidacloprid precipitation) in increased uptake by the roots and therefore increased efficacy.[10,44]

As new injection devices such as the one used in our assays can minimise mechanical injury to the palm stipe, our results point to this type of injection treatment as the most suitable for delivery of pesticides against *R. ferrugineus* in *P. canariensis*. Recommendations on the number of applications and doses should probably take into account the size of the palms as well as the bioecology of this pest. In the Northern Mediterranean Basin, oviposition and egg hatching periods extend from early April to mid-October/early November and from mid-March to mid/late October respectively.[42] Therefore, palms should be protected for about 7 months per season. As the effect of winter temperatures on the population dynamics of *R. ferrugineus* could be a synchronisation of the whole population,[41,48] a spray treatment targeting newly emerged adults in late March/early April could be followed by a stipe injection in mid/late April. These two treatments could effectively protect the palms for up to 2–3 months. Therefore, new action should be taken around late June/early July. A new injection at that time could cover the rest of the season but might be complemented by another spray targeting the second flying period around late September/early October. To minimise resistance risk, rotation of compounds with different modes of action should be implemented. When available, biological control agents[10,49,50] should be included in the sequence, as biological control does not favour the selection of pesticide-resistant strains. Finally, area-wide strategies aimed at reducing weevil populations such as mass trapping could prove to be highly effective if properly managed.[51]

ACKNOWLEDGEMENTS

The authors thank M Piquer (UJI), JM Barroso, LL Baronat and L Olivet (ENDOterapia Vegetal) and J Sancenón (Laboratorio Agroalimentario de Burjassot) for their help during the assays. C Bartra allowed us to use her nursery for our experiments. This research received funding from European FP7 (UJI and ENDOterapia Vegetal are partners of the project KBBE 2011-5-289566 Palmprotect consortium) and the Valencian Government (project IVIA-5611).

REFERENCES

1 Dembilio O and Jacas JA, Bio-ecology and integrated management of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in the region of Valencia (Spain). *Hell Plant Prot J* **5**:1–11 (2012).
2 Data sheets on quarantine pests. Rhynchophorus ferrugineus. *EPPO Bull* **38**:55–59 (2008).
3 EPPO Reporting Service 2009/002 – First record of *Rhynchophorus ferrugineus* in Curaçao, Netherlands Antilles, January 26. European and Mediterranean Plant Protection Organisation, Paris, France (2009).
4 Rugman-Jones PF, Hoddle CD, Hoddle MS and Stouthamer R, The lesser of two weevils: molecular-genetics of pest palm weevil populations confirm *Rhynchophorus vulneratus* (Panzer 1798) as a valid species distinct from *R. ferrugineus* (Olivier 1790), and reveal the global extent of both. *PLoS ONE* **8(10)**: e78379 (2013). doi:10.1371/journal.pone.0078379
5 Malumphy C and Moran H, Red palm weevil, *Rhynchophorus ferrugineus*. *Plant Pest Factsheet*. [Online]. (2009). Available: www.fera.defra.gov.uk/plants/publications/documents/factsheets/redPalmWeevil.pdf [2 May 2014].
6 Conselleria d'Agricultura, Pesca, Alimentació i Aigua. *Tríptico Picudo*. [Online]. Conselleria d'Agricultura, Pesca, Alimentació i Aigua (2013). Available: http://www.agricultura.gva.es/documents/170659/179073/Triptico+PICUDO+rev+ene2012.pdf/0cceeb8d-585b-4199-8bf4-3233272448bd [2 May 2014].
7 *Registro de Productos Fitosanitarios*. [Online]. MAGRAMA. Available: http://www.marm.es/es/agricultura/temas/medios-de-produccion/productos-fitosanitarios/registro/menu.asp [2 May 2014].
8 Hernandez-Marante D, Folk F, Sanchez A and Fernandez-Escobar R, Control of red palm weevil (*Rhynchophorus ferrugineus* Olivier) using trunk injections and foliar sprays. *Bol San Veg Plag* **29**:563–574 (2003).
9 *Insecticide Resistance Action Committee*. [Online]. Available: http://www.irac-online.org/teams/mode-of-action/ [3 May 2014].
10 Dembilio Ó, Llácer E, Martínez de Altube MDM and Jacas JA, Field efficacy of imidacloprid and *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in *Phoenix canariensis*. *Pest Manag Sci* **66**:365–370 (2010).
11 Abo-El-Saad MM, Elshafie HA and Bou-Khowh IA, Toxicity of bio-insecticide, Abamectin, on red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Int J Agric Sci Res* **2**:107–115 (2013).
12 El-Sebay Y and Abbas MK, Evaluation of high-pressure technique against red palm weevil *Rhynchophorus ferrugineus*. The 4th International Conf. *Egypt J Agric Res* **86(1a)**:215–223 (2008).
13 Speranza S, Uso dell'endoterapia nel controllo del *Rhynchophorus ferrugineus* (Olivier), *Atti della V Biennale Europea delle Palme – Dies Palmarum*, San Remo, Italy, pp. 1–6 (2008).
14 Tomlinson PB, *The Structural Biology of Palms*. Clarendon Press, Oxford, UK (1990).
15 Thomas R and de Franceschi D, Palm stem anatomy and computer-aided identification: the Coryphoideae (Arecaceae). *Am J Bot* **100**:289–313 (2013).
16 Doccola JJ, Bristol EJ, Sifleet SD, Lojko J and Wild PM, Efficacy and duration of trunk-injected imidacloprid in the management of hemlock woolly adelgid (*Adelges tsugae*). *Arbor Urb For* **33**:12–21 (2007).
17 Krupke CH, Hunt GJ, Eitzer BD, Andino G and Given K, Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* **7**(1):e29268 (2012).
18 Stein-Dönecke U, Führ F, Wienecke J, Hartwig J and Leicht W, Influence of soil moisture on the formation of dressing zones and uptake of imidacloprid after seed treatment of winter wheat. *Pfl Nachr Bayer* **45**:327–368 (1992).
19 Araki Y, Bornatsch W, Brauner A, Clark T, Dräger G, Kurogochi S *et al.*, Metabolism of imidacloprid in plants. *Proc IUPAC Congr Wash* **2B**:157 (1994).
20 Nauen R, Tietjen K, Wagner K and Elbert A, Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae). *Pest Sci* **52**:53–57 (1998).
21 Westwood F, Bean KM, Dewar AM, Bromilow RH and Chamberlain K, Movement and persistence of [14C] imidacloprid in sugar-beet plants following application to pelleted sugar-beet seed. *Pest Sci* **52**:97–103 (1998).
22 Köster J, Comparative metabolism of [pyridyl-14C-methyl] imidacloprid in plant cell suspension cultures. *Proc Brighton Crop Protection Conf – Pests and Diseases*, BCPC, Alton, Hants, UK, pp. 901–906 (1992).
23 Mullins JW, *Imidacloprid: a New Nitroguanidine Insecticide*. ACS Symposium Series, American Chemical Society, Washington, DC (1993).
24 Lankas GR and Gordon LR, Toxicology, in *Ivermectin and Abamectin*, ed. by Campbell WC. Springer-Verlag, New York, NY, pp. 89–112 (1989).
25 Lasota JA and Dybas RA, Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annu Rev Entomol* **36**:91–117 (1991).
26 Dembilio Ó, Jacas JA and Llácer E, Are the palms *Washingtonia filifera* and *Chamaerops humilis* suitable hosts for the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *J Appl Entomol* **133**:565–567 (2009).
27 Martín MM and García TC, Manejo de la cría del picudo rojo de la palmera, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera, Dryophthoridae), en dieta artificial y efectos en su biometría y biología. *Bol San Veg Plag* **32**:631–642 (2006).
28 Gesti Perich J, *El Poblament Vegetal dels Aiguamolls de l'Emporadà*. Institut d'Estudis Catalans, Barcelona, Spain (2006).
29 Servei Meteorològic de Catalunya. *Butlletí Climàtic Mensual*. [Online]. (2012). Available: http://www20.gencat.cat/docs/meteocat/Continguts/Climatologia/Butlletins%20i%20resums%20climatics/

Butlletins%20mensuals/2012/pdf/Butlleti_Maig12.pdf [30 July 2014].
30 Anastassiades M, Lehotay SJ, Štajnbaher D and Schenck FJ, Fast easy multiresidue method employing acetonitrile extraction portioning and dispersive solid phase extraction for determination of pesticide residues in produce. *JAOAC Int* **86**:412–431 (2003).
31 European Committee for Standardisation/Technical Committee 275 (Standards under development) Foods of plant origin: Determination of pesticide residues using GC-MS and/or LC–MS (/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE–QuEChERS method. European Committee for Standardisation, Brussels, Belgium (2007).
32 Kaplan EL and Meier P, Nonparametric estimation from incomplete data. *J Am Stat Ass* **53**:457–481 (1958).
33 Abbott WS, A method for computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
34 *Polo-PC, A User's Guide to Probit Or LOgit Analysis*. LeOra Software, Berkeley, CA (1987).
35 Robertson JL, Preisler HK, Frampton ER and Armstrong JW, Statistical analyses to estimate efficacy of disinfestation treatments, in *Quarantine Treatments for Pests of Fruit Plants*, ed. by Shrarp JL and Hallman GJ. Westview Press, Boulder, CO, pp. 47–65 (1994).
36 Ishaaya I, Kontsedalov S and Horowitz AR, Emamectin, a novel insecticide for controlling field crop pests. *Pest Manag Sci* **58**:1091–1095 (2002).
37 MacConnell JG, Demchak RJ, Preiser FA and Dybas RA, Relative stability, toxicity, and penetrability of abamectin and its 8,9-oxide. *J Agric Food Chem* **37**:1498–1501 (1989).
38 Feely WF, Crouch LS, Arison BH, Vanden Heuvel WJA, Colwell LF and Wislocki PG, Photodegradation of 4′-(epimethylamino)-4′-deoxyavermectin B1a thin films on glass. *J Agric Food Chem* **40**:691–696 (1992).
39 Cabello T, De la Peña J, Belda J and Barranco P, Laboratory test of imidacloprid and oxamyl against *Rhynchophorus ferrugineus*, new pest of palms in Spain. *Ann Appl Biol* **130**:2–3 (1997).
40 Kaakeh W, Toxicity of imidacloprid to developmental stages of *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera): laboratory and field tests. *Crop Prot* **25**:432–439 (2006).
41 Dembilio Ó and Jacas JA, Basic bio-ecological parameters of the invasive Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in *Phoenix canariensis* under Mediterranean climate. *Bull Entomol Res* **101**:153–163 (2011).
42 Dembilio Ó, Tapia GV, Téllez MM and Jacas JA, Lower temperature thresholds for oviposition and egg hatching of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in a Mediterranean climate. *Bull Entomol Res* **102**:97 (2012).
43 Estévez A, Ferry M and Gómez M, Endoterapia en palmeras: estudio de la eficacia y persistencia del tiametoxam en tratamientos preventivos contra el picudo rojo. *Phytoma* **226**:42–48 (2011).
44 Llácer E, Negre M and Jacas JA, Evaluation of an oil dispersion formulation of imidacloprid as a drench against *Rhynchophorus ferrugineus* (Coleoptera, Curculionidae) in young palm trees. *Pest Manag Sci* **68**:878–882 (2012).
45 Leicht W, Imidacloprid – a chloronicotinyl insecticide: biological activity and agricultural significance. *Pfl Nachr Bayer* **49**:71–83 (1996).
46 Elbert A, Overbeck H, Iwaya K and Tsuboi S, Imidacloprid, a novel systemic nitromethylene analogue insecticide for crop protection, in *Brighton Crop Protection Conf – Pests and Diseases*, BCPC, Alton, Hants, UK, pp. 21–28 (1990).
47 Scholz K and Spiteller M, Influence of ground cover of the degradation of imidacloprid in soil. *Proc Brighton Crop Protection Conf – Pests and Diseases*, BCPC, Alton, Hants, UK, pp. 883–888 (1992).
48 Abbas MST, IPM of the red palm weevil, *Rhynchophorus ferrugineus*, in *Integrated Management of Arthropod Pests and Insect Borne Diseases*. Springer, Dordrecht, The Netherlands, pp. 209–233 (2010).
49 Llácer E, Martinez de Altube MM and Jacas JA, Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in *Phoenix canariensis*. *BioControl* **54**:559–565 (2009).
50 Dembilio Ó, Karamaouna F, Kontodimas DC, Nomikou M and Jacas JA, Susceptibility of *Phoenix theophrasti* to *Rhynchophorus ferrugineus* (Col.: Curculionidae) and its control using *Steinernema carpocapsae*. *Span J Agric Res* **9**:623–626 (2011).
51 Guarino S, Bue PL, Peri E and Colazza S, Responses of *Rhynchophorus ferrugineus* adults to selected synthetic palm esters: electroantennographic studies and trap catches in an urban environment. *Pest Manag Sci* **67**:77–81 (2011).

