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Evaluation of the efficacy of insecticidal coatings based on teflutrin and chlorpyrifos against *Rhynchophorus ferrugineus*

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Summary

The date palm (*Phoenix dactylifera* L.) is an important economic resource for many nations worldwide, recently threatened by the presence of different insect pests, like the red palm weevil (RPW) *Rhynchophorus ferrugineus*. Different strategies to control the insect have been used so far worldwide, including insecticides, biological control, endotherapy and pheromones, but it is still causing serious damages to palms in several Countries. In the present study, a new approach has been used to control the insect, based on a non-phytotoxic and non-toxic coating material applied on palm trees to prevent the red palm weevil attacks. Two products, a glue (polyvinyl acetate) and an oil (raw linseed oil), have been used as coatings and applied together with a repellent and two insecticides (teflutrin and chlorpyrifos) at different dosages on 2 species of palm (*Phoenix dactylifera* and *Phoenix canariensis*). Phytotoxic effects of the treatments have been evaluated in greenhouse on 260 potted palms (130 *Phoenix dactylifera* and 130 P. *canariensis*) and no negative effects have been observed. Afterwards, a trial lasting 400 days has been carried out in a nursery located in Sicily (South Italy), treating 572 potted palm trees (286 *P.dactylifera* and 286 *P. canariensis*) with an average diameter at base of 18-20 cm. After 400 days, 48% of the untreated palms were infested, while only 3% of date palms and 7% of Canary palms treated with insecticide at lower dosages have been infested. The application of an insecticide based coating is a good strategy to control and prevent the red palm weevil infestation, in particular on date palms.

Keywords: *Rhynchophorus ferrugineus;* teflutrin; chlorpyrifos; polyvinyl acetate; coating.

Introduction

Rhynchophorus ferrugineus Olivier (Red Palm Weevil - RPW), a native pest of South and South East Asia, causes serious and important crop and landscape damage attacking date palms and other palm species in many parts of the world (EPPO, 2007). The male and female adults of this insect, member of *Coleoptera*: *Curculionidae*, are large reddish brown beetles about 3 cm long and with a characteristic long curved rostrum. Due to their strong wings, they are capable of undertaking long flights (Salama *et al*., 2009).

Damage to palms is produced mainly by the larvae. Adult females lay about 200 eggs at the base of young leaves or in wounds to the leaves and trunks; the grubs feed on the soft fibers and terminal bud tissues. They reach a size of more than 5 cm before pupation. Except just before pupating, they move towards inner tissues of the palm tree making tunnels and large cavities damaging its internal tissues. The larvae can be found anywhere within the palm, even in the very base of the trunk where the roots emerge. This burrowing weakens and eventually kills the tree. Although adult weevils can damage trees by feeding on them, larvae can cause greater damage by burrowing.

The complete life cycle of the insect takes an average of 82 days (Murphy and Briscoe, 1999), up to 93 (Salama et al., 2009) and 124 (Kaakeh, 2005), depending on different feeding substrate. They can be found in any place within the palm (Alkhazal *et al*., 2009) from the base of the trunk where the roots emerge up to the apical bud. Vertical distribution of infestations along the trunk of date palm trees generally showed that 70% of the attack occurred from the ground up to 1 -1.5 m. On other palm trees (i.e. *Phoenix canariensis*) the attacks occur principally near the apical bud (80-90%).

In 1994, this past had been captured in the south of Spain and it was observed in Italy later in 2004 (Vacante, 2013). Today, it can be found in almost all Mediterranean countries (EPPO, 2008). The spread of this pest occurs due to transporting infested young or adult date palm trees and offshoots from contaminated to uninfested areas.

The external symptoms on infested palm trees are a progressive yellowing of the leaf area, destruction of the rising leaf and necrosis in the flowers. Leaves begin to dry in ascending order in the crown; the apical leaf bends and eventually drops. Affected plant tissue turns foul, producing strong characteristic odours (EPPO, 2007).

Infested palms generally do not show any obvious early symptoms, and usually the weevil is detected only after most damage has been caused on the palm, and preventive measures are mostly indicated to control it (Kehat, 1999). Preventive and curative measures include: trunk injection with systemic insecticides; treatment of wounds with repellents; drenching of the crown of infested trees with insecticides (EPPO, 2008). Control of the red palm weevil is problematic and particularly challenging for several reasons, even with pesticides. Adults are mobile and easily bypass or evade containment barriers thereby expanding infestation outbreaks. Moreover, the pest is concealed deep inside palm trunks and consequently insecticides have to be applied frequently and over a long period (Faleiro, 2006; Llácer *et al*., 2010; Murphy and Briscoe, 1999). In addition, a restriction in the use of insecticides has occurred recently due to concerns about the side effects of chemical pesticides on the environment. Systemic insecticides may be the best chemicals available for controlling the RPW, also once palms are already infested (Llácer *et al*., 2012) and are also applied successfully with endoterapy and trunk injection, with concerns about residues on dates.

Natural enemies do not play an important part in controlling *R. ferrugineus* (Reginald, 1973) and at present there is no practical biological control. However, pheromones are increasingly used against *R. ferrugineus* and protocols for pheromone-based mass trapping of the weevil have been developed (Hallett *et al*., 1999).

Only in few cases, like in Israel, the eradication of the insect, detected in 1999, was achieved in 4 years by implementing several measures such as mass trapping, chemical treatment of infested palms, destruction of heavily infested palms and preventive measures (Hamburger *et al*., 2003). Similarly in Morocco and Tunisia (Vacante, 2013).

Considering the widespread presence of RPW and the difficulties to control it, a new approach has been applied to prevent palms infestation based on the use of non-phytotoxic and non-toxic coating material applied on palm trees to prevent the attacks.

Materials and methods

Treatments

Two products, a glue (polyvinyl acetate, Vinavil 59, Vinavil Spa, Italy) and an oil (raw linseed oil, Linoil, Chimica C.B.R. Spa, Italy), have been used as coatings and applied together with a repellent (camphor) and two insecticides, teflutrin (Teflustar, 0.2% p.a.) and chlorpyrifos (Zelig GR, 7.5% p.a.) at different dosages on 2 species of palm (*Phoenix dactylifera* and *Phoenix canariensis*).

Tefluthrin is one of the most toxic pyrethroids, while chlorpyrifos is a toxic crystalline organophosphate insecticide that inhibits acetylcholinesterase.

The mixtures of products applied in the trials are listed in Table 1.

Phytotoxicity evaluation

Seven years old *P. dactylifera* and 5 years old *P. canariensis* have been used and totally 260 potted palms (n. 130 *P. dactylifera* and n. 130 *P. canariensis*) have been treated and placed in greenhouse to assess the phytotoxicity of the selected products. The average diameter at the base was respectively 9.5±2.6 and 8.5±1.6 cm. Ten palm trees per species have been treated for each product mix. Products have been applied by painting, to avoid the drift of pesticides in an enclosed place (greenhouse).

Phytotoxic effects have been evaluated with a monthly visual assessment observing the presence/absence of leaves discoloration or necrosis (scale: 0= no symptoms; 1= light leaf chlorosis or necrosis; 2= leaf chlorosis or necrosis up to 25%; 3= 26-50% leaf chlorosis or necrosis; 4= 51-90% leaf chlorosis or necrosis; 5= plant death),.

At the same time of visual assessment other measurements have been carried out:

- Thickness measurements of the films applied (3 measurements/leaf on three leaves coated portion for each plant) with Positector 200-ultrasonic coating thickness gage.
- Indirect measurements of chlorophyll content (CCI) with Chlorophyll Content Meter 200 (Opti-Sciences). Chlorophyll has distinct optical absorbance characteristics that the CCM-200 plus exploits to non-destructively measure relative chlorophyll concentrations. Strong absorbance bands are present in the blue and red regions but not the green or infrared bands, hence the green appearance of a leaf. By measuring the amount of energy absorbed in the red band an estimate of the amount of chlorophyll present in the tissue is possible. Absorbance in the infrared band can be used to quantify and account for leaf thickness, so providing a more accurate CCI value. This device has optical absorbance in two different wavebands: 653 nm (Chlorophyll) and 931 nm (Near Infra-Red) providing CCI value.

<u>Efficacy evaluation</u>

The nursery activities took place between August 2013 and the end of November 2014.

After two months of acclimatization, treatments were carried out in October 2013 and repeated in November 2013 on 572 palm trees (286 *P. dactylifera* and 286 *P. canariensis*). The two species have been positioned in two separate blocks. The distance of each palm from the others was 1 meter. Date palms were from 9 to 13 years old, with average diameter at base 20.1±5.4 cm; Canary palms were from 7 to 8 years old with 17.8±1.8 cm diameter. Treatments have been carried out randomly inside the two blocks of palms at 14-16 October 2013, the coatings have been applied by brush and by spray (Black & Decker - SmartSelect HVLP Sprayer mod. BDPH400).

During the period November 2013 - November 2014 measurements of palms in open field (in Sicily) were carried out to characterize plant physiology and coating application, in order to assess the efficacy of the products to prevent RPW infestation and to test the efficacy of the curative effect.

Forty-four palm trees not treated (22 *P. dactylifera* and 22 *P. canariensis*) served as control. In the months of August and September 2013 irrigations were made every 2 days, in October every 4 days, from November 2013 to March 2014 every 7 days and from April to September 2014 every 3 days. Fertilizations have been made in October 2013, in May 2014 and in October 2014 using a ternary fertilizer (COMPO NPK Original Gold®, 5-10 g per pot).

The average temperature of whole period has been 18°C. Maximum temperatures were recorded in the months of July, August and September and were about 40 degrees.

At the end of November 2014 all the palms involved have been sectioned to verify the real infestation. Each palm tree has been cut by chainsaw at the base about 2-4 cm height.

Data analysis

About the phytotoxicity evaluation ANOVA with Tukey's HSD ($p \leq 0.05$) has been applied.

About the efficacy, infested palms were calculated as percentage, where healthy palm corresponds to 0 and infested palm to 100. ANOVA with Tukey's HSD ($p \leq 0.05$) has been performed.

Results and discussion

The average thickness at the end of trials was 0.257 ± 0.088 mm for vinyl acetate and 0.091 ± 0.023 mm for raw linseed oil in the greenhouse trial, 0.135±0.038 mm for vinyl acetate and 0.072±0.032 mm for raw linseed oil in the nursery trial.

At the end of phytotoxicity test, after 7 months, the absence of damages on treated potted palms were confirmed: no phytotoxic effects have been observed (Table 2). For each treatment and for each parameter no significant differences were observed during the months. In particular, no statistical difference have been observed comparing the treatments to the untreated control in terms of chlorophyll content (CCI), leaf discolouration and necrosis.

Regarding the trial in Sicily, 71 palms out of 572 were infested (36 date palms and 35 Canary palms) after 400 days from the treatments:

- 21 controls (not treated): 13 Date palms and 8 Canary palms;
- 32 palms treated with liquid suspensions insecticide-free;
- 11 with vinyl acetate based products (5 with Camphor and 6 without Camphor);
- 21 with raw linseed oil based products (10 with Camphor and 11 without Camphor) ;
- 3 palm treated with vynil acetate solution containing insecticide (Teflutrin) at low concentration;

- 2 palm treated with vynil acetate solution containing insecticide (Teflutrin) at high concentration;
- 4 palm treated with oil solution containing insecticide (Teflutrin) at low concentration;
- 2 palm treated with oil solution containing insecticide (Teflutrin) at high concentration;
- 3 palm treated with oil solution containing insecticide (Chlorpyrifos) at low concentration;
- 4 palm treated with oil solution containing insecticide (Chlorpyrifos) at high concentration.

Some infested palms were completely decayed or hollow and all stages of RPW were found (young and old larvae, adults).

The treatments containing insecticides reduced the infestations, but after 400 days they were losing their efficacy. The best results have been achieved with vinyl acetate and chlorpyrifos at both dosages (T5 and T6) and no infestation have been observed after 400 days. In the case of linseed oil and teflutrin or chlorpyrifos at high dosage (T7 and T9), no infestation have been observed after 323 days. For the combinations vinyl acetate and teflutrin at both dosages (T2 and T3), and linseed oil and teflutrin or chlorpyrifos at low dosage (T8 and T10) no infestation have been observed after 245 days.

After 400 days, 36 date palms were infested by RPW: 13 controls, 4 treated with vinyl acetate products (without insecticide), 1 treated with vinyl acetate product (with insecticide), 13 treated with raw linseed oil (without insecticide) and 5 treated with raw linseed oil (with insecticide) (Table 3). Vinyl acetate with teflutrin at higher dosage, vinyl acetate with chlorpyrifos at both dosages and raw linseed oil with teflutrin at higher dosage were able to prevent the infestations on date palms (Table 3).

After 400 days, 35 Canary palms were infested by RPW: 8 controls, 7 treated with vinyl acetate product (without insecticide), 4 treated with vinyl acetate product with insecticide, 8 treated with raw linseed oil with insecticide and 8 treated with raw linseed oil (without insecticide) (Table 3).

In the case of Canary palms, only the application of vinyl acetate with chlorpyrifos at both dosages was able to prevent the attacks on date palms (Table 3).

Comparing the results among the palms treated with linseed oil (with or without insecticides), vynil acetate (with or without insecticides) and untreated control, vinyl acetate generally reduced the infestation better than raw linseed oil (Tab. 4). Moreover, both vinyl acetate and linseed oil applied without insecticide were able to reduce the infestation (Tab. 4).

It seems that the vinyl coating without insecticide has a minimum repellent action, certainly greater than the coating with raw linseed oil. In fact, at the end of the trial , infested palms treated with raw linseed oil without insecticide are double respect those treated with vinyl (Tab. 5).

The vinyl acetate coating with insecticide had the best efficacy. Regarding the insecticides, Chlorpyrifos was more effective, but only if combined with the vinyl acetate. Tefluthrin also seems more effective combined with vinyl, especially in the long period (Tab. 6).

Conclusions

The treatments carried out had a positive effect on the health of the palms. After 400 days, 71 palm trees were infested by RPW: 21 controls and 50 treated. Only 9.4% of the treated palms were infested, compared to 47.7% of the untreated.

The first infestations on insecticide treated palms have been detected after 245 days, after that it begins to lose effectiveness. The efficacy of the treatment is variable and depending on the time, the presence and the type of active principle. The coatings with vinyl acetate and chlorpyrifos (T5 and T6) seem to be the most effective (100% up to 400 days in this test).

These products could be easily applied by airsprayer and the solution have been prepared and immediately used. Treatments on adult palms could leave small areas not covered (especially with not well pruned petioles) and therefore the possibility for RPW to penetrate would be higher compared to small plants. Based on information collected during the test, it can be assumed that a vinyl-based coating with an insecticide like chlorpyrifos could be used to significantly reduce the possibility of RPW infestation, providing a treatment every six months.

Similar results have been achieved by the use of the insecticidal paint Inesfly IGR FITO (Industrias Quõ´micas Inesba S.L., Paiporta, Spain), having as active ingredients 3.0% chlorpyrifos and 0.063% pyriproxyfen in a microencapsulated formulation (Llàcer *et al.*, 2010). The coating application confers the advantage of releasing the active ingredients slowly, so the treatments do not need to be applied frequently and the effect can last for a longer time (Lòpez *et al.*, 1999, Mosqueira *et al*. 2005, Amelotti *et al*. 2009). In our case, the coating was able to last 400 days, while the insecticidal paint Inesfly IGR FITO has been tested for 180 days (Llàcer *et al*., 2010).

According to our results, the coating without chlorpyrifos was also initially effective, but of course this active ingredient is the primary cause of the efficacy of the product. Chlorpyrifos is widespread used to control insect pests and it is recommended to control *R. ferrugineus* in several countries within the Europe. However, it is not possible to apply the coating as curative effect on already infested palms, because the target pest is protected inside the palm, and other products are recommended, such as imidacloprid (Kaakeh, 2006), or biological control agents (Llàcer *et al*., 2009).The product can be applied within an integrated pest management strategy for *R. ferrugineus* which includes surveillance, early detection, trapping using pheromones, protecting wounds, physical methods, sanitation, use of attractants and other chemicals (Hoddle *et al.*, 2013; Hussain *et* *al.*, 2013). In particular the adoption of early detection method (Rettori *et al*., 2015) integrated with vinyl acetate coating containing chlorpyrifos can be considered an optimal combination of methods for IPM.

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Table 1 – Different treatments applied in the trials.

Thesis	Coating	Solvent	Insecticide	Repellent
T1	Vinyl acetate 59 (100 g)	38.7 g H2O		5 g Camphor White oil
T2	Vinyl acetate 59 (100 g)	109.3 g H2O	40 g Teflustar (0.08 g teflutrin)	5 g Camphor White oil
T3	Vinyl acetate 59 (100 g)	109.3 g H2O	20 g Teflustar (0.04 g teflutrin)	5 g Camphor White oil
T4	Vinyl acetate 59 (100 g)	38.7 g H2O		
T5	Vinyl acetate 59 (100 g)	82.7 g H2O	30 g Zelig GR (2.25 g chlorpyrifos)	5 g Camphor White oil
T6	Vinyl acetate 59 (100 g)	46.7 g H2O	15 g Zelig GR (1.125 g chlorpyrifos)	5 g Camphor White oil
T7	Raw Linseed oil (100 g)	25 g Limonene	40 g Teflustar (0.08 g teflutrin)	5 g Camphor White oil
T8	Raw Linseed oil (100 g)	25 g Limonene	20 g Teflustar 0.04 g (~~0.08~~ g teflutrin)	5 g Camphor White oil
T9	Raw Linseed oil (100 g)	25 g Limonene	30 g Zelig GR (2.25 g chlorpyrifos)	5 g Camphor White oil
T10	Raw Linseed oil (100 g)	25 g Limonene	15 g Zelig GR (~~2.25 g~~ 1.125 g chlorpyrifos)	5 g Camphor White oil
T11	Raw Linseed oil (100 g)	25 g Limonene		
T12	Raw Linseed oil (100 g)	25 g Limonene		5 g Camphor White oil
T13	Untreated control			

Table 2 – Evaluation of phytotoxicity of coating treatments on date and canary palms.

Treatment	CCI		Discouloration**		Necrosis**	
	Phoenix dactylifera	***Phoenix canariensis***	***Phoenix dactylifera***	***Phoenix canariensis***	***Phoenix dactylifera***	***Phoenix canariensis***
T1	28.42±4.61*	53.37±15.52a	0.00±0.00a	0.00±0.00a	0.20±0.42a	0.50±0.53a
T2	35.13±11.11a	53.00±11.95a	0.00±0.00a	0.10±0.00a	0.10±0.32a	0.70±0.67a
T3	34.53±8.78a	45.37±10.46a	0.00±0.00a	0.10±0.32a	0.00±0.00a	0.40±0.52a
T4	30.41±9.49a	51.80±8.77a	0.00±0.00a	0.00±0.00a	0.10±0.32a	0.60±0.70a
T5	38.27±9.53a	44.85±15.13a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.10±0.32a
T6	36.55±5.95a	54.96±8.18a	0.00±0.00a	0.10±0.32a	0.10±0.32a	0.40±0.52a
T7	39.43±7.54a	46.26±11.01a	0.00±0.00a	0.10±0.32a	0.00±0.00a	0.40±0.70a
T8	41.78±8.57a	52.38±9.97a	0.00±0.00a	0.10±0.32a	0.00±0.00a	0.40±0.52a
T9	38.09±10.17a	52.32±10.64a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.10±0.32a
T10	43.29±10.83a	53.82±13.16a	0.00±0.00a	0.30±0.42a	0.10±0.32a	0.70±0.67a
T11	39.79±10.22a	48.25±12.70a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.50±0.53a
T12	41.73±8.45a	50.54±12.81a	0.00±0.00a	0.10±0.32a	0.00±0.00a	0.50±0.53a
Control	32.65±7.01a	58.11±15.33a	0.00±0.00a	0.00±0.00a	0.10±0.32a	0.30±0.42a
Total average	**36.93±9.48**	**51.16±12.21**	**0.00±0.00**	**0.07±0.23**	**0.0538±0.22**	**0.43±0.55**

* Tukey's HSD ($p \leq 0.05$)

**Discolouration/necrosis of leaves, scale: 0= no symptoms; 1= light leaf chlorosis/necrosis; 2= leaf chlorosis/necrosis up to 25%; 3= 26-50% leaf chlorosis/necrosis; 4= 51-90% leaf chlorosis/necrosis; 5= plant death.

Table 3 – Efficacy evaluation of coating treatments on date and canary palms, after 400 days. Infested palms were calculated as percentage, where healthy palm corresponds to 0 and infested palm to 100.

Treatment	**Valid cases**	***Phoenix dactylifera***	***Phoenix canariensis***	**Average**
		% of healthy palms		
T1	22	13.63±7.49ab*	9.09±6.27ab	11.36±4.84bc
T2	22	0±0a	9.09±6.27ab	4.54±3.17ab
T3	22	4.54±4.54ab	9.09±6.27ab	4.83±3.84ab
T4	22	4.54±4.54ab	22.73±9.14cd	13.64±5.23bc
T5	22	0±0a	0±0a	0±0a
T6	22	0±0a	0±0a	0±0a
T7	22	0±0a	9.09±6.27a	4.54±3.18ab
T8	22	9.09±6.27ab	9.09±6.27a	9.09±4.38bc
T9	22	9.09±6.27ab	9.09±6.27a	9.09±4.38bc
T10	22	4.54±4.54ab	9.09±6.27ab	6.82±3.84ab
T11	22	36.36±10.50c	13.64±7.49bc	25.00±6.60cd
T12	22	22.73±9.14bc	22.73±9.14cd	22.73±6.39cd
CONTROL	22	59.09±10.73c	36.36±10.50d	47.73±7.62e

* Tukey's HSD ($p \leq 0.05$)

Table 4 – Efficacy evaluation of treatments 1-4 (vinyl acetate without insecticide), 2-3-5-6 (vinyl acetate with insecticide), 7-8-9-10 (Raw Linseed oil with insecticide), 11-12 (Raw Linseed oil without insecticide) and control comparison on date and canary palms, after 400 days. Infested palms were calculated as percentage, where healthy palm corresponds to 0 and infested palm to 100.

Treatment	***Phoenix dactylifera***	***Phoenix canariensis***	**Average**
	% of healthy palms		
T2-T3-T5-T6 (vynil acetate based products with insecticide)	1.14±1.14a*	4.54±2.23ab	2.84±1.25a
T1-T4 (vynil acetate based products without insecticide)	9.09±4.38a	15.91±5.58ac	12.50±3.54b
T7-T8-T9-T10 (Raw Linseed based products with insecticide)	5.68±2.48a	9.09±3.08ab	7.39±1.98ab
T11-T12 (Raw Linseed based products without insecticide)	29.54±6.95b	18.18±5.88c	23.86±4.57c
Control	59.09±10.73c	36.36±10.49d	47.73±7.62d

* Tukey’s HSD ($p \leq 0.05$)

Table 5 - Percentage of infested palms related to products and insecticides

		110 days	152 days	180 days	210 days	245 days	285 days	323 days	400 days
Vinyl acetate	with insecticide	0,0%	0,0%	0,0%	0,0%	0,0%	1,1%	1,7%	2,8%
	without insecticide	2,3%	2,3%	3,4%	3,4%	4,5%	5,7%	10,2%	12,5%
Raw Linseed Oil	with insecticide	0,0%	0,0%	0,0%	0,0%	0,0%	1,1%	1,1%	7,4%
	without insecticide	3,4%	5,7%	8,0%	9,1%	12,5%	15,9%	18,2%	23,9%
Control		6,8%	6,8%	9,1%	15,9%	22,7%	29,5%	38,6%	47,7%

Table 6 - Percentage of infested palms related to insecticides.

		110 days	152 days	180 days	210 days	245 days	285 days	323 days	400 days
Teflutrin	Vinyl acetate	0,0%	0,0%	0,0%	0,0%	0,0%	2,3%	3,4%	5,7%
	Raw Linseed Oil	0,0%	0,0%	0,0%	0,0%	0,0%	1,1%	1,1%	6,8%
Chlorpyrifos	Vinyl acetate	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
	Raw Linseed Oil	0,0%	0,0%	0,0%	0,0%	0,0%	1,1%	1,1%	8,0%