Research Article

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Responses of *Rhynchophorus ferrugineus* adults to selected synthetic palm esters: electroantennographic studies and trap catches in an urban environment

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Abstract

BACKGROUND: The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, accidentally introduced into the European countries facing the Mediterranean basin, is becoming the most serious pest for ornamental palms in the urban environment. In the present work, tests were conducted on the electroantennogram (EAG) and behavioural responses of RPW adults to five selected synthetic palm esters, ethyl propionate, ethyl butyrate, ethyl isobutyrate, ethyl lactate and ethyl acetate, to assess the application of a mass trapping technique in an urban environment.

RESULTS: In the laboratory, EAG bioassays on RPW adults with the synthetic palm esters showed: (1) positive dose-dependent responses; (2) differences among the treatments; (3) a greater response to ethyl propionate; (4) a higher sensitivity of female antennae compared with male antennae. In the urban environment, RPW behavioural responses, evaluated over six biweekly observations on pheromone- and molasses- baited traps, showed that more adults were caught by traps supplemented with ethyl propionate and ethyl acetate than by traps supplemented with only ethyl propionate. Similar catches were recorded in the traps supplemented with ethyl propionate or ethyl acetate.

CONCLUSION: This study supports the application of the mass trapping implemented with synthetic palm esters as a potential tool for the management of RPW populations to protect ornamental palms in urban environments.

Keywords: red palm weevil; dose–response; mass trapping; ethyl acetate; ethyl propionate; EAG

1 INTRODUCTION

Rhynchophorus ferrugineus Olivier (Coleoptera: Curculionidae), commonly called the red palm weevil (RPW), is a pest accidentally introduced into several European countries of the Mediterranean Basin during the last 10 years as a consequence of commercial exchanges of date palm trees from contaminated areas of North Africa.[1–3] In Italy, the first palms killed by RPW were recorded in 2005 in Sicily.[4] Then, a few years later, the pest was found in most of the Italian regions facing the Mediterranean Sea, where it is killing mostly the Canary island date palm, *Phoenix canariensis* Hortorum ex Chabaud.[3,5]

In Asiatic and North African countries, RPW is a serious pest in plantations of date, oil and coconut palms, and its populations are efficiently controlled by IPM programmes that include cultural management, chemical control and semiochemical-based tools for monitoring and mass trapping.[6–8] In Italy, similarly to other European countries, RPW has assumed the role of a major pest of landscape palms, because palm trees are grown only for ornamental purposes.[9] Normally, landscape pests do not need to be managed constantly, as their damage should decrease through time.[10] By contrast, the RPW, which is included in the EPPO alert list,[11] requires strong control measures (quarantine, eradication), as it is lethal to palms. To date, the dominant strategies applied in the short term to prevent and/or reduce further RPW infestations are removing and destroying infested palm materials and applying insecticide treatments.[3,12] However, to avoid severe harm in urban environments, caused by an overuse of insecticides, the possibilities of adopting sustainable strategies to control RPW are under evaluation, with particular attention being paid to the possibility of developing a mass trapping method.[5] Mass trapping aims to catch enough target insects in order consistently to reduce their population to a level that results in a significant decrease in plant attacks as a consequence.[13] In semiochemical-based mass trapping, traps are baited with chemical lures such as pheromones and/or food attractants.[14–16] The mass trapping technique for *Rhynchophorus* spp. is often improved by the presence of synergistic palm volatiles in the pheromone-baited traps,[17,18] as palm tissues develop fermentation processes that produce volatiles that are synergistic to weevil aggregation

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pheromones.[19] In fact, pheromone-baited traps supplemented with host palm volatiles have been found to catch significantly more adults of *R. cruentatus* F., *R. palmarum* L., *R. phoenicis* L. and *R. ferrugineus* than pheromone alone.[20–23] Volatile chemicals produced from fermenting palm tissues, known as 'palm esters', such as ethyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate and ethyl lactate, and their perception by adult weevils have been proven by electroantennogram bioassays (EAG).[24,25] Several studies report that most of these compounds strongly enhance the attractiveness of pheromone-baited traps.[8,23] In the case of RPW, previous studies on mass trapping commonly incorporate the use of ethyl acetate as a synergistic kairomone of the aggregation pheromone,[5,8,26] but there is lack of data about the responses of the RPW to other palm esters.

In the present study an assessment is made of the responses of RPW adults to five selected palm esters, ethyl propionate, ethyl butyrate, ethyl isobutyrate, ethyl lactate and ethyl acetate, to evaluate their use as a synergistic kairomone in pheromone- and molasses-baited traps.

2 MATERIALS AND METHODS

2.1 Insects

RPW pupae were collected from damaged *P. canariensis* palms in Palermo and kept in an environmentally controlled room (25±1 °C, 70±10% RH, 16 : 8 h light : dark photoperiod) inside wooden cages (25 × 25 × 40 cm) with two 5 cm diameter mesh-covered holes for ventilation. After emergence, the adults were sexed and kept separated in different cages where they were fed with apples until used for experiments.

2.2 EAG bioassays

RPW antennae were cut off as close as possible to the base of the scape and were fixed on a plasticine surface by fine copper wire staples (4 mm). Glass capillaries (40 mm × 0.2 mm diameter) containing 0.1 M aqueous KCl solution were placed over silver wire electrodes attached to a signal acquisition system (IDAC-400; Synthech, Hilversum, The Netherlands). The recording electrode was inserted on the surface of the antennal club on which the olfactory sensilla were located, and the indifferent electrode was placed in the base of the antenna. The preparation was exposed to a constant air flow through a glass tube (2 cm diameter) positioned 3 cm away from the antenna. The glass tube presented one lateral hole that permitted the delivery of the stimulus puff inside the tube with the aim of a glass Pasteur pipette attached to Tygon tubes leading to an air source programmed to deliver a 3 s pulse at 1 L min^{-1}.

A series of EAG tests was conducted to produce dose–response curves with four synthetic selected palm esters, ethyl propionate, ethyl butyrate, ethyl isobutyrate and ethyl lactate (mixture of isomers). In addition, the response to ethyl acetate, the commonly used synergistic kairomone for this species, was also tested. All the chemicals were purchased from Sigma-Aldrich (Germany) and were >98% chemically pure. For this experiment, serial dilutions of tested compounds were made in HPLC-grade dichloromethane at concentrations ranging from 100 to 100 000 mg kg^{-1}. A quantity of 1 µL of solution of each tested compound was applied to filter paper strips (1 × 3 cm) in aliquots of active ingredient ranging from 0.1 to 1000 µg (pure compound), placed inside a Pasteur pipette (20 cm long). Control puffs were carried out using equal amounts of dichloromethane. The relative EAG response to each stimulus was normalised by dividing the amplitude of the response elicited from the test stimulus by the amplitude of the response elicited by the control. Seven replicates were carried out for each sex, using all the five doses tested. The sequence of exposure of each stimulus to each antenna was randomly defined.

2.3 Trap catches

The RPW response to synthetic palm esters was tested in an experiment using a total of 21 traps. Traps were located in two areas of Palermo, Caserma Scianna (16 ha) and Istituto Zooprofilattico (5 ha), with 12 traps located in Caserma Scianna and 9 traps in Istituto Zooprofilattico. The traps consisted of 10 L red plastic buckets with four openings (5 cm diameter) located equidistantly on the wall at about 5 cm below the upper rim of the bucket, and two openings (5 cm diameter) diametrically opposed on the lid. All traps were placed far from any palm trees actually present (at least 20 m) and, to facilitate weevil adult entrance, the traps were interred with their lower half in the ground, with the sideway openings at the ground level. Traps were baited with the male aggregation pheromone ferrorule released at 2–4 mg day^{-1} from polyethylene dispensers (Rhyfer 220; Intrachem Bio Italia SpA) and 2 L of a 10% v/v aqueous solution of sugar beet molasses obtained from *Beta vulgaris L. var. saccharifera L.* Only the palm esters that elicited the most evident responses in the EAG bioassays were tested as synergistic kairomones in the pheromone-baited traps. In this three-treatment experiment, pheromone + molasses-baited traps were tested in combination with 10% v/v emulsions of ethyl acetate, ethyl propionate or both in water. Seven traps per treatment were used. Release devices, containing 100 mL of the emulsion, were suspended about 10 cm below the trap lid. The traps were inspected every 2 weeks from 22 July 2009 to 29 September 2009. During the inspections, the adults were removed from the traps and sexed according to gender-specific external characteristics of the rostrum, as suggested from Booth *et al.*[27] Pheromone dispensers were replaced every 3 months, while synergistic kairomones and molasses were replaced every 2–4 weeks, depending on the temperatures and relative humidity of the period.

2.4 Statistical analysis

In EAG experiments, to obtain the dose–response curves, the data expressed as means of relative responses were analysed by repeated-measures ANOVA, followed by Fisher's LSD test, while, to evaluate the antennal sensitivity, the responses elicited by 0.1 µg doses, the lowest used in the EAG experiments, were compared with the solvent by *t*-test for independent samples. In field experiments, the numbers of adults captured with the traps were compared by multifactorial ANOVA, followed by Fisher's LSD test. Finally, the differences in the ratios from 1 for males and females captured were evaluated by χ^2. All the statistical analyses were performed using Statistica for Windows 6.0 (Stat Soft Italia, 1997).

3 RESULTS

3.1 EAG bioassays

RPW adults showed dose-dependent EAG responses, with increasing responses to increase in doses of the synthetic palm esters (Table 1, Fig. 1). The dose of 0.1 µg elicited the weakest response for all the treatments. At this dose, only ethyl propionate ($t = 2.69$; $P = 0.024$; *t*-test) and ethyl acetate ($t = 2.46$, $P = 0.035$; *t*-test)

Table 1. Repeated-measures ANOVA of the EAG response of RPW males and females (SS = sum of squares; df = degree of freedom; MS = mean squares; F = F-test; asterisks indicate that values differ statistically for $P < 0.05$)

Effect	SS	df	MS	F	P
Dose	649.9	5	130	10.31	0.000*
Esters	220.3	4	55.1	54.47	0.000*
Sex	146.2	1	146.2	11.59	0.001*
Dose*sex	75.4	5	15.1	1.2	0.32
Esters*dose	17.4	20	0.9	0.86	0.63
Esters*sex	10.6	4	2.6	2.61	0.036*
Esters*dose*sex	17.1	20	0.9	0.85	0.65

induced higher EAG responses than the solvent. Significant statistical differences in the EAG responses were observed among the synthetic palm esters (Table 1). The ester that elicited the strongest responses was ethyl propionate, while the weakest responses were recorded using ethyl lactate ($F = 54.47$; $P < 0.001$; df = 4; ANOVA). Overall, female antennae were more sensitive to the stimuli than male antennae ($F = 11.59$; $P < 0.001$; df = 1; ANOVA) (Table 1). The difference in the responses elicited between the genders was more evident in ethyl propionate, providing a significant sex*ester effect (Table 1).

3.2 Trap catches

The means (± SE) of adults captured per trap during each sampling period reached a peak of 49.5 ± 6.0 on the 9 August 2009; the lowest number of adults captured (22.9 ± 2.5) was registered on the 7 July 2009. Globally, the total number of adult RWP captured was 3544, with a proportion of male/female of 1/1.56 ($\chi^2 = 169.9$; $P < 0.001$). The number of females captured was consistently greater than the number of males in all treatments. The results of the catches using different synergistic kairomones are shown in Fig. 2. The means (± SE) of adults of RPW captured biweekly in pheromone + molasses traps complemented with ethyl acetate alone, ethyl propionate alone and ethyl acetate and ethyl propionate in combinations were respectively 33.05 ± 2.83, 28.65 ± 3.24 and 39.54 ± 4.14. The analysis of variance revealed that the pheromone + molasses traps complemented with the two esters in combination captured more adults than the pheromone + molasses traps complemented with ethyl propionate alone ($F = 2.51$; $P = 0.027$; df = 102). Traps complemented with only ethyl acetate caught a statistically intermediate number of weevils (Fig. 2).

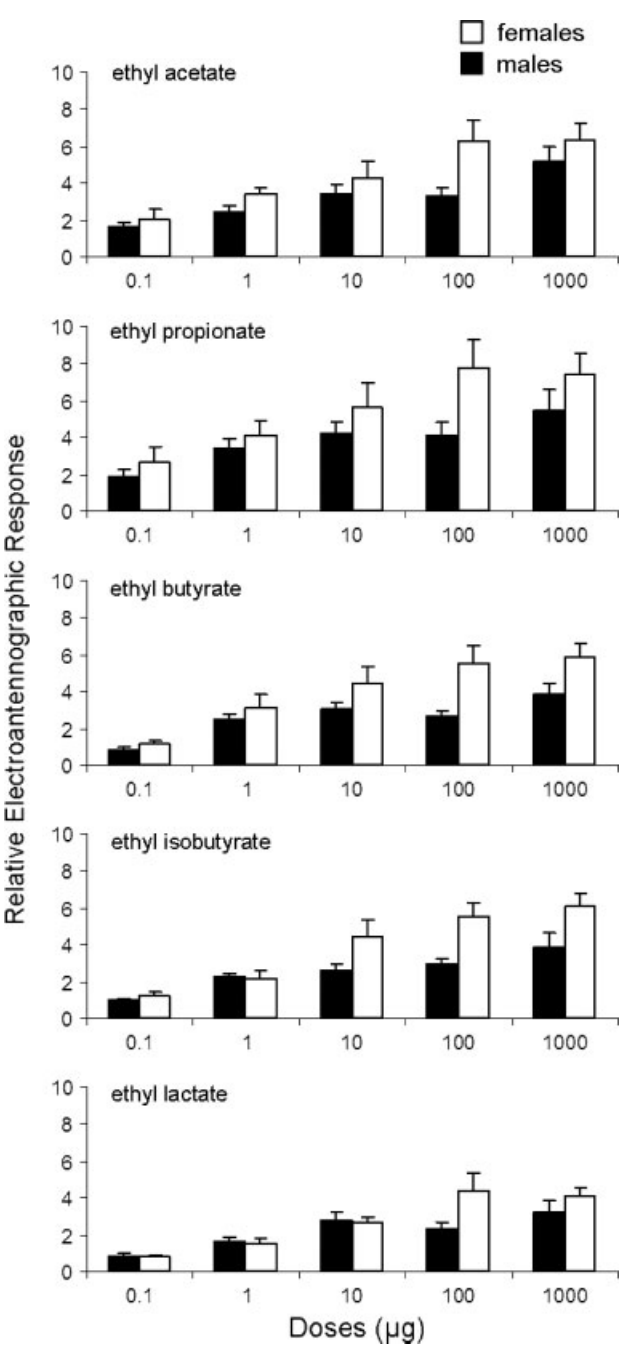

Figure 1. Dose–response (means ± SE) curves constructed from EAG responses of *Rhynchophorus ferrugineus* male and female adults ($n = 7$) to five synthetic palm esters, ethyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate and ethyl lactate, at five different doses.

4 DISCUSSION AND CONCLUSIONS

Under laboratory conditions, EAG bioassays on RPW adults revealed clear responses to all the synthetic palm esters tested. The most abundant responses were elicited from ethyl propionate, a compound already used successfully in trap catch experiments with the African palm weevil *R. phoenicis*,[23] while the lowest EAG responses were elicited from ethyl lactate, already used as a coattractant for *R. cruentatus*.[24] However, the relatively weak responses obtained with ethyl lactate could be explained by the characteristics of this compound: hygroscopic and much less volatile than the other esters tested. Significant EAG responses elicited by ethyl propionate and by ethyl acetate at the lowest dose (0.1 µg) could indicate the ability of RPW to detect the compounds at a greater distance from their source than the other esters screened in the EAG. The trap catch experiments using pheromone + molasses traps complemented with ethyl propionate recorded a number of catches similar to the one obtained using ethyl acetate. Moreover, the use of the two esters in combination determined more captures than ethyl propionate alone, suggesting that the blend of esters mimics more strongly the palm odour than the individual esters. Palm esters such as ethyl acetate and ethyl propionate have been identified by GC-MS from the volatiles of fermenting palm tissues.[25] Air collections and SPME head-space analysis already carried out from decaying tissues of *Elaeis* spp.,

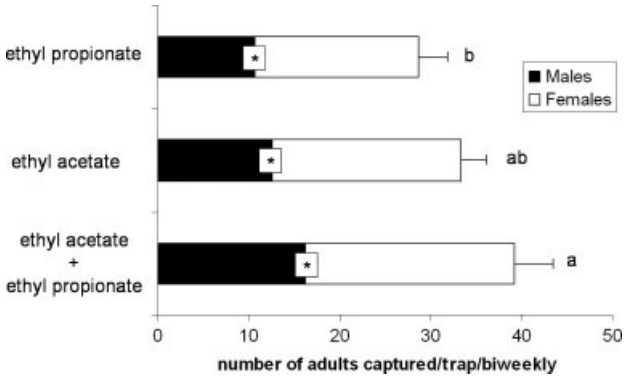

Figure 2. Numbers of *Rhynchophorus ferrugineus* males and females captured biweekly (from 22 July 2009 to 29 September 2009) per trap ($n = 21$). The traps were baited with the aggregation pheromone and sugar beet molasses and supplemented with ethyl propionate, ethyl acetate or the two esters in combination. Asterisks on the bars indicate that the sex ratio values differ statistically for $P < 0.01$ (*t*-test). Bars indicate the means $\pm$ SE of adults captured; no letters in common indicate that values differ statistically at $P < 0.05$ (ANOVA followed by Fisher LSD test).

Jacaratia spp., coconut palm and sugar cane showed that high percentages (60–90%) of the volatiles trapped comprise ethyl acetate and ethyl alcohol; nevertheless, other compounds such as ethyl propionate, ethyl butyrate and ethyl lactate were also found in smaller amounts.[23,25] These aspects support the commonly accepted fact that RPW is attracted by dying and damaged parts of palm.[26] In the case of the American palm weevil, *R. palmarum*, it has been demonstrated that the volatiles produced during the fermentation processes can play a role as kairomones triggering the primary attraction of insects to oil palms.[25] Moreover, literature evidence shows that molecules produced by young palm tissues could also be attractive to weevils. Gunawardena *et al.*,[28] working on volatiles produced from the coconut palm, *Cocos nucifera* L., found two compounds that showed short-range attractant properties for the RPW in laboratory bioassays: 4-nonalactone and 4-hydroxy-3-methoxystyrene.

In the trap catches, the number of captured females was greater than the number of males in all the treatments tested, with a proportion of 1/1.56 males/females. This value is different from the sex ratio of 1.08/1 males/females observed on infested palms of urban areas of Sicily.[29] The higher proportion of females captured in the traps complemented with ethyl acetate and ethyl propionate might be due to the higher sensitivity of females to these esters, as evidenced by the EAG experiments. Nevertheless, in this contest, the role of a pheromone and molasses complex in influencing the attractant action of the synthetic esters cannot be underestimated. The high number of adults captured demonstrates the sustainability of the use of the mass trapping technique in an urban environment, in accordance with the results of mass trapping experiments carried out in field conditions.[26]

In conclusion, the results of this study support the application of mass trapping implemented with palm esters as an important tool for the management of RPW populations and to prevent the infestation of ornamental plants in urban environments, where other techniques such as chemical control could have strong consequences on environmental pollution and human health. A better understanding of the attractive capacity of the palm ester mixtures and the relatively optimal doses could not only provide fundamental ecological knowledge but also make it possible to improve pheromone-baited trap efficiency in RPW control.

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