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Advances in the Use of Trapping Systems for *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae): Traps and Attractants

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Abstract

Given the social importance related to the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), efforts are being made to develop new control



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black pyramidal trap design (Picusan) has been verified in comparison with white and black buckets. In addition, the attractant and synergistic effect of ethyl acetate (EtAc) at different release levels has been evaluated under field conditions. The results show that Picusan traps captured 45% more weevils than bucket-type traps, offering significantly better trapping efficacy. The addition of water to traps baited with palm tissues was found to be essential, with catches increasing more than threefold compared with dry traps. EtAc alone does not offer attractant power under field conditions, and the release levels from 57 mg/d to 1 g/d have no synergistic effect with ferrugineol. Furthermore, significantly fewer females were captured when EtAc was released at 2 g/d. The implications of using EtAc dispensers in trapping systems are discussed.

red palm weevil ethyl acetate kairomone trap integrated pest management

Originally from South and Southeast Asia, the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), was first described on coconut palms by Lefroy in 1906 and later on date palm in India by Lal in 1917 (Kaakeh et al. 2001), but has recently colonized the Mediterranean basin and North America (Dembilio et al. 2010). Nowadays, it can be found in all continents, including Mediterranean basin, Madagascar, Aruba and Curaçao in South America, North America (United States and Mexico), Asia, and Australia, Papua New Guinea, Samoa, and Solomon Islands in Oceania (Fiaboe et al. 2012). *R. ferrugineus* was first detected in South Spain in 1995, although it did not expand to other regions in Spain until 2004 (Llácer et al. 2010). Apart from economic loss, the red palm weevil causes social concern, as the severity of its attack has a detrimental visual effect on landscape. In Spain, the main damage caused by this weevil is found in Canary Islands date palms [*Phoenix canariensis* (hort. ex Chabaud)] and date palms (*Phoenix dactylifera* L.), as they are the principal palm tree species cultivated for both ornamental and horticultural purposes.

The larval form is the most destructive stage of this weevil, as it penetrates deep into the stem and damages its internal tissues, which makes control difficult. Chemical control against the red palm weevil is based mainly on the repeated application of large quantities of synthetic insecticides for both preventive and curative procedures (Kaakeh 2006, Dembilio et al. 2010, Llácer et al. 2010). Other strategies include phytosanitation and biological methods, such as application of entomopathogenic nematodes (Dembilio et al. 2010) and fungus (Gindin et al. 2006). However, few studies targeting natural enemies have been conducted. Only a few larvae parasitoids (the wasp *Scolia erratica* Smith and calliphorid fly *Sarcophaga fuscicauda* Bottcher) and predators (the earwing *Chelisoches morio* F.) have been cited, although they do not seem to play an important part in the limitation of red palm weevil populations (Murphy and Briscoe 1999, Faleiro 2006).

The discovery of the red palm weevil aggregation pheromone composition, ferrugineol (4-methyl-5-nonanol) and ferruginone (4-methyl-5-nonanone) (Hallet et al. 1993), was essential to develop pheromone-based trapping and monitoring systems to manage this pest. The combination of these compounds has been used in mass-trapping programs throughout the Middle East, and the effectiveness of pheromone-based trapping for the



tool for controlling the *R. ferrugineus* in Israel. Using a trapping system is an efficient complementary technique to be included in an integrated pest management program. This system does not create resistances, reduces populations, allows population monitoring, and helps the efficacy of other control methods by optimizing the application of insecticides. Bucket-type traps have been traditionally used for weevils, and they became commercially available to capture the red palm weevil since it was first detected in Spain. However, it is still unknown if other designs could be more efficient than buckets, as some authors highlighted the importance of visual discrimination (trap color and silhouette) for weevils (Hallet et al. 1999).

Regarding attractants, Jaffé et al. (1993) reported that addition of ethyl acetate (EtAc) to traps baited with pheromone and sugarcane increases *Rhynchophorus palmarum* catches. Moreover, it has been proven that ethanol and EtAc blends have a moderate synergistic effect to the pheromone of *R. palmarum* (Rochat et al. 2000). However, the results published by Oehschlager (Oehschlager and González 2001, Oehschlager 2006) did not confirm this effect, as these authors found no significant differences between traps baited with pheromone or pheromone + EtAc. Regarding *R. ferrugineus*, El-Sebay (2003) reported a field trial where traps baited with EtAc captured more adults than those baited only with pheromone and food bait. However, this finding was not supported by statistical data. Thus, any significant effect of EtAc on red palm weevil trapping is still uncertain.

In this work, the efficacy of a new pyramidal trap to capture the red palm weevil has been tested and compared with standard bucket traps. In the same trial, the importance of water addition to traps was assessed. Furthermore, the effect of EtAc at different release rates has been tested in field conditions to evaluate the role of EtAc in red palm weevil trapping protocols.

Materials and Methods

Traps and Dispensers.

Traps. Two different commercially available devices were tested under field conditions in preliminary trials: the black pyramidal trap Picusan (supplied by Sansan Prodesing SL, Valencia, Spain) and the conventional white bucket trap (supplied by OpenNatur SL, Barcelona, Spain). Picusan consists of three parts: 1) a cylindrical base (25 cm in diameter, 6 cm in height); 2) a rough (1 mm between grooves) black pyramid with a 66% slope and a funnel inserted onto the upper side; and 3) a green cover on the top leaving a 4-cm aperture between the upper side of the pyramid and the top. This cover has a basket inserted into the center to place the pheromone dispenser. The OpenNatur bucket trap is a 12-liter traditional bucket-type trap, with 4 by 5-cm diameter holes in the lid and four more in the walls. Under the lid, pheromone or kairomone dispensers hang from a hook. In addition, the Picusan trap was also compared with black OpenNatur traps with the aforementioned features to avoid differences in efficacy because of the color factor.

Ferrugineol Dispensers. Pherocon RDPW 700 dispensers, supplied by Kenogard

4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferruginone). Commercial cotton wick-type dispensers loaded with 500 mg of aggregation pheromone (Biobest, Almería, Spain) were used in the trap evaluation trial carried out in 2011–2012. Once Picusan had become a completely commercial solution, Ferosan RF dispensers (Sansan Prodesing, Valencia, Spain) were used in the 2012 EtAc evaluation trials. These are low-density polyethylene vials loaded with 1 g of aggregation pheromone.

EtAc Dispensers. Different dispensing technologies were used to evaluate the effect of EtAc emission on red palm weevil captures. Plastic bag dispensers were used in the 2010 trials to evaluate the different EtAc emission levels; one of them provided by Rhynchonex kairomone (Rk) dispensers (Econex, Murcia, Spain), and the other two higher-emission levels were obtained by using one or two of the plastic bag dispensers developed in our laboratory (bag dispenser hereafter). These bags were 10 by 6 cm, made of high-density polyethylene (200 μm), coated with aluminum on one face, and loaded with 50 ml EtAc (99.5%, Scharlab SL, Barcelona, Spain).

To test the different EtAc emission rates in 2012, two types of polypropylene centrifuge tubes (Insulab SL, Valencia, Spain) were used: 1) 50-ml tube, 29 mm in diameter and 115 mm in height; and 2) 15-ml tube, 16.5 mm in diameter, and 120 mm in height. Tubes were loaded with 40 and 13 ml EtAc, respectively.

Trap Evaluation Trials.

Preliminary Trial 2010. A preliminary trial was carried out to compare the trapping efficacy of the Picusan prototype with the standard OpenNatur white bucket trap. It was designed as a randomized block assay with three replicates and was located in Godella (Valencia, Spain). The traps inside the blocks were separated by at least 30 m and the distance between blocks was at least 50 m. All the traps were baited with 700 mg Pherocon dispensers and they contained water on the base. They were revised and rotated every 3 or 4 d, and the number of females and males captured from 27 May 2010 to 14 June 2010 was recorded.

Trap Evaluation Trial 2011–2012. Later, improvements were introduced into both types of traps. The OpenNatur bucket traps were modified, according to laboratory observations and new recommendations, by including a plastic funnel inside the bucket to avoid weevils from escaping and a mesh on the outer walls to facilitate climbing. The modified Picusan trap included a new zip-tie locking system to avoid a space appearing between the container and the upper part of the trap that allows weevils to escape. The trapping efficacy of the improved traps was checked in this second trial carried out in Crevillente (Alicante, Spain). Here the effect of trap color was eliminated by using the black OpenNatur trap in the comparison made. In this trial, the effect of adding water inside the traps was also studied. The field trial was arranged with five replicates and four traps per block: 1) Picusan with water; 2) black OpenNatur with water; 3) dry Picusan; and 4) dry black OpenNatur. Traps were arranged in the vicinity of palm orchards, but never closer than 100 m to the orchard limits. Traps were placed 100 m apart inside each block, whereas blocks were positioned 200–300 m apart. Wet traps were filled with 4 liters of water, 1 liter of molasses (20% vol:vol, supplied by Biobest, Almería, Spain), 10 drops of polyethylene glycol, and Canary palm stem pieces, and were serviced biweekly. Dry traps

pheromone dispensers, which were replaced every 3 mo. Weevil captures were counted and traps were rotated clockwise every 2 wk from 25 January 2011 to 12 March 2012.

EtAc Field Evaluation.

The EtAc attraction power itself was checked in a field trial carried out in Godelleta (Valencia, Spain). This trial consisted in three replicates by comparing Picusan traps baited with the EtAc Rk dispenser and others baited with a Pherocon pheromone dispenser and decaying stem tissues of *P. canariensis*. Traps were placed in the field on 12 May 2010, and were revised and rotated every 3 d until 24 May 2010. Likewise, the synergistic effect of EtAc and pheromone was tested in a subsequent trial. The captures obtained in the Picusan traps baited with EtAc Rk and pheromone dispenser were compared with those obtained from the traps baited only with pheromone. The trial was carried out with three replicates from 24 May to 14 June 2010.

The red palm weevil adults' attraction to three different values of EtAc release rates was evaluated in a trial conducted in an 8-ha palm tree nursery located in Manises (Valencia, Spain). Three replicates of this trial were arranged, including four Picusan traps baited with a Ferosan pheromone dispenser and: 1) one EtAc Rk dispenser; 2) one EtAc bag dispenser; 3) two EtAc bag dispensers; and 4) only one with the pheromone dispenser as a reference. The emission levels corresponding to each baited trap were calculated according to the study of the release profiles described later. This trial started on 26 July 2010, and traps were revised and rotated every 3 d until 3 September 2010.

According to this result, two lower EtAc emission levels were tested in a new field trial, which were obtained with the Picusan traps baited with Ferosan and: 1) 1 by 15-ml centrifuge tube, or 2) 3 by 40-ml centrifuge tubes, as described before. One trap baited only with a Ferosan dispenser acted as the reference. Thus, this trial had four replicates of blocks with three traps, was carried out from 30 April to 12 June 2012, and traps were revised and rotated weekly.

EtAc Release Profiles.

The gravimetric method was used to determine the amount of EtAc released in relation to aging time. In parallel with the field trial, three dispensers of each type were aged under the same field conditions. Dispensers were placed inside the same trap type of traps used in the field trial. These dispensers were weighed weekly in the laboratory on a precision balance (A&D Instruments Ltd., Abingdon, United Kingdom). The weight differences over a period were referred as the active compound released from the dispenser.

Multiple regression was applied to study whether the emission was constant along the studied period by checking the significance of the quadratic effect ($P < 0.05$). When not significant, weight losses were fitted to a linear regression model ($y = ax + b$), where y is the weight of the dispensers (g) and x is the field exposure time (days), to obtain the emission level of each dispenser type.



The number of males, females, or total weevils recorded in each trapping period was divided by the number of days comprised in to calculate the weevils per trap and day index. Data according to the factors studied in each trial were analyzed by means of analysis of variance (ANOVA) (followed by a least significant difference test at $P < 0.05$). Before the analysis, the number of captures was transformed by \sqrt{x} to normalize the data. The Statgraphics Centurion XVI package was used to perform all the statistical analysis (Statpoint Technologies Inc., Warrenton, VA).

Results

Trap Evaluation.

Preliminary Trial 2010. The trapping performance of the Picusan trap was compared with the standard white bucket trap (Fig. 1), and the data collected were analyzed separately for each sex and total individuals trapped. The week and block factors were not significant in all cases ($P > 0.12$), but the trap factor had a significant effect on captures. The Picusan trap captured more total weevils than the standard bucket, and specifically more males (males: $F = 5.58$, $df = 1, 22$, $P = 0.03$; total: $F = 4.53$, $df = 1, 22$, $P = 0.04$), although the number of females captured was not significantly different ($F = 2.77$; $df = 1, 22$; $P = 0.11$).

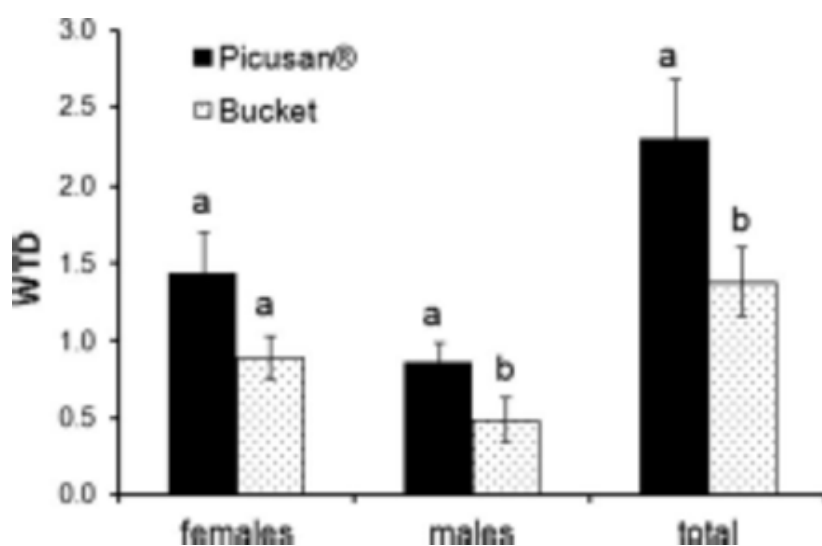


Fig. 1

Mean number of weevils per trap and day (WTD) captured in black Picusan and traditional white bucket traps (OpenNatur) baited with Pherocon ferrugineol dispensers in the preliminary trial 2010. Number of females, males, and total weevils was analyzed separately; bars labeled with the same letter are not significantly different (least significant difference [LSD] test at $P > 0.05$). WTD values refer to the mean total trap catch over the course of the experiment.

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palm weevils captured in dry traps. These differences are clearly shown in Fig. 2 for both types of traps. Thus, the addition of water to traps is essential for trapping efficacy, as confirmed by the multifactor ANOVA ($F = 204.06$; $df = 1, 316$; $P < 0.001$). This fact led us to separately analyze efficacy of traps with and without water. In any case, the mean number of weevils captured biweekly over the course of the experiment with dry Picusan did not significantly differ from that obtained with dry black OpenNatur traps, as depicted in Fig. 3 (males: $F = 0.85$; $df = 1, 201$; $P = 0.36$; females: $F = 0.66$; $df = 1, 201$; $P = 0.42$; total: $F = 0.80$; $df = 1, 201$; $P = 0.37$). However, the Picusan traps were significantly more effective than the OpenNatur black bucket type when baited with pheromone and filled with water, molasses, and palm stem tissues (Fig. 3) in terms of number of males, females, or total weevils captured (males: $F = 10.82$; $df = 1, 202$; $P = 0.001$; females: $F = 12.39$; $df = 1, 202$; $P < 0.001$; total: $F = 13.21$; $df = 1, 202$; $P < 0.001$).

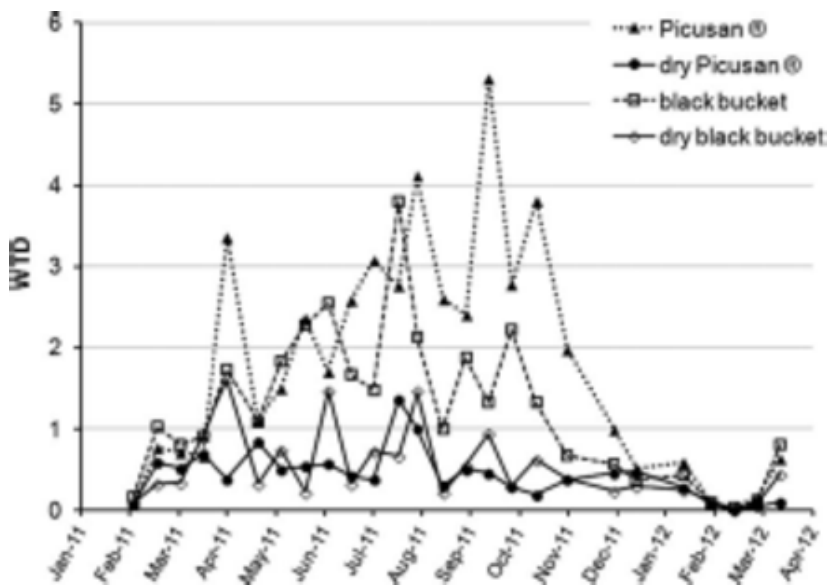


Fig. 2 Population dynamics of red palm weevil in Picusan and black bucket traps filled with and without water recorded in trial 2011–2012. WTD values are average of five blocks for each type of trap on each trapping period.

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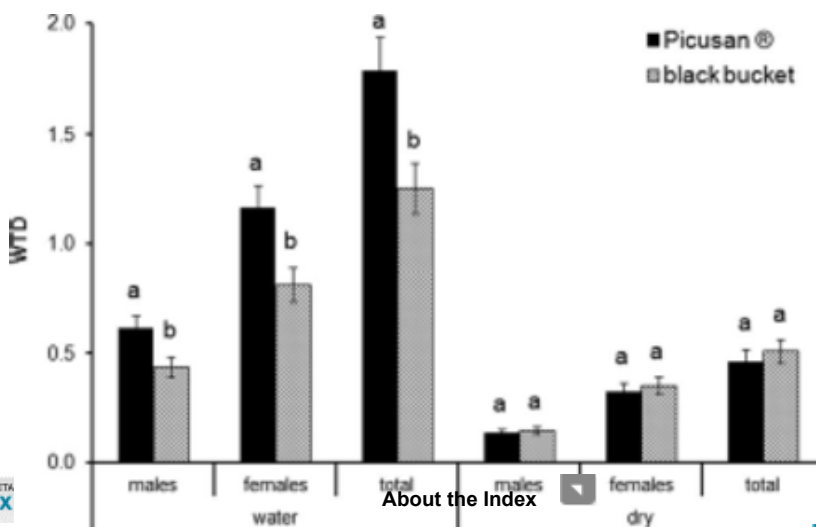
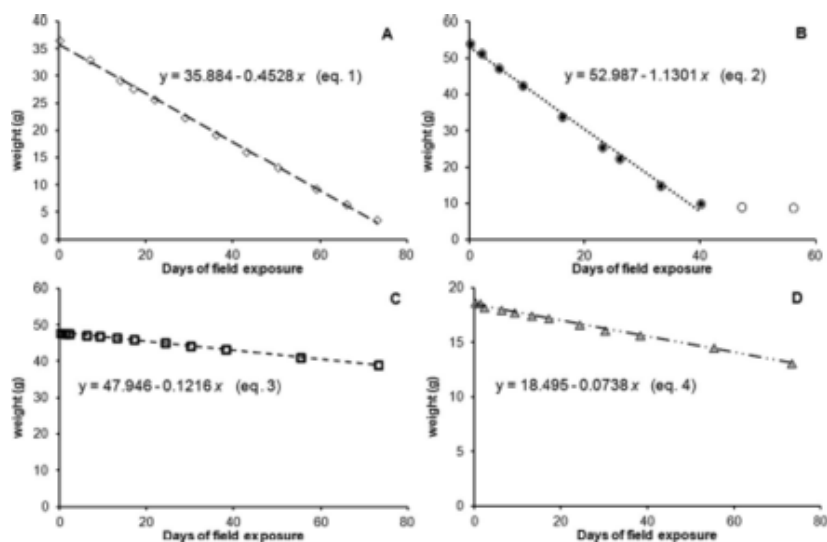


Fig. 3

Number of WTD captured during the trap evaluation trial 2011 in dry modified black Picusan and black bucket traps (OpenNatur), and the same ones filled with water and molasses. All traps were baited with Ferosan ferrugineol dispensers. Number of females, males, and total weevils was analyzed separately; bars labeled with the same letter are not significantly different (LSD test at $P > 0.05$). WTD values refer to the mean total trap catch over the course of the experiment.

EtAc Release Profiles.

The results of release profiles of EtAc are shown in **Fig. 4**. Loss of weight of the Rk dispenser was fitted to the linear model given by equation 1 (**Fig. 4A**) ($R^2 = 0.99$); thus, EtAc was released at a constant rate of ≈ 0.45 g/d, given by the slope of the fitted line, for at least 60 d. Quadratic term was therefore not significant for Rk dispenser data; however, the multiple regression showed the significance of this term in the complete release profile (0–60 d) of the bag dispenser (**Fig. 4B**). Actually, loss of weight was virtually zero for this dispenser after 40 d of exposure. Thus, bag dispenser was considered to be depleted after this period, and release profile fitted the linear model according to equation 2 ($R^2 = 0.98$). Consequently, pheromone release was constant and equal to 1.1 g/d for 40 d.

**Fig. 4**

EtAc release profiles from the four different dispensers used in field trials: (A) commercial Rk dispenser, (B) handmade bag dispenser, (C) 40-ml centrifuge tube, and (D) 15-ml centrifuge tube, as dispensers' weight (g) versus the time of field exposure (days). All models fitted to linear equations ($R^2 > 0.90$).

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In the subsequent trial carried out in 2012, the 40- and 15-ml centrifuge tubes achieved the desired lower emission levels of 121.6 and 73.8 mg/d, respectively, given by the linear release profiles depicted in **Fig. 4C** and **D** (equations 3 and 4; $R^2 = 0.97$ and 0.92,

EtAc Field Evaluation.

The data obtained in the first trial demonstrated the negligible effect of EtAc as an attractant itself, which was compared with the effect of pheromone combined with the decaying stem pieces of canary palm. Only three individuals were captured in the traps baited with EtAc, whereas 145 red palm weevil adults were captured in those baited with pheromone and plant material throughout the trial.

The synergistic effect of adding EtAc to pheromone was also evaluated. **Figure 5** shows that all the captures with the (pheromone + EtAc Rk dispenser) combination did not significantly differ from pheromone alone ($F = 0.01$; $df = 1, 37$; $P = 0.91$). The same result was obtained by analyzing the data for males and females separately ($F = 0.43$; $df = 1, 37$; $P = 0.52$ and $F = 0.00$; $df = 1, 37$; $P = 0.96$, statistical values for males and females, respectively). It is important to highlight that these results correspond to the previously calculated emission value given by the Rk EtAc dispenser (0.45 g/d).

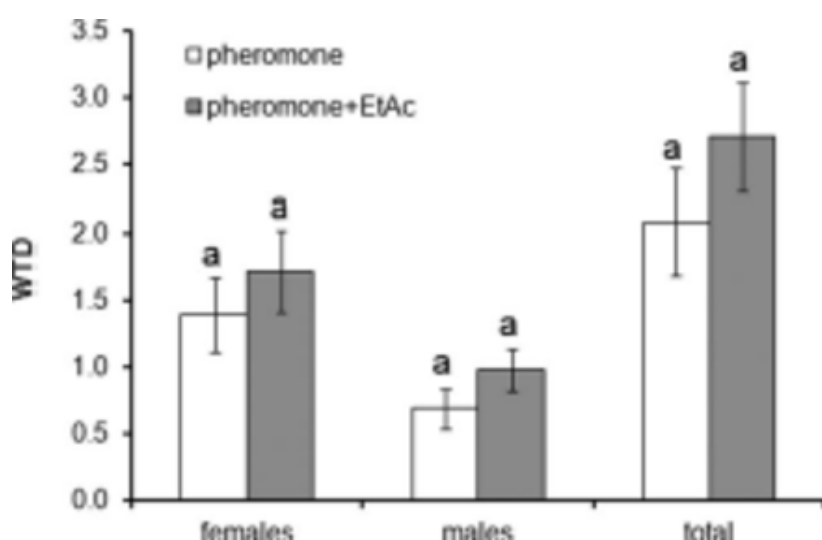


Fig. 5

Number of WTD captured in traps baited only with ferrugineol and with ferrugineol + commercial EtAc Rk dispenser (0.46 g/d). Number of females, males, and total weevils was analyzed separately; bars labeled with the same letter are not significantly different (LSD test at $P > 0.05$). WTD values refer to the mean total trap catch over the course of the experiment.

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Three EtAc emission levels combined with pheromone were subsequently evaluated against the traps baited with only pheromone as a control (**Fig. 6**). Regarding the number of adults trapped, the emission levels of 0.45 and 1.1 g/d did not significantly differ from the control ($F = 2.01$; $df = 3, 116$; $P = 0.12$), but a higher EtAc release rate resulted in fewer captures, and there were even significant differences found between the traps baited with EtAc dispensers releasing 0.45 and 2.2 g/d. The same result was obtained when analyzing male captures separately ($F = 1.01$; $df = 3, 116$; $P = 0.39$). However, females' behavior differed somewhat: the highest EtAc emission level (2.2 g/d) had a detrimental effect on female captures, as demonstrated by the significant differences

only with the pheromone dispenser.

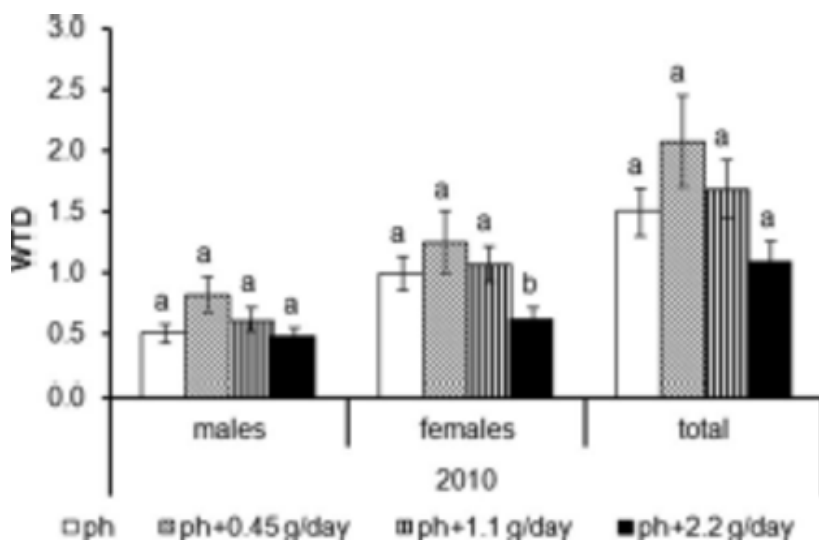
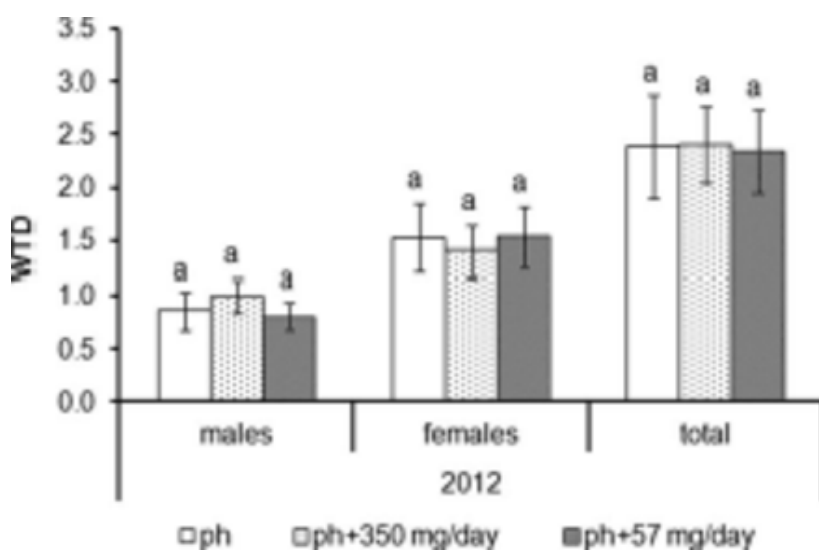


Fig. 6

Number of WTD captured in traps baited with ferrugineol dispensers and three different EtAc emission levels, 0.45, 1, and 2 g/d. Number of females, males, and total weevils was analyzed separately; bars labeled with the same letter are not significantly different (LSD test at $P > 0.05$). WTD values refer to the mean total trap catch over the course of the experiment.

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According to these results, two lower EtAc emission levels were tested (**Fig. 7**), which were previously calculated as 350 and 57 mg/d. Similarly, these emission rates had no significant effect on pheromone attraction for female, male, or total weevil catches (males: $F = 0.88$; $df = 2, 59$; $P = 0.42$; female: $F = 0.17$; $df = 2, 59$; $P = 0.84$; total: $F = 0.35$; $df = 2, 59$; $P = 0.71$). All the described trials had a nonsignificant effect of EtAc on captures and evidence that EtAc alone cannot substitute the synergistic effect of palm odor on attracting the red palm weevil.



emission levels, 57 and 350 mg/d. Number of females, males, and total weevils was analyzed separately; bars labeled with the same letter are not significantly different (LSD test at $P > 0.05$). WTD values refer to the mean total trap catch over the course of the experiment.

Discussion

Bucket-type traps have been traditionally used for trapping weevils, and they were the first option after the red palm weevil invaded the Mediterranean region. Since its first detection in Spain, different bucket trap designs have become commercially available, but further studies were needed to study if other designs can prove more effective. For *R. palmarum*, buckets have been reported to be significantly more effective than other trap designs (Oehlschlager et al. 1993). Nonetheless, Hallet et al. (1999) highlighted the importance of visual discrimination for weevils with regard to trap color and silhouette. This phenomenon was previously observed with pyramid traps, which were originally designed for the pecan weevil, *Curculio caryae* (Horn), and which also provided an attractive visual stimulus for plum curculio by mimicking tree trunks (Mulder et al. 1997). Hallet et al.'s (1999) experiment, which compared the trapping efficacy of buckets of different colors and orientations, reported that significantly more red palm weevils were captured in black inverted bucket traps than in white bucket traps, either inverted or not. This means that gentle slopes play an important role because of *R. ferrugineus*' crawling behavior and that dark colors help host or trap location by highlighting their silhouettes against the background. The importance of using dark colors has been also recently reported in relation to bucket traps by Al-Saoud et al. (2010) and Abuagla and Al-Deeb (2012). By assuming these hypotheses, in this work, the efficacy of a new black pyramidal trap design (Picusan) to capture *R. ferrugineus* was compared with the standard bucket trap. Results confirm the suitability of pyramidal trap designs to maximize red palm weevil trapping efficacy. Ávalos and Soto (2013) disagree with the effectiveness of Picusan, as they found that bucket traps were more efficient in a choice test. They arranged four different traps 1.2 m away from each other. Interference between traps was obvious and therefore the study only provided information about which trap was more likely to catch insects when they are attracted to the proximity of traps.

When water was introduced as a study factor, the total number of weevils captured was significantly larger in those traps filled with water than in dry traps. Traps contained palm stem pieces in both cases; thus, the results confirm that addition of water to traps is essential for trapping performance, as it takes part in the fermentation of palm material when used as a synergistic source of odor.

Many works showed a strong synergistic effect between aggregation pheromones and natural plant odor sources. There are reports that the introduction of host plant tissues into weevil traps increases the number of captures with the aggregation pheromones of *Rhynchophorus cruentatus* F. (Weissling et al. 1994), *R. palmarum* (Jaffé et al. 1993), *Rhynchophorus phoenicis* F. (Gries et al. 1994), and *R. ferrugineus* (Hallet et al. 1999).

fermentation volatiles of different palm tissues, palm oils, coconut, and pineapple are attractive to *Rhynchophorus* weevils (Nagnan et al. 1992, Jaffé et al. 1993, Rochat et al. 1993, Giblin–Davis et al. 1994), which include the so-called “palm esters,” such as EtAc. The identification of the key odorants present in host palm volatiles is crucial to help develop a synthetic red palm weevil pheromone synergist to replace plant material in weevil traps. Despite knowing that EtAc elicits significant electroantennographic responses, its role in field trapping performance has not been clearly demonstrated for *R. ferrugineus*. Negative responses for it attracting *R. palmarum* to EtAc itself have been reported; however, the traps baited with rhynchophorol, EtAc, and sugarcane have shown much higher catches than the same ones without EtAc (Jaffé et al. 1993). Some studies have pointed out that *R. ferrugineus* adults' attraction increases under field conditions when EtAc is added to pheromone and food bait (Oehlschlager and González 2001, El-Sebay 2003), although the results are not supported by any statistical analysis.

The trials included in this work suggest that EtAc emission rates <1 g/d do not significantly increase the efficacy of the traps baited with only pheromone. Furthermore, the highest EtAc release rate tested (2 g/d) captured significantly less female weevils. This result might be related to the locomotory behavior bioassays performed by Schmidt–Büsser et al. (2010), which found that females are oriented later and less to 1 ng ferrugineol mixed with 10 µg EtAc. Recently, the kairomone effect of the two-ester blend, EtAc and ethyl propionate, has been described for *R. ferrugineus* (Guarino et al. 2011). However, this statement does not provide sound evidence because the number of captures in the traps baited without esters, as a control, remains unknown, as do the data on the ester release rates.

Despite this work demonstrating the efficacy of the pyramidal Picusan trap for *R. ferrugineus*, the synthetic pheromone synergists issue remains uncertain. The obtained results coincide with some authors who state that EtAc alone cannot be used as a substitute for natural palm odor (Faleiro 2006, Schmidt–Büsser et al. 2010). However, efforts are being made to find the volatiles that are missing in the synergistic mixture to be formulated to replace the cumbersome use of plant material in red palm weevil trapping systems.

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