Lures for red palm weevil trapping systems: Aggregation pheromone and synthetic kairomone

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Running title: Lures for Red Palm Weevil trapping systems

Abstract

BACKGROUND: The optimization of the lure is essential for the implementation of trapping systems to control insect pests. In this work, the response of the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, to increasing emission rates of its aggregation pheromone (ferrugineol) and the efficacy of a convenient synthetic kairomone based on fermentation odors (ethyl acetate and ethanol) have been evaluated in different years and locations along the Mediterranean basin.

RESULTS: In general, although capture data and emission had noticeable variability among locations, significantly less RPW were captured in pyramidal Picusan® traps with the lowest ferrugineol emission rates tested (0.6-3.8 mg/day^{-1}). Captures increase rapidly with ferrugineol emission up to 4-5 mg day^{-1}; then, higher emission rates did not improve nor decrease captures, up to the highest emission rate tested of 50.9 mg day^{-1}. Thus, there is no evidence of an optimum release rate corresponding with a maximum of RPW catches. Traps baited with the synthetic kairomone (1:3 ethyl acetate/ethanol) captured from 1.4 to 2.2 times more total weevils than traps baited only with ferrugineol. Moreover, in most of the locations, the synthetic blend was at least as effective as the local co-attractants used (plant material + molasses).

CONCLUSIONS: Ferrugineol emission rate can vary in a wide range without affecting significantly RPW response. Co-attractants based on fermenting compounds, ethyl acetate and ethanol, are able to improve the attractant level of ferrugineol and could be employed to replace non-standardized natural kairomones in RPW trapping systems after further optimization of their proportions and doses.

Keywords *Rhynchophorus ferrugineus*, 4-methyl-5-nonanol, ethyl acetate, ethanol, mass trapping, monitoring

1 INTRODUCTION

The use of trapping systems is an efficient technique to be included in any integrated pest management program (IPM) to control the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, by means of preventive and curative measures. Early detection and monitoring are essential to plan further actions against infestations, whereas mass trapping helps reducing population levels. Management of RPW by this mean has been widely employed throughout the Middle East and the effectiveness of the pheromone-based trapping for RPW was demonstrated.[1] Later, results presented by Soroker et al.[2] indicated that mass trapping could serve as a tool for controlling RPW in Israel and it is a key part of the IPM carried out in Saudi Arabia to protect palm crops.[3] Semiochemical-based trapping systems for weevils had three main components: trap, aggregation pheromone and kairomone (co-attractant). Buried bucket traps are traditionally employed for these purposes but improvements (color, surface, retention system) and even new trap designs have been introduced in the last years.[1,4-5] Regarding the attractant, Hallett et al.[6] first reported identification and activity (both in electrophysiological and field tests) of the main compound of the RPW aggregation pheromone, 4-methyl-5-nonanol (ferrugineol). A second compound with electrophysiological activity, 4-methyl-5-nonanone (ferruginone), was also identified in the volatile extracts but field tests did not evidence pheromonal activity. The aggregation pheromone is emitted by the males of the species and attracts both sexes, with bias towards females, which is highly favorable for the mass trapping technique. The second component for weevil attraction is the kairomone; it has been demonstrated that natural palm baits have poor attractant power by themselves but strongly synergize the effect of the aggregation pheromone.[7] The fermentation volatile compounds emitted by different host plant tissues have been studied through electrophysiological bioassays and have revealed that RPW antennae are responsive to many compounds, including the so called 'palm esters'.[8-9] RPW attraction to these compounds has been also tested in field trials: Guarino et al.[8] observed that a blend of the esters ethyl acetate and ethyl propionate improved catches in traps baited with pheromone and molasses, better than the individual esters. More recently, Vacas et al.[9] found that RPW catches increased two-fold with the 1:3 ethyl acetate/ethanol blend compared to aggregation pheromone alone, even achieving 76% efficacy if compared to the total weevil catches obtained with a kairomonal co-attractant composed by *P. canariensis* palm stem and sugar molasses.

In general, sensitivity of pheromone-based monitoring and efficacy of mass trapping strategies is highly determined by pheromone emission rates. It has been widely described that pheromone release rate must

be controlled because insect response could decrease below and above an optimum value.[10-14] Besides, pheromone cost is a key parameter for the implementation of this kind of control methods; thus, optimum pheromone emission rates should be known to avoid pheromone waste. The pheromone dose-dependent behavior of RPW has been early evaluated by Hallet et al.[6] in field experiments, in which bucket traps releasing 3 mg day^{-1} of ferrugineol captured significantly more weevils than traps with emission rate of 0.3 and 1 mg day^{-1}.

Available literature dealing with RPW lures is focused on studies in a particular location and no common protocols are then described for implementation anywhere. The present work reports results obtained with the aim of establishing trapping protocols valid for most of the areas where RPW is present or susceptible to be invaded. For this purpose, the field tests reported herein were conducted with standard protocols in five different countries along the Mediterranean basin, covering a large geographical area. The response of the RPW to increasing ferrugineol emission rates was evaluated in field trials by comparing the number of weevils captured in pyramidal Picusan® traps baited with different types and numbers of ferrugineol dispensers. Similarly, to evaluate the potential of synthetic lures to replace the use of plant material to boost trap attractiveness, the efficacy of the synthetic co-attractant suggested in Vacas et al.,[9] a blend of ethyl acetate and ethanol, has been assessed relative to ethyl acetate alone or local reference co-attractants (palm pieces and/or molasses).

2 MATERIALS AND METHODS

2.1 Traps and dispensers

The new design of pyramidal trap Picusan® (Sansan Prodesing SL, Náquera, Valencia, Spain), described in Vacas et al.,[5] was employed in all the field trials, the base of which was filled with 1.5-2 L water. Aggregation pheromone dispensers employed in our trials only used ferrugineol as aggregation pheromone due to the lack of evidences for pheromonal activity of ferruginone in the literature available[6] and our own experience. The standard commercial aggregation pheromone dispenser employed in all the trials as reference was Pherosan RF (Sansan Prodesing SL, Náquera, Valencia, Spain), which is a polyethylene (PE) vial (18 mm diam. x 35 mm h.) loaded with 1 g of ferrugineol (98% purity, sum of enantiomers). The response of RPW to different ferrugineol emission rates was studied by baiting traps with different types or numbers of pheromone dispensers. The lowest emission rate was provided in 2012 by 5-ml Nalgene™ low-density polyethylene vials (LD-PE) (20 mm diam. x 25 mm h.) (Fisher Scientific

SL, Madrid, Spain) loaded with 1 g of ferrugineol (98% purity). In the following trials, the Pherosan RF dispenser was modified by different experimental means to slow down its emission and provide the lower emission rates. In particular, in 2013 the dispenser was modified with an adhesive tape coating (mod-RF 1), and in 2014, the dispenser was inserted inside a 12-ml Nalgene™ LD-PE vial (23 mm diam. x 36 mm h.) (Fisher Scientific SL, Madrid, Spain) (mod-RF 2). Previous to field installation, it was ascertained that Pherosan RF emission rate was effectively reduced by the mentioned modifications by studying the release profile of laboratory aged dispensers. The highest emission rates tested in each trial were obtained by baiting traps with 2, 3 or 4 Pherosan RF dispensers as described below.

Synthetic kairomone dispensers (K) were 100-mL LD-PE bottles (Kartell SPA, Noviglio, Italy), loaded with 30 mL of the 1:3 ethyl acetate/ethanol blend in all the trials except in trial K3, where loading was mistaken and the 1:2 ratio was accidentally tested. Active ingredients were emitted through a 100 gauge LD-PE sheet attached to the top of the bottle. Same type of dispensers was loaded with 30 mL of ethyl acetate (EtAc) to test this compound alone in the kairomone trials.

2.2 Release profile studies

In parallel to the field trials, release profiles of the pheromone and kairomone dispensers were studied in each location. The gravimetric method was employed to assess the amount of ingredients released in relation to the aging time. Three additional dispensers of each type were aged under the same field conditions inside the same type of trap in each location and were weighed weekly in the laboratory on a precision balance (0.0001 g). Dispensers were aged during the corresponding study periods, from the beginning to the end of each field trial, according to dates in Tables 2 and 3. This was not performed in the kairomone trial conducted in Egypt (trial K1) due to technical difficulties. The weight differences over a period were referred to as the amount of ferrugineol or kairomone released from the dispenser. To obtain the mean emission level for each dispenser, recorded weights (y) were fitted by polynomial regression with the independent variable x, number of ageing days, and its linear and quadratic effects were studied. When effect of the quadratic term was not significant (F test at $P > 0.05$), recorded weights fitted linear regression models, $y = a + bx$; thus, the slope of the linear model gave the mean release rate of the corresponding dispenser, which was assumed constant throughout the study period.

In the case of the aggregation pheromone, it was previously ascertained by gas chromatography (GC-FID) that dispenser weight losses corresponded effectively to ferrugineol emission and not to degradation

products. Similarly, for the ethyl acetate/ethanol dispensers, we checked the ratio in which the compounds were emitted. For this purpose, a GC/FID analysis of the remaining kairomone contained in the dispensers employed in some trials was performed and compared with the GC/FID analysis of the initial kairomone blends. All GC/FID analysis used a Clarus500 gas chromatograph from PerkinElmer (Wellesley, MA, USA) and injections were made onto a ZB-5MS column (30 m × 0.25 mm × 0.25 µm; Phenomenex Inc., Torrance, CA, USA). Carrier gas was helium at 1.2 ml/min and detector temperature was set at 250 ºC.

2.3 Trials

All of the trials were designed as randomized block assays and were carried out in the locations described in Table 1 and pointed out in Fig. 1. The revision of catches and rotation of traps were performed on a weekly basis. In all of the trials, the traps within each block were separated by at least 50 m and the distance between blocks was at least 200 m.

2.3.1 Aggregation pheromone trials

In each trial (Table 2), each block consisted in four traps with different baits to provide four different ferrugineol emission rates. In trial P1, traps were baited with: (1) one 5-ml Nalgene^{TM} LD-PE vial, (2) one Pherosan RF, (3) two Pherosan RF, and (4) three Pherosan RF dispensers. In trial P2, traps were baited with: (1) one mod-RF 1, (2) one Pherosan RF, (3) two Pherosan RF, and (4) four Pherosan RF dispensers. For the trials carried out during 2014 (trials P3-P6), traps were baited as described for trial P2, except for trap (1) which contained one mod-RF 2.

2.3.2 Kairomone trials

Four plots were arranged in each trial (Table 3) to test the synthetic kairomone blend ethyl acetate/ethanol, each of them consisting of 4 or 5 traps with the fifth corresponding to the use of the reference co-attractant employed in the local protocols (palm tissues and/or molasses). All traps were baited with one standard Pherosan RF dispenser, which were assumed to emit ferrugineol at release rates $\geq$ 4 mg day^{-1} according to previous studies. In general, each block included a trap baited with: (1) only one Pherosan RF dispenser (ph); (2) ph + 1 dispenser with the synthetic kairomone (ph+1K); (3) ph + 2 dispensers with the synthetic kairomone, to have a higher emission (ph+2K); (4) ph + 1 ethyl acetate dispenser (ph+EtAc); (5) ph + reference local co-attractant (the one which is commonly used in each location, as detailed in Table 3). Replacement of water and co-attractants was done every 5 weeks (after

one complete trap rotation) in all locations except in Egypt, where it was done every two weeks due to a higher evaporation rate.

2.4 Statistical analysis

The number of total weevils captured in each trap recorded during each trapping period was divided by the number of days between dates to calculate the weevils per trap and day (WTD) index. Although more females than males were caught in general (female/male ratios > 1), there was not remarkable difference in the responses by either sex and, thus, statistical analysis was performed with the total number of weevils captured.

To deal with non-homogeneous variance and data overdispersion, we used generalized linear model (GLM) techniques assuming quasi-Poisson error variance[15] to compare the mean number of WTD captured in each trap. Once each model was fitted, the validity of the assumptions made was evaluated with the *plot*(glm.model) function by checking residuals distribution and the existence of patterns and outliers. For each trial, we constructed different models with the number of WTD as the dependent variable and the emission rate (trap), sampling date, block and their interactions as the explanatory variables (interaction trap x date x block was not significant in all cases). The significance of the explanatory variables was assessed by backward elimination from the model and subsequent comparison of the two models using the F test statistic. When significant effects were found the *glht* function in the multcomp package[16] was used to perform Tukey HSD tests for post-hoc pairwise comparisons. All these statistical analyses were conducted with R (R version 3.1.0).[17]

To draw a general conclusion and study the existence of an optimum ferrugineol emission rate corresponding to a relative maximum of RPW captures, we followed the methodology employed in Vacas et al.[12] Briefly, we applied a two-factor ANOVA (location and sampling date) using the log(x+1)-transformed captures of the whole data set (trials P1-P6). The residuals of this model still account for the variability of captures caused by ferrugineol emission, as this factor was not included in the ANOVA. Thus, these residuals were saved and used in a subsequent multiple regression analysis to study the existence of a relative maximum by checking the significance of the quadratic effect of the ferrugineol emission in a polynomial model. These analyses were performed using the Statgraphics Centurion XVI 16.2 package (StatPoint Technologies Inc., Warrenton, VA, USA).

198 **3 RESULTS**

199 **3.1 Aggregation pheromone trials**

200 Ferrugineol dispensers employed in the different trials provided the mean emission rates given in Table 4.
201 As can be noticed, emission was variable among locations probably due to the local environmental
202 conditions. For example, trial P3 which began earlier in the season was affected by lower mean
203 temperatures, obtaining the lowest ferrugineol emission rates tested.
204 Results of the trial conducted in Manises (Spain) in 2012 (trial P1), showed that ferrugineol emission
205 significantly affected RPW captures (Table 5). Significantly less captures were obtained with the lowest
206 emission rate tested (2.6 mg day^{-1}) relative to the rest ($P < 0.04$, Tukey HSD test). Emission rates over 4.2
207 mg day^{-1} did not significantly improve the attractiveness to RPW (Fig. 2- P1). Same result was obtained
208 in Valencia (Spain) during summer 2013 (trial P2). When aggregation pheromone was released at rates
209 from 5.5 up to 44.6 mg day^{-1} (Table 4), the emission rate did not have a significant effect on weevil
210 captures (Table 5) (Fig. 2- P2).
211 When the same type of experiment was conducted in different locations during spring-summer 2014
212 (trials P3-P6), results showed that, in general, all the lowest pheromone release rates attracted
213 significantly fewer weevils in the traps. In the trial conducted in Greece (trial P3), the emission rate had a
214 significant effect on weevil captures (Table 5) and the lowest emission rate tested, 0.6 mg day^{-1}, achieved
215 significantly lower weevil captures (Fig. 2- P3). No significant differences were observed between
216 emission rates ranging 2.7-10.8 mg day^{-1} in trial P3 ($P > 0.20$, Tukey HSD test). This result also agrees
217 with the experiment conducted in Israel (Fig. 2- P5), where RPW response was not significantly affected
218 by ferrugineol emission rates ranging 2.1-32.4 mg day^{-1} (Table 6). In contrast, in Italy (trial P4) and Spain
219 (trial P6), the response threshold was somewhat different, as significantly lower total captures were
220 obtained by emitting 2.6-3.8 mg day^{-1} of ferrugineol ($P < 0.04$, Tukey HSD test) (Fig. 2 –P4 and P6).
221 Besides ferrugineol emission, RPW catches were in general strongly affected by the other factors studied,
222 block and date (Table 5), which is explained by the natural dispersion and seasonality of RPW.
223 Although data variability was remarkable, same trend was observed in all trials for RPW response to the
224 different ferrugineol emission rates. When trying to draw a general conclusion gathering the whole
225 available data (trials P1-P6), multiple regression analysis showed that there is no definite optimum
226 ferrugineol emission rate. After removing data variability due to time (date) and location by means of the
227 two-way ANOVA (date: $F_{26,654} = 4.97$, $P < 0.001$; location $F_{4,654} = 41.83$, $P < 0.001$), multiple regression

228 analysis performed with the residuals of the ANOVA as the dependent variable showed that the quadratic
229 term of the emission did not have a significant effect (P = 0.28; model: R^2 = 0.27) and, thus, there was no
230 evidence of an optimum release rate corresponding with a maximum of RPW catches. However, the trend
231 significantly fitted the logarithmic model depicted in Fig. 3 (P = 0.013; model R^2 = 0.51), suggesting that
232 captures increase rapidly up to release rates of about 4-5 mg day^{-1} and slowly over this threshold.
233
234 **3.2 Kairomone trials**
235 Kairomone dispensers provided the mean emission rates listed in Table 4, which varied among trials
236 attending to local environmental conditions. GC-FID analysis also revealed that ethyl acetate/ethanol
237 were emitted in a 2:1 ratio in trial K3, where K dispensers were loaded with 1:2 ethyl acetate/ethanol
238 blend. By contrast, ratio was approximately 2:3 in the samples analyzed from the rest of locations where
239 1:3 ethyl acetate/ethanol blend was employed to load the K dispensers.
240 As mentioned above for ferrugineol trials, RPW captures were in general strongly affected by the factors
241 block and time (date) and the addition of the synthetic kairomone ethyl acetate/ethanol blend (factor trap)
242 to ferrugineol-baited traps also had significant effects on RPW captures (Table 6). In general, traps baited
243 with ph + 1 dispenser with the 1:3 synthetic kairomone (ph+1K) performed significantly better than
244 ferrugineol alone, improving trap efficacy (Fig. 3). Besides, in most cases, there was no need for a higher
245 emission using 2 kairomone dispensers (ph+2K), as captures obtained were not significantly different
246 from those obtained by using 1 kairomone dispenser (P > 0.28, Tukey HSD tests). On the other hand, the
247 use of ethyl acetate alone did not significantly improve the attractant power of ferrugineol, except in trial
248 K5 (P = 0.014, Tukey HSD test). The 1:3 ethyl acetate/ethanol blend was at least as effective as the
249 reference local co-attractant in trials K1, K4 and K5. In trial K3, neither the 1:2 ethyl acetate/ethanol
250 blend nor ethyl acetate alone achieved improved trapping efficacy compared to the use of ferrugineol
251 alone (Fig. 2- K3), while the local co-attractant molasses+EtAc provided significant increase in RPW
252 catches relative to the rest of the co-attractants tested.
253
254 **4 DISCUSSION**
255 The dose-dependent response of RPW to its aggregation pheromone has been previously reported in the
256 literature. In accordance with Hallett et al.,[1,6] ferrugineol released at 3 mg day^{-1} captured 1.5 times more
257 adults than at 1 mg day^{-1}, but the authors did not test higher emission rates. Later, Rochat and Avand-

Faghih[18] observed that release rates over 5 mg day^{-1} gave no significant differences in RPW attraction. Results reported herein of the trials conducted in Spain (P1 and P2) showed that pheromone emission rates ranging from 4.2 to 12.6 mg day^{-1} did not significantly affect both female and male RPW responses. Even high emission rates up to 44.6 mg day^{-1} did not have any significant effect (negative or positive) on weevil captures.

Our results were further supported by field trials conducted in different locations in the Mediterranean basin during spring-summer 2014 (trials P3-P6), thus covering varied environmental conditions. But it is precisely for this reason, wide range of microclimate and landscape conditions, that capture data and emission had noticeable variability among experimental sites. Indeed, the distribution of RPW populations is usually clumped and not homogenous,[19] and it is affected over the year by the availability of hosts and the microclimate conditions of each particular location. Dembilio and Jacas[20] found a strong relationship between mean annual temperature and the RPW development, which determines the seasonality and the number of generations to be expected in each geographical area. On the other hand, as can be noted in the tables reported, even using common protocols and dispensers, ferrugineol emission varied over a wide range, for instance, from 0.6 to 3.8 mg day^{-1} in the case of dispenser mod-RF 2. It is documented that emission rates of dispensers based on polyethylene membranes increased exponentially with temperature[21] and local temperatures, even at the level of trap (e.g. different insolation), were affecting and causing this variability. In spite of this, analyzing each trial separately, we found that traps baited with the lowest emission rates (0.60-3.85 mg day^{-1}) trapped in general significantly fewer weevils, and increasing emission rates did not improve efficacy (up to 50.92 mg day^{-1}). In the global analysis with all the available data (trials P1-P6), the multiple regression analysis did not find a significant quadratic effect of ferrugineol emission on captures; as a consequence, we cannot report an optimum ferrugineol emission rate within the studied range corresponding with a maximum level of captures. Instead, the trend fitted a logarithmic model, in which captures increase rapidly with emission rate up to a threshold (4-5 mg day^{-1}) and then slow down reaching a plateau. Thus, trap catches are not reduced above an optimum emission rate, as described for the response to sex pheromones in other insect orders, such as Lepidoptera,[22] Diptera[14] or Hemiptera.[11] Lack of optimum pheromone release rate has already been described for the related species *Rhynchophorus palmarum* L., the South American palm weevil. Oehlschlager et al.[4] reported that aggregation pheromone emission could range between 0.3 – 200 mg day^{-1} without significantly affecting *R. palmarum* catches. Actually, antagonistic or saturation effects

have never been reported to be caused by an aggregation pheromone. For example, the nitidulid beetle *Carpophilus hemipterus* (L.) responded to its pheromone at all doses between 15 - 15000 µg without significant differences.[23] Based on our results, although both the emission rate and the response of the weevils were affected by the environmental conditions of each location, we can generally conclude that ferrugineol emission rate can vary in a wide range without affecting significantly RPW catches. Accordingly, any commercial dispenser designed to emit ferrugineol at mean release rates near 4-5 mg day^{-1}, will be suitable for RPW trapping systems. Higher release rates do not provide significantly higher captures but have an impact on the longevity of the dispensers and subsequently on the cost of system, as more frequent replacements will be required.

As synergizing component of trapping systems, the next step to improve efficacy and optimize cost is to replace the use of natural kairomones. Our results showed that the 1:3 ethyl acetate/ethanol blend suggested in Vacas et al.[9] is able to improve trap efficacy and perform significantly better than ferrugineol alone, capturing from 2.2 to 1.4 times more total weevils. However, when the ratio was modified to 1:2 (ethyl acetate/ethanol) (trial K3), captures were not significantly improved. Although mean release rates of the blend were similar (Table 4), GC/FID analysis of the remaining content in the dispensers revealed that ethyl acetate/ethanol were emitted in a 2:1 ratio in trial K3 samples, whereas ratio was approximately 2:3 in the rest of the samples analyzed from trials K2, K4 and K5 (same as reported in Vacas et al.[9]). This is suggesting the importance of ethanol in the synthetic kairomone blend that should be released even in a higher proportion than ethyl acetate. However, the synthetic blend achieved higher mean captures than ethyl acetate alone but not significantly in all cases, which indicates that blend proportions and dose still need adjustments.

The use of molasses as part of the local co-attractants is mainly providing the ethanol needed for the kairomonal effect, whereas palm pieces provide fermenting odors, being ethyl acetate and ethanol the main compounds.[8,9] Thus, the present work supports the potential of a simple and convenient synthetic co-attractant to improve the efficacy of ferrugineol-baited traps and this is demonstrated on a broader geographical scale than earlier reported.[9] However, results indicate that the blend still needs optimization. Proportion of compounds in the blend and the dose are crucial to improve trapping performance. More exhaustive studies measuring the ethyl acetate/ethanol quantities and proportions released from the most successful local co-attractants are needed to develop controlled-release dispenser for synthetic

kairomones. This would allow reducing the hand-labor required to service the traps in order to maintain attractant activity and standardize the attractant for monitoring purposes.

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Tables

Table 1. Description of the experimental areas

Country	Location	Coordinates	Elev. (m)[a]	Surrounding area	Host palms available	Trial[b]
Egypt	Ismaïlia (Ismaïlia)	30°42'00" N; 31°48'0" E	10	date palms and mango orchards	*P. dactylifera*	K1
Greece	Lavrio (Attiki)	37°43'20" N; 24°3'5" E	3	urban/rural: great number of palms in houseyards, median strips and gardens	mainly *P. canariensis*; few *P. dactylifera*, *W. filifera* and *C. humilis*	P3, K2
Israel	Almagor (Jordan Valley)	32°54"46' N; 35°35'54" E	3	avocado orchards, olive orchards and open areas.	-	P5
Israel	Rehovot (Center District)	31°54'24" N; 34°48'17" E	60	many scatered palms in gardens with a variety of ornamental plants	mainly *P. canariensis* and *P. dactylifera*; few *W. filifera* and *S. romanzoffiana*	K3
Italy	Grottammare (Ascoli Piceno)	42°59′20″ N; 13°52′05″ E	4	urban area - scattered palms and nursery near one of the plots	*P. canariensis*	K4
Italy	Palermo (Sicily)	38°06'25" N; 13°21'07" E	43	urban area/ park	*P. canariensis*	P4, K5
Spain	Manises (Valencia)	39°30'17" N; 0°30'33" O	52	industrial/urban area - palm nursery	mainly *P. canariensis* and *P. dactylifera*; also *C. humilis* and *W. filifera*	P1, P6
Spain	Valencia (Valencia)	39°29'2" N; 0°20'27" O	6	urban area, gardens and herbaceous crops	mainly *P. canariensis* and *P. dactylifera*; some *W. filifera*	P2

[a] Elevation (meters above sea level).

[b] Code of the trials carried out at each location (See Tables 2 and 3).

393 Table 2. Details of the trials carried out to test different ferrugineol emission rates

Trial[a]	T mean (ºC)	T max (ºC)	T min (ºC)	RH mean (%)	Start	End	Blocks[b]
P1	22	33.3	9.2	70.7	07-09-12	16-10-12	3
P2	25	36.4	13.9	70.5	26-07-13	20-09-13	4
P3	19.3	29.7	9.3	68	04-04-14	02-06-14	4
P4	22	37.6	12.3	55	07-05-14	02-07-14	4
P5	27.4	40.3	9.6	57.8	14-05-14	09-07-14	4
P6	25.1	36.6	14.9	67	13-06-14	08-08-14	4

394 [a] Code of the trials according to Table 1.

395 [b] Number of blocks arranged.

396

Table 3. Details of the trials carried out to compare ferrugineol co-attractants, including K: a mixture of ethyl acetate and ethanol.

Trial[a]	T mean (ºC)	T max (ºC)	T min (ºC)	RH mean (%)	Start	End	Blocks[b]	Local co-attractant[c]
K1	28.7	42.9	16.4	55	08-05-14	10-07-14	4	*P. dact.* + molasses
K2	27.4	35.1	20.8	58	26-06-14	22-08-14	4	-
K3	27.2	40.2	10.8	64	19-08-14	04-11-14	4	molasses + EtAc
K4	16.3	26.2	6.6	81	17-09-14	26-11-14	4	*P. can.* + molasses
K5	25.1	35	14	56	04-08-14	13-10-14	4	*P. can.* + molasses

[a] Code of the trials according to Table 1.

[b] Number of blocks arranged.

[c] Local co-attractant included in the comparison: (*P. dact.*) *Phoenix dactylifera* stem pieces, (*P. can.*) *Phoenix canariensis* petioles and/or molasses. Water and co-attractants were renewed every 5 weeks, except in Trial K1 (2 weeks).

405 Table 4. Release profiles of the dispensers employed and corresponding mean emission rates of the traps
406 included in each trial

Trial[a]	Dispenser model[b]	no. units	R^{2} [c]	Mean emission (mg day^{-1})
P1	LD-PE vial	1	0.99	2.6
	Pherosan RF	1	0.99	4.2
	Pherosan RF	2	-	8.4
	Pherosan RF	3	-	12.6
P2	mod-RF 1	1	0.99	5.5
	Pherosan RF	1	0.98	11.2
	Pherosan RF	2	-	22.3
	Pherosan RF	4	-	44.6
P3	mod-RF 2	1	0.95	0.6
	Pherosan RF	1	0.99	2.7
	Pherosan RF	2	-	5.4
	Pherosan RF	4	-	10.8
P4	mod-RF 2	1	0.90	3.8
	Pherosan RF	1	0.89	12.7
	Pherosan RF	2	-	25.4
	Pherosan RF	4	-	50.9
P5	mod-RF 2	1	0.95	2.1
	Pherosan RF	1	0.98	8.1
	Pherosan RF	2	-	16.2
	Pherosan RF	4	-	32.4
P6	mod-RF 2	1	0.98	2.6
	Pherosan RF	1	0.99	12.6
	Pherosan RF	2	-	25.2
	Pherosan RF	4	-	50.4
K2	K[d] LD-PE bottle	1	0.99	165.2
	K LD-PE bottle	2	-	330.4
	EtAc LD-PE bottle	1	0.99	623.7
K3[e]	K LD-PE bottle	1	0.95	133
	K LD-PE bottle	2	-	266
	EtAc LD-PE bottle	1	0.99	517
K4	K LD-PE bottle	1	0.99	110
	K LD-PE bottle	2	-	220
	EtAc LD-PE bottle	1	0.99	316.2
K5	K LD-PE bottle	1	0.99	155
	K LD-PE bottle	2	-	310
	EtAc LD-PE bottle	1	0.99	341

407 [a] Code of the trials according to Table 1.

408 [b] Dispenser model: (LD-PE vial) 5-ml vial loaded with ferrugineol; (Pherosan RF) standard commercial
409 ferrugineol dispenser; (mod-RF 1) Pherosan RF modified with an adhesive tape coating; (mod-RF 2)
410 Pherosan RF inserted inside a 12-ml LD-PE vial; (K) 100-ml LD-PE bottle loaded with ethyl
411 acetate/ethanol blend; (EtAc) 100-ml LD-PE bottle loaded with ethyl acetate.

[c] Correlation coefficient of the linear model fitted to weight losses of the unit dispenser with the number of aging days – indicates that the corresponding emission by the number of units is an estimate based on the value for an elementary dispenser.

[d] K is the synthetic co-attractant composed by a 1:3 (ethyl acetate/ethanol) blend.

[e] K dispensers were accidentally loaded with 1:2 (ethyl acetate/ethanol) blend in trial K3.

Table 5. Results of the trials carried out to compare ferrugineol emission rates: Weevil captures and contribution of the explanatory variables evaluated by analyses of variance using generalized linear models.

Trial[a]	Total RPW[b]	Ratio F/M[c]	Trap	Date	Block	trap x date	trap x block	date x block
P1	611	1.4	$F_{3,42} = 7.23$; $P < 0.001$	$F_{4,42} = 14.96$; $P < 0.001$	$F_{2,42} = 21.92$; $P < 0.001$	$P = 0.26$	$P = 0.82$	$F_{8,42} = 2.68$; $P = 0.018$
P2	350	2.4	$F_{3,114} = 0.19$; $P = 0.91$	$F_{7,114} = 1.86$; $P = 0.08$	$F_{3,114} = 12.13$; $P < 0.001$	$P = 0.64$	$P = 0.66$	$P = 0.96$
P3	1600	2.7	$F_{3,109} = 15.69$; $P < 0.001$	$F_{7,109} = 5.55$; $P < 0.001$	$F_{3,109} = 9.96$; $P < 0.001$	$P = 0.12$	$P = 0.71$	$P = 0.29$
P4	354	1.8	$F_{3,114} = 9.52$; $P < 0.001$	$F_{7,114} = 7.56$; $P < 0.001$	$F_{3,114} = 9.75$; $P < 0.001$	$P = 0.65$	$P = 0.73$	$P = 0.84$
P5	292	1.6	$F_{3,85} = 0.75$; $P = 0.53$	$F_{7,85} = 2.23$; $P = 0.035$	$F_{3,85} = 4.91$; $P = 0.003$	$P = 0.28$	$P = 0.11$	$F_{21,85} = 1.71$; $P = 0.045$
P6	285	1.9	$F_{3,112} = 6.78$; $P < 0.001$	$F_{7,112} = 2.52$; $P = 0.02$	$F_{3,112} = 15.45$; $P < 0.001$	$P = 0.83$	$P = 0.14$	$P = 0.52$

[a] Code of the trials according to Table 1.

[b] Total number of weevils captured in each trial (females + males).

[c] Mean ratio females/males (F/M) of weevils captured in each trial.

Table 6. Results of the trials carried out to compare various ferrugineol co-attractants including a mixture of ethyl acetate/ethanol: Weevil captures and contribution of the explanatory variables evaluated by analyses of variance using generalized linear models.

Trial[a]	total RPW[b]	ratio F/M[c]	trap	date	block	trap x date	trap x block	date x block
K1	810	2.1	$F_{4,183} = 15.2$; $P < 0.001$	$F_{9,183} = 3.93$; $P < 0.001$	$F_{3,183} = 2.46$; $P = 0.06$	$P = 0.84$	$P = 0.27$	$P = 0.93$
K2	1795	2.3	$F_{3,114} = 3.04$; $P = 0.03$	$F_{7,114} = 1.06$; $P = 0.39$	$F_{3,114} = 2.60$; $P = 0.05$	$P = 0.62$	$P = 0.48$	$P = 0.98$
K3	2100	1.4	$F_{4,178} = 8.72$; $P < 0.001$	$F_{9,178} = 10.7$; $P < 0.001$	$F_{3,178} = 18.7$; $P < 0.001$	$P = 0.15$	$P = 0.27$	$P = 0.44$
K4	3059	1.8	$F_{4,156} = 8.10$; $P < 0.001$	$F_{9,156} = 44.8$; $P < 0.001$	$F_{3,156} = 19.1$; $P = 0.001$	$P = 0.06$	$P = 0.07$	$F_{27,156} = 2.04$; $P = 0.004$
K5	830	1.6	$F_{4,183} = 10.62$; $P < 0.001$	$F_{9,183} = 3.94$; $P < 0.001$	$F_{3,183} = 15.1$; $P < 0.001$	$P = 0.37$	$P = 0.46$	$P = 0.31$

[a] Code of the trials according to Table 1.

[b] Total number of weevils captured in each trial (females + males).

[c] Mean ratio females/males (F/M) of weevils captured in each trial.

Figure captions

Fig. 1 Locations where the field trials have been conducted along the Mediterranean basin. Description of experimental areas and code of trials according to Table 1.

Fig. 2 Mean (± SE) number of weevils captured per trap and per day in pyramidal Picusan® traps deployed in the trials P1-P6 (see Tables 2 and 5) aimed at evaluating the dose of ferrugineol emitted. For each trial, bars labelled with the same letter are not significantly different (Tukey HSD tests, $P > 0.05$).

Fig. 3 Mean (±SE) residuals from the ANOVA performed with factors date and location using the whole data set of aggregation pheromone trials (P1-P6). Multiple regression analysis performed to correlate the dependent variable residuals with the factor emission fitted the logarithmic model depicted (discontinuous line; $P = 0.013$, $R^2 = 0.51$).

Fig. 4 Mean (± SE) number of weevils captured per trap and per day in pyramidal Picusan® traps deployed in the kairomone trials: K1-K5 (see Tables 3 and 6) aimed at comparing various ferrugineol (ph) co-attractants. All traps contained ph. The trials included no co-attractant (none), only ethyl acetate (EtAc), a local co-attractant (local C; Table 3; absent in K2), and K: a 1:3 mixture of ethyl acetate/ethanol using 1 or 2 dispensers (1K and 2K, respectively). For K3, the K dispensers were accidentally loaded with a 1:2 ratio. For each trial, bars labelled with the same letter are not significantly different (Tukey HSD tests, $P > 0.05$).