



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

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

This is a pre print version of the following article:

Original

Lures for red palm weevil trapping systems: Aggregation pheromone and synthetic kairomone / Vacas, Sandra; Melita, Ourania; Michaelakis, Antonios; Milonas, Panagiotis; Minuz, ROXANA LUISA; Riolo, Paola; Abbass, Mohamed Kamal; Lo Bue, Paolo; Colazza, Stefano; Peri, Ezio; Soroker, Victoria; Livne, Yaara; Primo, Jaime; Navarro Llopis, Vicente. - In: PEST MANAGEMENT SCIENCE. - ISSN 1526-498X. - ELETTRONICO. - 73:1(2017), pp. 223-231. [10.1002/ps.4289]

Availability:

This version is available at: 11566/236167 since: 2022-05-26T18:08:23Z

Publisher:

Published

DOI:10.1002/ps.4289

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

1 **Lures for Red Palm Weevil trapping systems: aggregation pheromone and**
2 **synthetic kairomone**

3

4 Sandra Vacas¹, Ourania Melita², Antonios Michaelakis², Panagiotis Milonas², Roxana Minuz³,
5 Paola Riolo³, Mohamed Kamal Abbass⁴, Paolo Lo Bue⁵, Stefano Colazza⁵, Ezio Peri⁵, Victoria
6 Soroker⁶, Yaara Livne⁷, Jaime Primo¹, Vicente Navarro-Llopis¹

7

8 ¹ Centro de Ecología Química Agrícola - Instituto Agroforestal del Mediterráneo, Universitat Politècnica
9 de València, Valencia (Spain)

10 ² Department of Entomology & Agricultural Zoology, Benaki Phytopathological Institute, Kifissia
11 (Greece)

12 ³ Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche,
13 Ancona (Italy)

14 ⁴ Plant Protection Research Institute, Agricultural Research Center, Giza (Egypt)

15 ⁵ Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Palermo (Italy)

16 ⁶ Agricultural Research Organization, the Volcani Center, Bet-Dagan (Israel)

17 ⁷ Eden Experimental Farm, Emeq Beit Sheaan (Israel)

18

19 Correspondence: Sandra Vacas, Centro de Ecología Química Agrícola - Instituto Agroforestal del
20 Mediterráneo, Universidad Politécnica de Valencia. Camino de Vera s/n, edificio 6C, 5ª planta. 46022,
21 Valencia (Spain). E-mail: sanvagon@ceqa.upv.es; tel: +34 963879058; fax: +34 963879059

22

23 **Running title:** Lures for Red Palm Weevil trapping systems

24

25 **Abstract**

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

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

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

43

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

46

47

48 1 INTRODUCTION

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

78 be controlled because insect response could decrease below and above an optimum value.¹⁰⁻¹⁴ Besides,
79 pheromone cost is a key parameter for the implementation of this kind of control methods; thus, optimum
80 pheromone emission rates should be known to avoid pheromone waste. The pheromone dose-dependent
81 behavior of RPW has been early evaluated by Hallet et al.⁶ in field experiments, in which bucket traps
82 releasing 3 mg day⁻¹ of ferrugineol captured significantly more weevils than traps with emission rate of
83 0.3 and 1 mg day⁻¹.

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

95

96 **2 MATERIALS AND METHODS**

97 **2.1 Traps and dispensers**

98 The new design of pyramidal trap Picusan[®] (Sansan Prodesing SL, Náquera, Valencia, Spain), described
99 in Vacas et al.,⁵ was employed in all the field trials, the base of which was filled with 1.5-2 L water.
100 Aggregation pheromone dispensers employed in our trials only used ferrugineol as aggregation
101 pheromone due to the lack of evidences for pheromonal activity of ferruginone in the literature available⁶
102 and our own experience. The standard commercial aggregation pheromone dispenser employed in all the
103 trials as reference was Pherosan RF (Sansan Prodesing SL, Náquera, Valencia, Spain), which is a
104 polyethylene (PE) vial (18 mm diam. x 35 mm h.) loaded with 1 g of ferrugineol (98% purity, sum of
105 enantiomers). The response of RPW to different ferrugineol emission rates was studied by baiting traps
106 with different types or numbers of pheromone dispensers. The lowest emission rate was provided in 2012
107 by 5-ml NalgeneTM low-density polyethylene vials (LD-PE) (20 mm diam. x 25 mm h.) (Fisher Scientific

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

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

121

122 **2.2 Release profile studies**

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

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

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

145

146 **2.3 Trials**

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

151 *2.3.1 Aggregation pheromone trials*

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

158 *2.3.2 Kairomone trials*

159 Four plots were arranged in each trial (Table 3) to test the synthetic kairomone blend ethyl
160 acetate/ethanol, each of them consisting of 4 or 5 traps with the fifth corresponding to the use of the
161 reference co-attractant employed in the local protocols (palm tissues and/or molasses). All traps were
162 baited with one standard Pherosan RF dispenser, which were assumed to emit ferrugineol at release rates
163 $\geq 4 \text{ mg day}^{-1}$ according to previous studies. In general, each block included a trap baited with: (1) only
164 one Pherosan RF dispenser (ph); (2) ph + 1 dispenser with the synthetic kairomone (ph+1K); (3) ph + 2
165 dispensers with the synthetic kairomone, to have a higher emission (ph+2K); (4) ph + 1 ethyl acetate
166 dispenser (ph+EtAc); (5) ph + reference local co-attractant (the one which is commonly used in each
167 location, as detailed in Table 3). Replacement of water and co-attractants was done every 5 weeks (after

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

170

171 **2.4 Statistical analysis**

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

177 To deal with non-homogeneous variance and data overdispersion, we used generalized linear model
178 (GLM) techniques assuming quasi-Poisson error variance¹⁵ to compare the mean number of WTD
179 captured in each trap. Once each model was fitted, the validity of the assumptions made was evaluated
180 with the *plot(glm.model)* function by checking residuals distribution and the existence of patterns and
181 outliers. For each trial, we constructed different models with the number of WTD as the dependent
182 variable and the emission rate (trap), sampling date, block and their interactions as the explanatory
183 variables (interaction trap x date x block was not significant in all cases). The significance of the
184 explanatory variables was assessed by backward elimination from the model and subsequent comparison
185 of the two models using the F test statistic. When significant effects were found the *glht* function in the
186 *multcomp* package¹⁶ was used to perform Tukey HSD tests for post-hoc pairwise comparisons. All these
187 statistical analyses were conducted with R (R version 3.1.0).¹⁷

188 To draw a general conclusion and study the existence of an optimum ferrugineol emission rate
189 corresponding to a relative maximum of RPW captures, we followed the methodology employed in Vacas
190 et al.¹² Briefly, we applied a two-factor ANOVA (location and sampling date) using the $\log(x+1)$ -
191 transformed captures of the whole data set (trials P1-P6). The residuals of this model still account for the
192 variability of captures caused by ferrugineol emission, as this factor was not included in the ANOVA.
193 Thus, these residuals were saved and used in a subsequent multiple regression analysis to study the
194 existence of a relative maximum by checking the significance of the quadratic effect of the ferrugineol
195 emission in a polynomial model. These analyses were performed using the Statgraphics Centurion XVI
196 16.2 package (StatPoint Technologies Inc., Warrenton, VA, USA).

197

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 $4\text{-}5 \text{ mg day}^{-1}$ 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-

258 Faghih¹⁸ observed that release rates over 5 mg day⁻¹ gave no significant differences in RPW attraction.
259 Results reported herein of the trials conducted in Spain (P1 and P2) showed that pheromone emission
260 rates ranging from 4.2 to 12.6 mg day⁻¹ did not significantly affect both female and male RPW responses.
261 Even high emission rates up to 44.6 mg day⁻¹ did not have any significant effect (negative or positive) on
262 weevil captures.

263 Our results were further supported by field trials conducted in different locations in the Mediterranean
264 basin during spring-summer 2014 (trials P3-P6), thus covering varied environmental conditions. But it is
265 precisely for this reason, wide range of microclimate and landscape conditions, that capture data and
266 emission had noticeable variability among experimental sites. Indeed, the distribution of RPW
267 populations is usually clumped and not homogenous,¹⁹ and it is affected over the year by the availability
268 of hosts and the microclimate conditions of each particular location. Dembilio and Jacas²⁰ found a strong
269 relationship between mean annual temperature and the RPW development, which determines the
270 seasonality and the number of generations to be expected in each geographical area. On the other hand, as
271 can be noted in the tables reported, even using common protocols and dispensers, ferrugineol emission
272 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
273 documented that emission rates of dispensers based on polyethylene membranes increased exponentially
274 with temperature²¹ and local temperatures, even at the level of trap (e.g. different insolation), were
275 affecting and causing this variability. In spite of this, analyzing each trial separately, we found that traps
276 baited with the lowest emission rates (0.60-3.85 mg day⁻¹) trapped in general significantly fewer weevils,
277 and increasing emission rates did not improve efficacy (up to 50.92 mg day⁻¹). In the global analysis with
278 all the available data (trials P1-P6), the multiple regression analysis did not find a significant quadratic
279 effect of ferrugineol emission on captures; as a consequence, we cannot report an optimum ferrugineol
280 emission rate within the studied range corresponding with a maximum level of captures. Instead, the trend
281 fitted a logarithmic model, in which captures increase rapidly with emission rate up to a threshold (4-5
282 mg day⁻¹) and then slow down reaching a plateau. Thus, trap catches are not reduced above an optimum
283 emission rate, as described for the response to sex pheromones in other insect orders, such as
284 Lepidoptera,²² Diptera¹⁴ or Hemiptera.¹¹ Lack of optimum pheromone release rate has already been
285 described for the related species *Rhynchophorus palmarum* L., the South American palm weevil.
286 Oehlschlager et al.⁴ reported that aggregation pheromone emission could range between 0.3 – 200 mg
287 day⁻¹ without significantly affecting *R. palmarum* catches. Actually, antagonistic or saturation effects

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

297 As synergizing component of trapping systems, the next step to improve efficacy and optimize cost is to
298 replace the use of natural kairomones. Our results showed that the 1:3 ethyl acetate/ethanol blend
299 suggested in Vacas et al.⁹ is able to improve trap efficacy and perform significantly better than ferrugineol
300 alone, capturing from 2.2 to 1.4 times more total weevils. However, when the ratio was modified to 1:2
301 (ethyl acetate/ethanol) (trial K3), captures were not significantly improved. Although mean release rates
302 of the blend were similar (Table 4), GC/FID analysis of the remaining content in the dispensers revealed
303 that ethyl acetate/ethanol were emitted in a 2:1 ratio in trial K3 samples, whereas ratio was approximately
304 2:3 in the rest of the samples analyzed from trials K2, K4 and K5 (same as reported in Vacas et al.⁹). This
305 is suggesting the importance of ethanol in the synthetic kairomone blend that should be released even in a
306 higher proportion than ethyl acetate. However, the synthetic blend achieved higher mean captures than
307 ethyl acetate alone but not significantly in all cases, which indicates that blend proportions and dose still
308 need adjustments.

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

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

319

320 **Acknowledgements**

321 The research leading to these results has received funding from the 7th European Union Framework
322 Programme under Grant Agreement FP7 KBBE 2011-5-289566 (PALM PROTECT). Authors want to
323 thank Yaakov Nakashe, Ami Landau for field assistance in the trials conducted in Israel, and Gikarakis
324 Dimitrios and Fokas Vlasias in Greece trials.

325 **References**

- 326 1. Hallett RH, Oehlschlager AC and Borden JH. Pheromone trapping protocols for the Asian palm weevil,
327 *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Int J Pest Manag* **45**:231-237 (1999).
- 328 2. Soroker V, Blumberg D, Haberman A, Hamburguer-Rishard M, Reneh S, Talebaev S, Anshelevich L
329 and Harari AR. Current status of red palm weevil infestation in date palm plantations in Israel.
330 *Phytoparasitica* **33**:97-106 (2005).
- 331 3. Faleiro JR, El-Saad MA and Al-Abbad AH. Pheromone trap density to mass trap *Rhynchophorus*
332 *ferrugineus* (Coleoptera: Curculionidae/Rhynchophoridae/ Dryophthoridae) in date plantations of
333 Saudi Arabia. *Int J Trop Insect Sci* **31**:75-77 (2011).
- 334 4. Oehlschlager AC, Chinchilla CC, González LM, Jirón LF, Mexzon R and Morgan B. Development of a
335 pheromone-based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *J*
336 *Econ Entomol* **86**:1381–1392 (1993).
- 337 5. Vacas S, Primo J and Navarro-Llopis V. Advances in the use of trapping systems for *Rhynchophorus*
338 *ferrugineus* (Coleoptera: Curculionidae): Traps and attractants. *J Econ Entomol* **106**:1739-1746
339 (2013).
- 340 6. Hallett RH, Gries G, Gries R and Borden JH. Aggregation pheromones of two Asian palm weevils,
341 *Rhynchophorus ferrugineus* and *R. vulneratus*. *Naturwissenschaften* **80**:328–331 (1993).
- 342 7. Giblin-Davis RM, Oehlschlager AC, Pérez A, Gries G, Gries R, Weissling TJ, Chinchilla CM, Peña
343 JE, Hallett RH, Pierce HD and González LM. Chemical and behavioral ecology of palm weevils
344 (Curculionidae: Rhynchophorinae). *Fla Entomol* **79**:153–167 (1996).
- 345 8. Guarino S, Lo Bue P, Peri E and Colazza S. Responses of *Rhynchophorus ferrugineus* adults to
346 selected synthetic palm esters: electroantennographic studies and trap catches in an urban
347 environment. *Pest Manag Sci* **67**:77–81 (2011).
- 348 9. Vacas S, Abad-Payá M, Primo J and Navarro-Llopis V. Identification of pheromone synergists for
349 *Rhynchophorus ferrugineus* trapping systems from *Phoenix canariensis* palm volatiles. *J Agr Food*
350 *Chem* **62**:6053-6064 (2014).
- 351 10. Anshelevich L, Kehat M, Dunkelblum E and Greenberg S. Sex pheromone traps for monitoring the
352 European vine moth, *Lobesia botrana*: Effect of dispenser type, pheromone dose, field aging of
353 dispenser, and type of trap on male captures. *Phytoparasitica* **22**:281–290 (1994).

- 354 11. Zhang A and Amalin D. Sex pheromone of the female pink hibiscus mealybug, *Maconellicoccus*
355 *hirsutus* (Green) (Homoptera: Pseudococcidae): Biological activity evaluation. *Environ Entomol*
356 **34**:264–270 (2005).
- 357 12. Vacas S, Alfaro C, Navarro-Llopis V, Zarzo M and Primo J. Study on the optimum pheromone
358 release rate for attraction of *Chilo suppressalis* (Lepidoptera: Pyralidae). *J Econ Entomol* **102**:1094-
359 1100 (2009).
- 360 13. Vacas S, Alfaro C, Zarzo M, Navarro-Llopis V and Primo J. Effect of sex pheromone emission on the
361 attraction of *Lobesia botrana*. *Entomol Exp Appl* **139**:250–257 (2011).
- 362 14. Navarro-Llopis V, Alfaro C, Primo J and Vacas S. Response of two tephritid species, *Bactrocera*
363 *oleae* and *Ceratitis capitata*, to different emission levels of pheromone and parapheromone. *Crop*
364 *Prot* **30**:913-918 (2011).
- 365 15. O’hara RB and Kotze DJ. Do not log-transform count data. *Meth Ecol Evol* **1**:118–122 (2010).
- 366 16. Hothorn T, Bretz F and Westfall P. Simultaneous Inference in General Parametric Models. *Biom J*
367 **50**:346–363 (2008).
- 368 17. R Development Core Team. The R Foundation for Statistical Computing. URL [http://www.R-](http://www.R-project.org)
369 [project.org](http://www.R-project.org) (2014).
- 370 18. Roachat D and Avand-Faghih A. Trapping of red palm weevil (*Rhynchophorus ferrugineus*) in Iran
371 with selective attractants, in *Practice oriented results on use and production of neem ingredients*
372 *and pheromones IV*, ed. by Kleeberg H and Zebitz CPW, Druck & Graphic, Giessen, Germany, pp.
373 219-224 (2000).
- 374 19. Faleiro JR, Kumar JA and Rangnekar PA. Spatial distribution of red palm weevil *Rhynchophorus*
375 *ferrugineus* Oliv.(Coleoptera: Curculionidae) in coconut plantations. *Crop Prot* **21**:171-176 (2002).
- 376 20. Dembilio Ó and Jacas JA. Basic bio-ecological parameters of the invasive Red Palm Weevil,
377 *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in *Phoenix canariensis* under
378 Mediterranean climate. *Bull Entomol Res* **101**:153-163 (2011).
- 379 21. Torr SJ, Hall DR, Phelps RJ and Vale GA. Methods for dispensing odour attractants for tsetse flies
380 (Diptera: Glossinidae). *Bull Entomol Res* **87**:299-311 (1997).
- 381 22. Vacas S, López J, Primo J and Navarro-Llopis V. Response of *Tuta absoluta* (Lepidoptera:
382 Gelechiidae) to Different Pheromone Emission Levels in Greenhouse Tomato Crops. *Environ*
383 *Entomol* **42**:1061-1068 (2013).

384 23. Bartelt RJ, Vetter RS, Carlson DG and Baker TC. Influence of pheromone dose, trap height, and
385 septum age on effectiveness of pheromones for *Carpophilus mutilatus* and *C. hemipterus*
386 (Coleoptera: Nitidulidae) in a California date garden. *J Econ Entomol* **87**:667-675 (1994).
387

388 **Tables**389 **Table 1.** Description of the experimental areas

Country	Location	Coordinates	Elev. (m) ^a	Surrounding area	Host palms available	Trial ^b
Egypt	Ismailia (Ismailia)	30°42'00" N; 31°48'0" E	10	date palms and mango orchards	<i>P. dactylifera</i>	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 <i>P. canariensis</i> ; few <i>P. dactylifera</i> , <i>W. filifera</i> and <i>C. humilis</i>	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 scattered palms in gardens with a variety of ornamental plants	mainly <i>P. canariensis</i> and <i>P. dactylifera</i> ; few <i>W. filifera</i> and <i>S. romanzoffiana</i>	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	<i>P. canariensis</i>	K4
Italy	Palermo (Sicily)	38°06'25" N; 13°21'07" E	43	urban area/ park	<i>P. canariensis</i>	P4, K5
Spain	Manises (Valencia)	39°30'17" N; 0°30'33" O	52	industrial/urban area - palm nursery	mainly <i>P. canariensis</i> and <i>P. dactylifera</i> ; also <i>C. humilis</i> and <i>W. filifera</i>	P1, P6
Spain	Valencia (Valencia)	39°29'2" N; 0°20'27" O	6	urban area, gardens and herbaceous crops	mainly <i>P. canariensis</i> and <i>P. dactylifera</i> ; some <i>W. filifera</i>	P2

390 ^a Elevation (meters above sea level).391 ^b Code of the trials carried out at each location (See Tables 2 and 3).

392

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

Trial ^a	T mean (°C)	T max (°C)	T min (°C)	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 ^aCode of the trials according to Table 1.

395 ^bNumber of blocks arranged.

396

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

Trial ^a	T mean (°C)	T max (°C)	T min (°C)	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	<i>P. dact.</i> + 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	<i>P. can.</i> + molasses
K5	25.1	35	14	56	04-08-14	13-10-14	4	<i>P. can.</i> + molasses

399 ^aCode of the trials according to Table 1.

400 ^bNumber of blocks arranged.

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

404

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 ^{2c}	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.

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

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

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

417

418

419 Table 5. Results of the trials carried out to compare ferrugineol emission rates: Weevil captures and
 420 contribution of the explanatory variables evaluated by analyses of variance using generalized linear
 421 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	$F_{3,112} = 6.78$; $P < 0.001$	$F_{7,112} = 2.52$; $P = 0.02$	$F_{3,112} = 15.45$; $P < 0.001$	$P = 0.83$	$P = 0.14$	$P = 0.52$

422 ^a Code of the trials according to Table 1.

423 ^b Total number of weevils captured in each trial (females + males).

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

425

426 Table 6. Results of the trials carried out to compare various ferrugineol co-attractants including a mixture
 427 of ethyl acetate/ethanol: Weevil captures and contribution of the explanatory variables evaluated by
 428 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	$F_{4,183} = 10.62;$ $P < 0.001$	$F_{9,183} = 3.94;$ $P < 0.001$	$F_{3,183} = 15.1;$ $P < 0.001$	$P = 0.37$	$P = 0.46$	$P = 0.31$

429 ^a Code of the trials according to Table 1.

430 ^b Total number of weevils captured in each trial (females + males).

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

432

433

434 **Figure captions**

435

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

438

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

442

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

447

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

455