# Abstract

Improved trap efficacy is crucial for implementing control methods for red palm weevil, *Rhynchophorus ferrugineus* (Olivier; Coleoptera: Dryophthoridae), based on trapping systems, such as mass trapping, attract and infect or attract and sterilize techniques. Although new trap designs have been proposed and aggregation pheromone dispensers have been optimized, aspects such as the use of co-attractants (molasses) and trap placement are still not well defined and standardized. The efficacy of three concentrations of molasses and different formulations to reduce water evaporation in traps was studied in different field trials to improve trapping systems and to prolong trap servicing periods. In addition, the performance of installing groups of traps or single traps was also evaluated with the aim of improving the attracted/captured weevils ratio. Our results showed that captures increased when molasses were added at 15% to the water contained in the trap and that a thin layer of oil, created by adding 2–3% of paraffinic oil to water, was able to effectively reduce evaporation and prolong trap servicing periods. Moreover, 3.5-fold more weevils were captured when placing five traps instead of one at the same trapping point. Results obtained allow improved efficacy and may have an impact in the economic viability of trapping systems and, therefore, in integrated pest management programs.

Red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae), is an important pest of coconut (*Cocos nucifera* L. [Arecales: Arecaceae]) and date palms (*Phoenix dactylifera* L. [Arecales: Arecaceae]) in south and southeast Asia from where it is native (Dembilio and Jacas 2011). It was first detected in the United Arab Emirates in 1985 (Faleiro et al. 2010), and spread rapidly to all the countries of the Gulf region and Egypt. It was reported for the first time in Europe in 1994 in Spain (Barranco et al. 1996) although it is currently present in all European Mediterranean countries, North Africa, the Caribbean, continental United States and southern China (Giblin-Davis et al. 2013). In date palm growing areas, such as Saudi Arabia or Israel, this pest has a severe economic impact; annual loss owing to the eradication of severely infested palms in Saudi Arabia has been estimated to range from US\$1.74 to 8.69 million at 1–5% infestation, respectively (El-Sabea et al. 2009).

RPW females lay their eggs at the base of the fronds in separate holes made with their rostrum. Neonate larvae bore into the palm meristem and, on completion of development, move back to the base of the fronds to pupate. A new generation emerges, and these adults may remain within the same host or leave to another one. If the palm still contains fresh tissues, many of the newly emerged remain and reproduce until the palm eventually dies. Subsequently, adults will move and look for a new palm host (Dembilio et al. 2010a).

The control methods available for RPW include drench application of neonicotinoid insecticide to the crown of the palm (Llácer et al. 2012), entomopathogenic fungi (Dembilio et al. 2010b) or nematodes (Llácer et al. 2009), injections into the trunk of systemic insecticides (Dembilio et al. 2015) or mass trapping (Soroker et al. 2005, Faleiro et al. 2006, Giblin-Davis et al. 2013). With the currently available traps and lures, mass trapping is not able to protect palms against RPW by itself, but has been considered an essential tool for integrated pest management (IPM) programs. These programs have been implemented to suppress this pest in several date palm plantations of Saudi Arabia and other Middle East countries (Abraham et al. 2000, Vidyasagar et al. 2000, Oehlschlager 2010, Faleiro et al. 2011). The traditional traps employed for mass trapping are bucket traps with lateral and upper holes baited with RPW aggregation pheromone, a 9:1 mixture of ferrugineol ((4S, 5S)-4-methyl-5-nonanol) and ferrugineone (4S-methyl-5-nonanone) (Hallett et al. 1993), and a coattractant based on dates and/or sugar cane molasses and/or ethyl acetate (Soroker et al. 2005). However, it has been recently reported that pyramidal traps can achieve higher captures compared to bucket traps and that addition of ethyl acetate, with no other kairomone cue, does not significantly improve catches compared to traps baited only with ferrugineol (Vacas et al. 2013, 2014). However, trap catches increase when ethyl acetate is added to the traps that contain ferrugineol+molasses or plant tissues (palm stems or fruits). This indicates the importance of other kairomone compounds in addition to ethyl acetate (Vacas et al. 2014, 2017; Abdel-Azim et al. 2017).

Improvements to both trap design and attractant are crucial for trapping systems to succeed, but trap position also determines the system's efficacy in terms of protecting palm specimens. In general, currently used traps do not effectively capture all the attracted insects and the captured/attracted ratio does not usually exceed 50% (Rubio et al. 2011). Those weevils attracted but not caught could potentially infest palms neighboring pheromone-baited traps (Faleiro 2006). Although trap placement protocols based on distancing traps and palms could reduce this issue, it is not always possible, for example inside plantations. Thus, improving the captured/attracted ratio is crucial to avoid the side effects of these trapping systems.

Mass trapping is being used in more than 4,000 ha as part of IPM programs in Saudi Arabi Al-Hassa Oasis (Al-Shawaf et al. 2012), which involves the placement of 8,000 bucket traps baited with 1 liter water, 200 g of dates and ferrugineol. Presence of water in traps increases captures of R. ferrugineus (Vacas et al. 2013) and is, therefore, important to maintain water in traps, to avoid them from completely evaporating. In the Mediterranean region of Spain (Elche, Valencia), more than 3,200 ha have been treated with mass trapping at three different densities, one trap per ha, one trap each 2 ha and one trap each 4 ha, depending on the density of palms and weevil population pressure. Pyramidal Picusan traps (Vacas et al. 2013) were employed in this region and were baited with a ferrugineol dispenser, 2 liters water with 5% molasses and pieces of Phoenix canariensis (hort. ex Chabaud; Arecales: Arecaceae) palm tissues (TRAGSA, personal communication). During the warmer season, these traps should be serviced monthly by replacing molasses/palm tissues and refilling water to avoid traps from drying. In warmer places, trap servicing is even more frequent; e.g., traps are serviced weekly and water is replaced as needed in Saudi Arabia (Al-Shawaf et al. 2012, Hoddle et al. 2013) or every 1-2 wk in Israel (Soroker et al. 2005). Fermenting material, such as molasses, is renewed every 2 mo in Israel or every 6 wk in Saudi Arabia (Soroker et al. 2005, Faleiro et al. 2010). In both cases, increasing the efficacy of traps and baits and prolonging the lifespan of the attractant are key points to ensure this method's economic viability.

The trials reported herein aimed to improve the different parameters involved in the efficacy of RPW trapping systems. One of them is the use of co-attractants. For this purpose, the trapping efficacy of three concentrations of molasses was studied, as was the effect of palm tissues added to traps when molasses were employed. Trap servicing is crucial for implementing mass trapping. Thus, different formulations to reduce water evaporation have also been proposed and tested to prolong servicing periods without reducing attractant power. The influence of sun exposure on the efficacy of trapping has also been evaluated. In addition, as recent studies have demonstrated that increasing the number of pheromone dispensers in the same trap does not increase the number of captures (Vacas et al. 2017), we studied the trapping efficacy of installing groups of traps or single traps to evaluate whether these groups were able to improve the attracted/captured weevils ratio.

## **Materials and Methods**

#### **Traps and Pheromone Dispensers**

Black pyramidal trap Picusan (Sansan Prodesing SL, Náquera, Valencia, Spain), as described in Vacas et al. (2013), was employed in all the field trials, the base of which can contain up to 3 liters water. The standard commercial aggregation pheromone dispenser employed in all cases was Pherosan RF, also supplied by Sansan Prodesing SL (Náquera, Valencia, Spain). This is a plastic vial (18 mm diam. × 35 mm h.) loaded with 1 g of ferrugineol (98% purity, sum of enantiomers),

with an approximate lifespan of 100 d. Release rate of the Pherosan RF dispensers was previously studied and ranged 4.2–12.6 mg of pheromone per day (Vacas et al. 2017).

## Trial 1-Molasses Concentration

The performance of three different concentrations of sugar beet molasses was tested in field. For this purpose, Picusan traps were baited with Pherosan RF dispensers and filled with 2-liter water solutions that contained 0, 5, and 15% sugar beet molasses with 70-75% dry residue (~27% disaccharide, ~15.5% polysaccharide, 34.5% monosaccharide) (Dadmel 55 supplied by Dadelos SL, Valencia, Spain). A fourth thesis was tested which comprised a 5% molasses water solution, plus 8 g of regular baking powder (potassium bitartrate + soda bicarbonate) to reinforce the release of  $CO_2$ . Thus, four blocks of four traps were arranged following a randomized complete block design in the municipality of Elche (Alicante, Spain; coordinates: 38.246270°, -0.693530°), in a 200-ha area with mixed palm (30%), pomegranate (25%), and olive (10%) orchards, as well as other herbaceous crops. Gardens and backyards represented less than 5% of the area, although most of them include isolated ornamental palm trees. Palm species cultivated in the area are P. dactylifera (70%), Washingtonia robusta H. Wendl. (Arecales: Arecaceae) (15%), P. canariensis (10%) and others (5%). Palm tree orchards are usually treated once (in spring) or twice (spring and autumn) per year with chlorpirifos (48%) and/or imidacloprid (20%). Blocks were always installed in palm tree orchards. Traps within each block were separated by 30 m. This distance was considered enough to avoid direct competition between traps meanwhile avoiding great population differences due to the natural clumped distribution of RPW. The distance between blocks was at least 200 m. They were installed on 19 April 2013 and weevil catches were recorded every 14 d for 6 mo until 23 September (12 records). Males and females were distinguished. Traps were emptied and refilled with a new solution and rotated clockwise within each block after counting the number of captured weevils. In this way, each tested solution was placed in the same position three times during the trial.

### Trial 2—Water evaporation

Prior to testing field trapping performance, different formulations were studied to check their potential to reduce trap water evaporation under summer conditions between 30 June and 27 August 2013 (average temperature in July and August of 25 and 25.5°C, respectively). The formulations tested in this preliminary trial included water solutions with: 1) 50% propylene glycol (PG); 2) 1% paraffinic oil (83%, Araoil supplied by Agrofit, Valencia, Spain); 3) 3% paraffinic oil; 4) 6% paraffinic oil; 5) 20% glycerin. Traps were filled with 2 liters of each formulation and weighed at the beginning and then periodically for 2 mo to evaluate weight loss. Weight differences between periods indicate the amount of water loss. The study was performed in triplicate and traps were placed in the Universitat Politècnica de València campus (Valencia, Spain) to be exposed to environmental conditions.

The best formulations obtained by the preliminary study were tested in the field for trapping performance purposes in the municipality of Elche (Alicante, Spain) described in the section 'Trial 1—Molasses concentration'. Thirty-one blocks of four traps were deployed in field to test four formulations: 1) water + 2% paraffinic oil, 2) water + 2% paraffinic oil + 15% molasses, 3) water + 2% paraffinic oil + 15% molasses + three pieces of palm stem tissues (PST) (15 × 15 × 15 cm), 4) water + 3% PG + 15% molasses + three pieces of PST (15 × 15 × 15 cm). Preliminary test indicated that when an oil layer totally covers the surface of the water container, evaporation was dramatically reduced. Thus, 2% of paraffinic oil was enough to reduce water evaporation in this trap

design. The formulation that contained 3% PG was included in the trial as it is a common practice in Spain to reduce trap water evaporation. The traps in each block were separated by at least 30 m and the distance between blocks was at least 200 m. Traps were deployed on 29 July 2014 and visited after 8 wk to count the number of captured males and females and to review trap status, by checking if some liquid remained inside the trap. Each trap's sun exposure was also recorded and traps were classified accordingly as: high insolation (when exposed to midday sun, traps totally unprotected), medium insolation (traps under a tree, protected from midday sun but exposed to the sun during morning or evening), and low insolation (when trap remained in the shade, protected from the sun all day long under several trees or a roof).

## Trial 3-Sun Exposure

As sun exposure influences temperature inside the trap, and consequently water evaporation and performance, we studied this effect during two different periods: a warmer period from July to mid-September (daily average temperature between 20.5 and 31.1°C) and a period with mild temperatures from the last week of September to the last week of November (daily average temperature between 10.9 and 25.4°C). For this trial, 124 Picusan traps were installed during each period in a 124-ha area in Elche (Alicante, Spain), by placing traps in a grid separated by 100 m. Each trap was classified according to the sun exposure grades defined above: 88 traps remained in the shade for most of the day (low), 44 traps were directly insolated at midday (high) and 116 traps were exposed to sun only during morning or evening (medium). Traps were baited with a Pherosan RF dispenser and were filled with water, 15% sugar beet molasses Dadmel 55 (Dadelos SL, Valencia, Spain) and 2% paraffinic oil. Traps were checked only once, 2 mo after being installed. All the captured RPW were counted distinguishing by sex. In order to study trap's internal temperature, one Microlite USB data logger (resolution 0.1°C, accuracy 0.3°C; Fourtec, United States) was placed inside a highly-insolated trap and another one inside a shaded trap. Location of these traps was distanced only 100 meters to avoid microclimate differences.

## Trial 4—Number of Traps per Trapping Point

In order to improve the captured/attracted RPW ratio, we compared the captures obtained when 1, 3, or 5 traps were placed at the same point. The trial was placed in the same area in Elche (Alicante, Spain) and the three different blocks of traps were separated 2 km. In each block we deployed a single trap (one trap per point), one set of three traps separated 1 m in a triangle arrangement (three traps per point) and one set of five traps in a 1-m square arrangement with a trap in the center (five traps per point), each set separated 100 m. Traps were serviced fortnightly during 1 yr, from March 2014 to February 2015 (24 records). Catches were counted by distinguishing between males and females and sets of traps were rotated clockwise (each set of traps was in the same position eight times). All the traps contained a Pherosan RF dispenser and were baited with 2-liter 15% molasses water solution.

An additional trial was carried out during 32 wk in 2016 in the same trial field to compare captures in sets of 1, 3, or 5 traps baited with one pheromone dispenser regard a set of one trap baited with four dispensers. This trial was conducted in order to study the effect of higher pheromone emission in trap catches.

### **Statistical Analysis**

For all the trials, the number of total weevils captured in each trap recorded during each trapping period was divided by the number of days between the dates to calculate the value of weevils per trap and day (WTD). Although more females were caught than males in all trials (68 vs. 32%), no remarkable difference was found in responses by either sex. Consequently, results of the statistical analysis performed with the total number of captured weevils are presented herein.

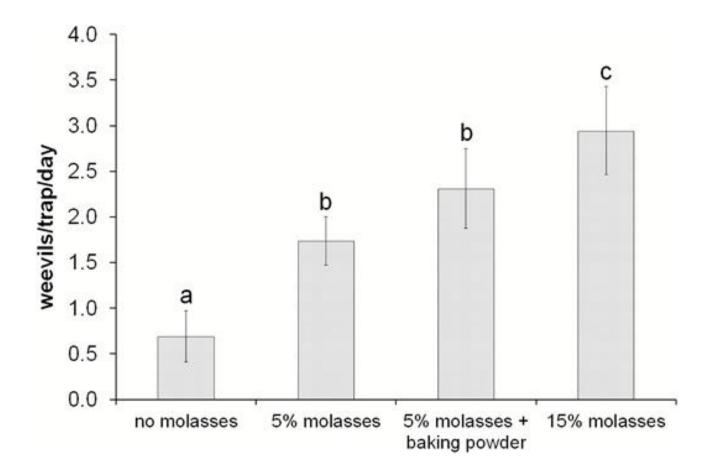
Data according to the factors considered in each trial were analyzed by means of analysis of variance (ANOVA) to compare the mean number of WTD captured in each trap. Data were log(x+1)-transformed to homogenize variance prior to applying the ANOVA, except in trial 4 when data were sqrt(x)-transformed. When significant effects were found, a least significant difference (LSD) test at P < 0.05 was employed for multiple range comparisons. The Statgraphics Centurion XVI package was used to perform all the statistical analysis (Statpoint Technologies Inc., Warrenton, VA).

## Results

## Trial 1-Molasses Concentration

The concentration of molasses employed in the traps had a significant effect on RPW captures (Fig. 1) ( $F_{3,143} = 29.25$ ; P < 0.001). The factor sampling date was also considered and was significant due to natural RPW population dynamics ( $F_{11,143} = 6.27$ ; P < 0.001), whereas the interaction (concentration of molasses × sampling date) was not significant ( $F_{33,143} = 1.41$ ; P = 0.09). Total weevil captures were significantly higher when molasses were employed at 15% (2.94 ± 0.51 RPW per trap and day) compared to the 5% concentration (1.74 ± 0.24 RPW per trap and day) or water without molasses (0.69 ± 0.15 RPW per trap and day). Adding baking powder to a 5% molasses water solution increased average captures compared to using only 5% molasses, but not significantly (Fig. 1).

Fig. 1.



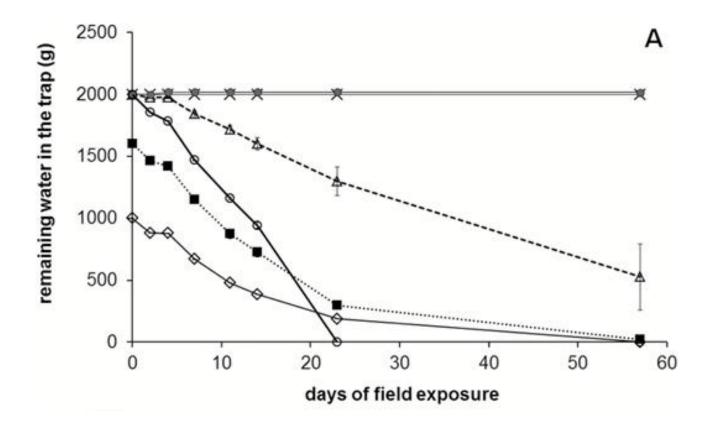
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Mean ( $\pm$ SE) weevils captured per trap and day in traps baited with aggregation pheromone and different concentrations of sugar beet molasses. Bars labeled with different letters are significantly different (multiple range test, LSD intervals at *P* < 0.05).

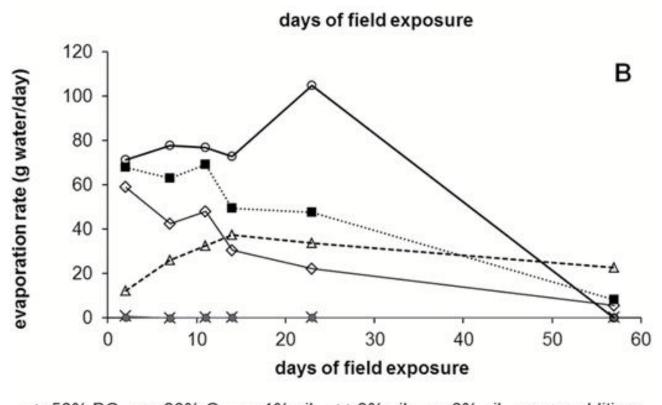


The preliminary study showed that all the traps that did not contain paraffinic oil were dry 23 d after exposure to environmental conditions (Fig. 2A). Although 50% PG and 20% glycerin lowered the evaporation rate (Fig. 2B), the quantity of water that remained in the trap after 1 mo was below 15% of the initial quantity of water in both formulations. Only 2% water-loss occurred after 2 mo when 3 or 6% paraffinic oil was added to the water in the traps. However, 1% oil was not enough to reduce water evaporation and 75% of water was lost after 2 mo of field exposure (Fig. 2A).

Fig. 2.

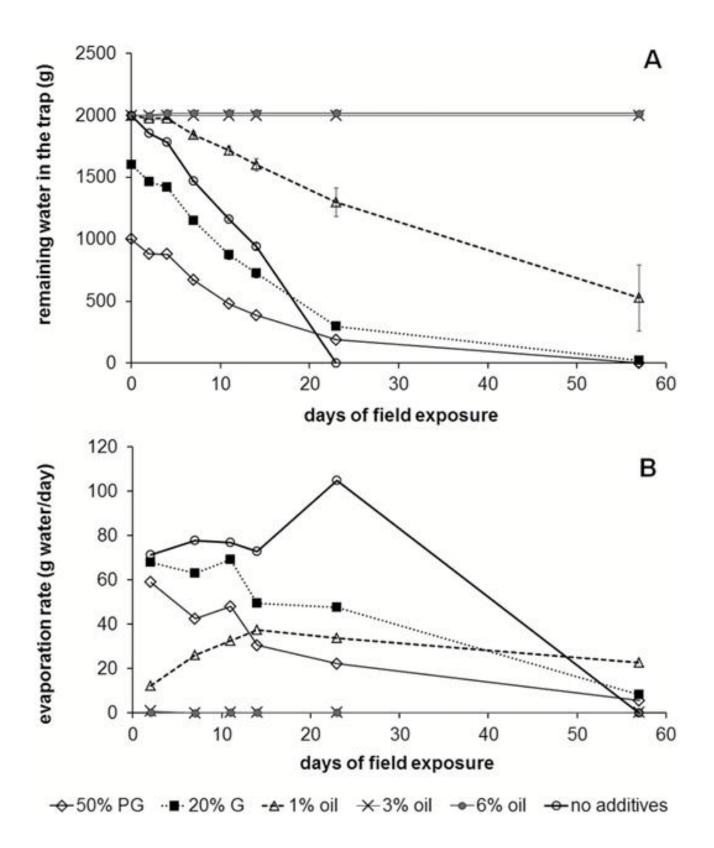


- + 50% PG ... = 20% G - A- 1% oil - + 3% oil - - 6% oil - + no additives



→ 50% PG ···· 20% G · A 1% oil · 3% oil · 6% oil · no additives <u>View largeDownload slide</u>

(A) Remaining amount of water (g) in traps exposed to environmental conditions using different formulations to reduce evaporation: 50% PG, 20% glycerin, 1, 3, and 6% paraffinic oil as additives.(B) Water evaporation profile in traps exposed to environmental conditions using different formulations to reduce evaporation: 50% PG, 20% glycerin, 1, 3, and 6% paraffinic oil as additives.



The results of the field trapping trial testing formulations to reduce water evaporation in traps with different sun exposures (Table 1) revealed that the formulation had a significant effect ( $F_{4,140} = 10.18$ ; P < 0.001) and that the sun exposure was only marginally significant ( $F_{2,140} = 2.62$ ; P = 0.056), but their interaction was not significant ( $F_{8,140} = 0.58$ ; P = 0.79). Therefore, high-insolated traps captured significantly fewer weevils than the traps placed in the shade, regardless of the formulation type contained in the trap, 0.48 versus 0.68 RPW per trap and day, respectively (in the multiple range test with LSD intervals at the 95% confidence level).

#### Table 1.

Total RPW captures ( $\pm$ SE) recorded in traps baited with different formulations to reduce water evaporation in field trapping conditions

#	T :	N	Weevils/trap/day (mean ± SE) <sup>b</sup>				Water
#	Liquid composition <sup>a</sup>		Global	High	Medium	Low	loss
1	Water + 2% oil	31	0.20 ± 0.03a	0.14 ± 0.06a	0.21 ± 0.03a	0.36 ± 0.16a	31 %
2			0.83 ± 0.08c	0.82 ± 0.07c	0.78 ± 0.08b	0.95 ± 0.15b	34 %
5	+ PS1		0.49 ± 0.06b	0.30 ± 0.08a	0.50 ± 0.06ab	0.75 ± 0.14ab	96 %
4	+ PS1		0.65 ± 0.09b	0.57 ± 0.08b	0.71 ± 0.09b	0.60 ± 0.16ab	77 %
5	Water + 2% oil + K	31	0.57 ± 0.06b	0.55 ± 0.07b	0.56 ± 0.06b	0.69 ± 0.18ab	36 %

<sup>*a*</sup>Oil: paraffinic oil; molasses: Dadmel 55 sugar beet molasses; PG: Propylene glycol; PST: Palm stem tissue (*Phoenix canariensis*); K: dispenser with 40 ml of the synthetic co-attractant composed by 1:3 ethyl acetate/ethanol.

<sup>*b*</sup>Mean (±SE) number of weevils captured per trap and day in all the traps (global) and in traps with high, medium, and low insolation separately. For each sun exposure level and global data, means with different letter were significantly different in ANOVA-LSD test at P < 0.05.

<sup>c</sup>Mean percentage of water loss at the end of the trial (8 wk).

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Regarding liquid composition, the traps baited with the mixture #2 (water + 2% oil + 15% molasses) obtained significantly more catches than the rest of mixtures, and all the formulations that

contained co-attractant (#2–5) were significantly more attractive than those without molasses in the LSD test (Table 1). Specifically, the traps with a dispenser of synthetic kairomone (#5) captured significantly more weevils than those without co-attractant (#1), but do not reach the level of catches obtained with 15% molasses (#2; Table 1).

After 53 d of field exposure, water had almost completely dried in the traps without oil (#3), while the formulations that contained paraffinic oil were able to retain water. The effect of oil became even more evident when only the sun-exposed traps were considered (Table 1). The trap that contained PG instead of oil captured almost half of the weevils obtained in the traps with 2% oil, both with PST (#3 and 4), when exposed to high insolation ( $F_{1,17} = 5.07$ ; P = 0.038). However, no significant differences were obtained between these baits when traps were placed in the shade ( $F_{1,10}$ = 0.43; P = 0.526) or under medium insolation ( $F_{1,29} = 0.34$ ; P = 0.214). It must be highlighted that the addition of PST to the traps with the formulation (water + 2% oil + 15% molasses) promoted water evaporation (77 vs. 34% water loss, with and without PST, #4 and #2 respectively) and, consequently, yielding significantly reduced global RPW catches (0.65 vs. 0.83 weevils per trap per day, with and without PST, #4 and #2 respectively). Given that traps were visited for weevil counting 8 wk after their deployment, the higher water evaporation in traps with PST probably produced a premature loss of trapping efficacy, whereas traps without PST maintained efficacy even after this period.

### Trial 3—Sun Exposure

Sun exposure grades had a significant effect on weevil captures (Table 2). The traps totally exposed to the sun (high insolation) captured significantly fewer weevils than those under low insolation when summer and autumn captures (global) were analyzed together ( $F_{2,241} = 5.32$ ; P = 0.005). The factor season was also considered in the ANOVA and had a significant effect ( $F_{1,241} = 42.60$ ; P < 0.001) and we decided to analyze data separately for each season. The interaction (sun exposure × season) was also considered but was not significant ( $F_{2,241} = 0.32$ ; P = 0.72).

#### Table 2. Mean (±SE) weevils captured per trap and day depending on sun exposure of the trap

Sup ovposition	Season <sup>b</sup>			
Sun exposure <sup>a</sup>	Summer	Autumn	Global	
High	$0.48 \pm 0.05a$	$0.27 \pm 0.04a$	$0.42 \pm 0.04a$	

Medium	$0.56 \pm 0.04$ ab	$0.39 \pm 0.03b$	$0.52 \pm 0.03$ ab
Low	$0.68 \pm 0.07$ b	$0.46 \pm 0.05b$	$0.61 \pm 0.05b$

<sup>a</sup>Sun exposure levels.

<sup>*b*</sup>Results considering data from summer season (July–mid-September), autumn season (end-September to end-November) or global data. For each season and global data, values labeled with different letters are significantly different in ANOVA-LSD test at P < 0.05.

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When analyzing data separately, the effect of sun exposure on captures was similar during the two study periods (Table 2). In summer, although marginally significant (factor sun exposure:  $F_{2,112} = 2.01$ ; P = 0.14), the traps at low insolation captured significantly more weevils than those at high insolation, with nonsignificant differences noted when traps were submitted to intermediate sun exposure (Table 2). The factor block was significant ( $F_{3,112} = 4.52$ ; P = 0.005), probably due to natural RPW population dynamics and clumped distributions, whereas the interaction (sun exposure × block) was not significant ( $F_{6,112} = 0.80$ ; P = 0.57). In autumn, significant differences were once again observed with lowest captures in the most exposed traps compared to shaded and intermediate insolation (factor sun exposure:  $F_{2,111} = 3.72$ ; P = 0.027; factor block:  $F_{3,111} = 1.25$ ; P = 0.30; interaction:  $F_{6,111} = 0.66$ ; P = 0.68). On the whole, the traps located in the shade throughout the trial (from July to end of November) captured 50% more weevils than those placed in the most exposed positions. The high temperatures inside sunny traps might explain this effect, so the temperature inside the trap was measured (Table 3). The most evident effect of insolation was observed on the maximum temperature in summer in sunny traps, which peaked at 60.8 for 1 h, 17°C more than traps in the shade.

+				
+				

Table 3.

Temperatures recorded inside traps with different sun exposure levels during summer and autumn

C	Position	Temperature (°C)			
Season		Min	Max	Average	
Summer	Sunny	17.7	60.8	33.0	
	Shaded	18.4	43.6	29.9	
A	Sunny	1.0	45.8	21.0	
Autumn	Shaded	1.8	40.7	20.1	

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## Trial 4—Number of Traps

The total weevils captured per set of traps and day increased with the number of traps located in a same trapping point (trap set factor:  $F_{2,188} = 43.44$ ; P < 0.001; date factor:  $F_{23,188} = 7.28$ ; P < 0.001; block factor:  $F_{2,188} = 48.69$ ; P < 0.001) (Table 4). This suggested that not all the weevils attracted to a location were effectively captured in a single trap. When took into account the number of traps per point, the ratio captures per day and per trap was significantly different from placing sets of one or set of three or five traps (trap set factor:  $F_{2,188} = 21.14$ ; P < 0.001; date factor:  $F_{23,188} = 10.21$ ; P < 0.001). The interaction (trap set × date) was significant ( $F_{46,92} = 1.67$ ; P = 0.019), which indicates that the trapping efficacy of the different trap sets was affected by the natural population dynamics. Interaction plot showed that captures in sets of 1, 3, or 5 traps were not significantly different when weevil population was very low (January–February) ( $F_{2,28} = 1.21$ ; P = 0.167); however, significant differences were found in the rest of the trial period and this could explain the interaction significance.

#### Table 4.

Mean RPW captures ( $\pm$ SE) recorded when different numbers of traps are employed at the same trapping point

# traps per point	Weevils/point/day	Weevils/trap/day
1	$0.96 \pm 0.09a$	$0.96 \pm 0.09a$
3	$1.70 \pm 0.18b$	$0.57 \pm 0.06b$
5	$2.49 \pm 0.27c$	$0.50 \pm 0.05b$

Means with different letter in the same column were significantly different in ANOVA-LSD test at P < 0.05. Untransformed data are presented.

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Moreover, captures did not significantly increased in traps baited with four dispensers (0.65  $\pm$  0.17) regard traps baited with only one dispenser (0.54  $\pm$  0.12) in ANOVA test ( $F_{3,60} = 1.69$ ; P = 0.179), which clearly indicates that the higher captures in the set of three or five traps was not due to the larger number of attractants at the same point.

## Discussion

Improved trap efficacy is crucial for implementing mass trapping, attract and infect or attract and sterilize techniques. Although aggregation pheromone dispensers have been optimized and the

optimum release rate has been recently reported (Vacas et al. 2017), the use of co-attractants is essential for increasing captures (Giblin-Davis et al. 1996a). Considerable efforts have been made to find synthetic kairomones (Vacas et al. 2014, Guarino et al. 2011) and Vacas et al. (2017) suggested that a standardized mixture of ethanol and ethyl acetate can replace the use of 5% molasses added to water as co-attractant, which was the common practice in Spain, following experiences carried out in United Arab Emirates (TRAGSA, personal communication). The results reported herein suggest that adding 5% molasses to water, in Picusan traps baited with ferrugineol, increased by 2.5-fold the number of weevils captured regard traps without molasses. However, captures increased even more when molasses were added at 15%, obtaining 69% more captures than by using the 5% concentration. Later on, traps baited with pheromone + water + 2% oil + 15% molasses captured significantly more weevils than those including the synthetic kairomone (K) instead of molasses (0.83 vs. 0.57 weevils per trap per day). Thus, the synthetic kairomone previously reported (Vacas et al. 2017) still needs improvement. The effect of molasses concentration can be associated with the volatiles produced during sugar fermentation, which takes place inside traps. Short-chain alcohols and esters have been described as kairomones for RPW (Zada et al. 2002, Guarino et al. 2011, Vacas et al. 2014). Some of these compounds are produced during sugar fermentation and it is intuitively obvious that their release increase with the quantity of sugars provided. However, it must be taken into account that sugar concentrations over 15-20% can reduce yeast growth (Gray 1945). Therefore, we cannot expect a higher production of fermentation products with concentrations of molasses over this level. Our results demonstrated that adding molasses to the water in pheromone-baited traps significantly increased captures and this increment was even higher if ethyl acetate was also provided. Thus, while improving synthetic kairomones, the use of molasses still appears as an effective co-attractant for R. ferrugineus, which can be standardized based on composition parameters, such as dry residue or saccharide content.

Another product released during fermentation is  $CO_2$ , which is known to play a role in the foraging and oviposition behavior of hematophagous and phytophagous insects (Guerenstein and Hildebrand 2008). In our trial, we tested the effect on trap captures of adding sodium bicarbonate (baking powder) to increase the release of this gas. Although the emission rate was not controlled, given that pKa of sodium bicarbonate is 8.2 and, in this case, the pH of water was 7.9, we expected a slow decomposition in  $CO_2$  and a sodium salt. However, results were not conclusive, captures increased regarding using 5% molasses alone but not significantly. Probably,  $CO_2$  emission rate and its effect was shorter than expected. Thus, further studies are needed to evaluate more precisely the effect of promoting  $CO_2$  emission on RPW trap captures.

The most serious drawback for trapping systems is that frequent trap servicing is necessary to maintain attractant power. Indeed, it is the highest cost of mass trapping in the region of Valencia (Spain), and even represents over 50% of the total cost of the technique, including traps and attractants. For this reason, it is extremely important to reduce servicing costs and to prolong the lifespan of attractants. It has been demonstrated that using water is essential for increasing trap efficacy (Vacas et al. 2013) and, therefore, controlling water evaporation is necessary to reduce trap servicing. For this purpose, we proposed several formulations that included addition of paraffinic oil, PG or glycerin, but only a thin layer of oil, created by adding 2-3% of oil to water, was able to effectively reduce water evaporation. The main problem of reducing evaporation is to reduce the level of weevil attraction or its efficacy in retaining them. When using molasses, the addition of substances to reduce water evaporation might be also affecting their fermentation process. Furthermore, water loss means increasing concentration of the substance employed to reduce evaporation, which might also affect trap attractiveness. This phenomenon has not been precisely evaluated in the present work but, in the case of adding 2% oil to (water + 15% molasses), those traps yielded the highest trapping efficacy, suggesting that fermentation process is not significantly affected during the studied period (8 wk), although this would need to be checked in comparison with traps baited with (water + 15% molasses) without oil. In the reported conditions, evaporation

rate was reduced with this formulation to values that allow trap servicing every 3 mo under Mediterranean climate. Therefore, these results indicated that only slight evaporation is needed to attract and capture weevils, and that the water consumed can be reduced, which implies good savings in trap servicing costs.

Also related to water evaporation and trap servicing, a study on the best location of traps was included in the present work. It was found that, generally, traps exposed to sun radiation at midday caught significantly fewer weevils than shaded traps. RPW preferably flies when temperatures are moderate and relative humidity is at its highest (Faleiro 2006). This preference for mild temperatures could explain why weevils were caught in the traps with lower inner mean temperatures. It has been described that temperature inside traps covered with aluminum foil is at least 6°C lower than in insolated traps (Nakamura et al. 1999). By directly measuring the temperatures, but these differences can increase up to 17°C in the maximum insolation hours between sunny and shaded traps, with a temperature peak of over 60°C inside traps. This high summer temperature might explain the fewer captures in the sunny traps. In autumn, when temperatures in the shaded traps were only 5°C below those in the sunny ones, the same reduction in captures was observed. Consequently, whenever possible, avoiding installing traps in the most sun-exposed positions is recommended for better trap efficacy and to prolong servicing periods.

It is well known that most trap designs are not able to capture all the insects attracted to their vicinity because some of them are able to escape and others just finally do not go into the trap. This phenomenon has been widely demonstrated for fruit flies. In studies reported by Aluja et al. (1989), only 31% of the Anastrepha individuals that landed on the exterior of the trap were finally caught, whereas Perea-Castellanos et al. (2015) reported that 2-30% of Mexican fruit flies (A. ludens (Loew; Diptera: Tephritidae)) that entered the trap managed to escape. Likewise, escape ratios ranging 2-43% were also observed for coleopterans, such as sweetpotato weevil, Cylas formicarius (Fabricius; Coleoptera: Brentidae), when comparing different trap designs (Jansson et al. 1992) or different trap efficacies regarding their area for landing and crawling to capture West Indian sugarcane weevil, Metamasius hemipterus sericeus (Olivier; Coleoptera: Curculionidae) (Giblin-Davis et al. 1996b). The same effects were observed for Picusan traps by Rubio et al. (2011), reporting weevil trapped/attracted ratios even below 50% in this kind of traps. In this regarding, placing a single trap near a palm tree to prevent isolated plants to be attacked by RPW is not generally recommended because those weevils attracted, but not effectively captured in the trap, will probably infest the palm tree. Increased infestations in palms near weevil traps has been observed and reviewed by several authors (Hunsberger et al. 2000, Faleiro 2006, Abdel-Azim et al. 2017). The presence of isolated palm trees, as ornamental plants, is very common in Spain and, thus, using traps to protect these palms is highly controversial. However, this strategy could be applied if we could effectively catch all the RPW attracted to traps. For this reason, we tested if one trap with four pheromone dispensers or more than one trap at the same trapping point was able to attract and capture more RPW than single traps. The results showed that the traps baited with multiple ferrugineol dispensers did not capture more weevils than those with only one dispenser. However, 3.5-fold more weevils were captured when placing five traps instead of one at the same trapping point. Therefore, our results suggested that isolated single traps caught 30% of attracted insects at the most and by installing several traps in a same trapping point we are increasing the probability of capturing the attracted insects. In commercial date palm plantations, e.g., this effect could be milder given that many traps can be evenly distributed over a large area. In ornamental isolated palms, however, we could consider installing several traps per point to improve the captured weevils ratio and to help reduce palm infestations. In line with this, it is important to calculate the cost of installing and serving traps together or separately. A density of two traps per ha increase total RPW captures by threefold to fourfold compared to a density of 0.5 traps per ha (Vidyasagar et al. 2016), when traps are distributed homogenously in the plot. However, our

experiment indicated that almost the same increase of captures was obtained when several traps were placed together. Although the cost of the traps and lures is the same whether they are installed homogenously distributed or clumped, the maintenance cost is totally different. Servicing four or five traps together could lead to major savings in labor costs and transportation. In this regarding, the daily cost for a person and transportation is  $203.53 \in /d$ . We have observed that a single person can service 38 traps per day if they are deployed in a 100-m grid. However, when deployed in a 173-m grid (three traps per point), a single person serviced 74.12 traps per day, whereas 87.5 traps per day can be serviced in a grid of 223 m (five traps per point). Therefore, the cost of maintenance can be reduced 49% when traps are placed in groups of three traps, and 57% when placed in groups of five. On the other hand, considering that the number of captured weevils per trap is higher when using single traps, we have calculated the cost of capturing one weevil with the three deployment strategies. By means of this calculation, placing three or five traps per point means a saving of 9 and 12% respectively. In the case of using paraffinic oil to prolong servicing periods (mainly for water supply), we have demonstrated that traps can be serviced every 3 mo and not 1.5 mo in the warmer season, which means a reduction from four to two visits for servicing in summer, and doing the same number of maintenance visits during winter. Then, total servicing visits per year can be reduced from six to four, a 33% saving, from 42.85 to 28.57 € per trap and year. Meanwhile, the cost of paraffinic oil is limited to  $1.71 \in$  per liter (only  $0.07 \in$  per trap) and the cost of molasses is 0.53 cts per liter. Therefore, the cost of increasing from 5 to 15% molasses concentration only means increasing cost from 2.6 to 7.9 cts per trap.

As a concluding remark, the efficacy of mass trapping or monitoring techniques improve by using the suitable composition of attractants and avoiding traps from high sun exposure, and using more than one trap at the same point when protecting single palms. Savings in servicing costs obtained by reducing evaporation may result in the economic viability of mass trapping when the main cost for implementation is manual labor.

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