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# Study of chromatic attraction of the red palm weevil, *Rhynchophorus ferrugineus* using bucket traps

**Juan Antonio ÁVALOS, Antonia SOTO**
*Instituto Agroforestal Mediterráneo (IAM), Universitat Politècnica de València, Spain*


## Abstract

*Rhynchophorus ferrugineus* (Olivier) (Coleoptera Dryophthoridae) is at present considered the biggest threat to palm trees worldwide. Due to the current trend towards the use of environmentally-friendly control measures, increasing interest is being shown in trapping as a way of dealing with this pest. The present study assessed the influence of chromatic attraction on the capture of *R. ferrugineus* adults and identified possible reasons for the better performance of pheromone traps. Two tests were carried out using coloured bucket traps; in the first the traps were baited with male aggregation pheromone and kairomone from *R. ferrugineus,* while the second involved traps without olfactory attractants. The spectral reflectance of the colours tested and of leaves and external trunk fibres from the *Phoenix canariensis* (Hort. ex Chabaud) were measured by spectrophotometer. The internal climatic conditions of the coloured traps were also analysed. Black traps, both with and without olfactory attractants, captured significantly more *R. ferrugineus* adults than red and white traps. The higher efficacy of the black traps was mainly due to chromatic attraction and not only to the possibly higher emission of olfactory attractants. The olfactory attractants used to bait traps are responsible for the female-biased captures, a phenomenon found to occur with all the colours tested. This study provides useful information for improving current management strategies against *R. ferrugineus*.


**Key words:** *Rhynchophorus ferrugineus*, palm tree, chromatic attraction, pheromone trapping, spectrophotometer, sex ratio.

## Introduction

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Dryophthoridae) is one of the most invasive and destructive palm tree (Arecaceae) pests worldwide (Faleiro, 2006). The larval stages feed inside the trunk and frequently destroy the apical growth area, causing the death of the palm (Murphy and Briscoe, 1999; EPPO/OEPP, 2008). Infestations are usually detected after the palm tree has been seriously damaged (Blumberg, 2008), and only well-trained technicians can detect early symptomatology, so that the numbers of plants lost and the consequent financial impact is considerable. In the Valencia Region in Eastern Spain, around 20,000 palms were killed by *R. ferrugineus* between 2004 and 2009, at a cost of approximately €27 million (Dembilio and Jacas, 2010). The pest has a broad range of hosts and is able to breed in a wide variety of climatic conditions (Murphy and Briscoe, 1999), thus increasing its invasive ability and potential for damage.

*R. ferrugineus* is native to southern Asia and Melanesia, where it is a key pest of the coconut, *Cocos nucifera* (L.) (Arecaceae), and oil palm, *Elaeis guineensis* (Jacquin) (Arecaceae) (Wattanapongsiri, 1966; Misra, 1998). During the first decade of the 21$^{st}$ century, it spread all over the Mediterranean Basin, also being detected in Australia (EPPO/OEPP, 2008; 2013). The pest was recorded for the first time in some countries of Northeast Asia such as China in 1999, and Japan in 2000 (Li *et al.*, 2000; Yoshitake *et al.*, 2001). The massive shipments of infested palm trees to countries previously free of the pest contributed to its rapid spread (Abraham *et al.*, 1998). It was first detected in the American continent in 2009, on the island of Curaçao, in the Caribbean (EPPO/OEPP, 2009).

Different methods are currently being used to control *R. ferrugineus*. Preventive and curative applications of chemical pesticides (Faleiro, 2006). An extended alternative to the use of chemical insecticides involves the application of entomopathogenic nematodes, principally *Steinernema carpocapsae* (Weiser) (Nematoda Steinernematidae) (Abbas *et al.*, 2001; Llácer *et al.*, 2009). Other natural enemies of this insect have been identified but have poor efficacy against *R. ferrugineus* (Mazza *et al.*, 2014). Cultural methods are also used, such as the early detection and destruction of infested plant material (EPPO/OEPP, 2008) and the removal of the damaged parts of the palm (known as vegetal surgery) (La Mantia *et al.*, 2008). Finally, a pheromone/food-based trapping system is also used for monitoring and mass trapping of the pest (Hallett *et al.*, 1999; Abbas *et al.*, 2006). In the European Union, pest management in urban green areas is dictated by the guidelines on pesticides issued in 2009, which restricts the use of chemical plant protection products (European Parliament and Council, 2009). Therefore, mass trapping constitutes an environmentally-friendly method of integrated management against *R. ferrugineus*, based on the use of different semiochemicals designed to attract and capture the weevils (El-Sayed *et al.*, 2006). Moreover, several studies carried out in different regions of Saudi Arabia show that pheromone traps, used as a part of an integrated pest management program, reduced infestation levels and captures rates of the pest (Abraham *et al.*, 2000; Vidyasagar *et al.*, 2000).

The colour of the trap is a key factor in attracting and trapping many insect species (Cross, 1973; Prokopy and Owens, 1983). For example, the New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval) (Coleoptera Curculionidae) is attracted to russet-brown colours in field conditions (Reddy *et al.*, 2011). The Apple blossom weevil, *Anthonomus pomorum* (L.) (Coleoptera Curculionidae) is attracted to blue (Hausmann *et al.*, 2004), while the sweet potato weevil *Cylas formicarius* (F.)

(Coleoptera Brentidae) is attracted to light red (Reddy *et al.*, 2012).

Many studies have examined the trap colour preferences of *R. ferrugineus,* but the results have been discordant (Hallett *et al.*, 1999; Abdallah and Al-Khatri, 2005; Faleiro, 2005; Kalleshwarswamy *et al.*, 2006; Martínez *et al.*, 2008; Al-Saoud, 2010; Al-Saoud *et al.*, 2010; Tapia *et al.*, 2010), although others identified black as the insects' favourite colour (Abuagla and Al-Deeb, 2012; Al-Saoud, 2013; Vacas *et al*., 2013; Abdel-Azim *et al*., 2014). Although the aforementioned studies analysed weevil chromatic attraction, there is a lack of knowledge about the effect of the colour as the main factor in their captures, and the possible reasons for this colour preference.

The aim of the present study was to analyse the importance of the chromatic factor in captures of *R. ferrugineus* adults. In order to confirm the effect of chromatic attraction on the number of captures, tests were carried out on the attractiveness of traps baited with and without olfactory lures. The efficacy of coloured traps were also assessed in relation to internal trap climatic conditions (temperature and relative humidity), and the spectral reflectance of the leaves and external fibres of the trunk of the Canary Island date palm, *Phoenix canariensis* (Hort. ex Chabaud) (Arecaceae). The sex ratio of the natural populations of this weevil was analysed and a check was made to determine whether trap colour had any influence on the female/male ratio of captured *R. ferrugineus* adults. The results obtained provide information that should be helpful in improving the control of *R. ferrugineus* by mass trapping systems.

## Materials and methods

### Trap characteristics

In both tests the traps consisted of: (1) a 15.6 litre plastic bucket, with four 6 × 4 cm holes cut below the upper rim, at a distance of 21 cm from the base; (2) a funnel, placed inside the bucket to prevent the weevils escaping; (3) a lid for the bucket, with four holes similar to those in the bucket, with a piece of steel wire to hang the chemical attractants on (pheromone and kairomone dispensers); and (4) a plastic mesh on the outside of the bucket to help the insects climb into the trap. All external surfaces were painted in a certain colour of commercial acrylic spray paints (Tkrom Spray®, Eupinca Inc., Murcia, Spain). The traps were placed on the ground, as according to Oehlschlager (2006), in this position, the insect have a better landing surface, and consequently the traps capture more weevils.

### Test with olfactory attractants

The traps baited with olfactory attractants were tested in eight 7-year-old palm tree groves highly infested by *R. ferrugineus*, near the town of Torrente in eastern Spain (39°25'37.6"N 00°27'28.9"W; 17 m elevation) from beginning May to end of June 2009. The traps were coloured either red (Red fire gloss, RAL 3000), white (White gloss, RAL 9010) or black (Black gloss, RAL 9005). They contained: (1) a *R. ferrugineus* male aggregation pheromone dispenser (Ferrolure+) (Rhynchonex® Pheromone, Econex Ltd, Murcia, Spain) composed of 4-methyl-5-nonanol and 4-methyl-5-nonanone (9:1), containing 700 mg of 95% pure active ingredients; (2) a kairomone dispenser (Rhynchonex® Kairomone, Econex Ltd, Murcia, Spain), containing 40 ml of 95% pure ethyl acetate (El-Sebay, 2003); (3) a piece of infested *P. canariensis* petiole (sized 8 × 5 cm); and (4) four litres of water containing propylene glycol (5-10 ml/l). The pheromone and kairomone dispensers had a usable life of 3 months (Econex, 2013a; 2013b). The experimental design consisted of a total number of 24 traps, distributed in eight replications with three treatments per replication (one of each colour under study). Within each replication, the distance between treatments was 1.2 m. The short distance between treatments, together with the high infestation levels in the study area, cause a high rate of olfactory compounds concentrated around traps, so that the trap colour was the main attractant factor. The traps were inspected weekly during the experimental period, carrying out eight countings in which the number and sex of captured adult weevils were registered. Trap position within a repetition was rotated clockwise after each count, to minimize the influence of trap position on the results.

A data logger (Delta® OHM, HD226-1, Padova, Italy) was placed inside the black and white traps, without affecting the insect entrance. Internal temperature and relative humidity (RH) were registered every 30 min over five consecutive days, to compare the internal climatic conditions in the aforementioned traps, as these are the colours with the maximum and minimum light absorption.

### Test without olfactory attractants

This test was carried out in semi-field conditions without olfactory attractants at the Universitat Politècnica de València (Spain), from January to March 2010. The goal was to determine whether the insect captures under field conditions in the previous trial were due to chromatic attraction or to a factor related to the release of olfactory attractants. This trial was conducted inside a secure enclosure (2 × 6 × 2 m), with double entry door and constructed of iron wire mesh with a small hole size (0.5 × 0.5 cm), to prevent the adult weevils from escaping. The coloured traps were tested under semi-field conditions, according to the results obtained from the test with olfactory attractants. First we compared the black and red traps, which had the largest captures in the field assay. Next, the coloured trap with the highest captures (black) was compared with the coloured trap with the lowest number of captured adults (white) in the field test. Each pairwise comparison (black *vs.* red and black *vs.* white) consisted of six replications. The traps to be tested were arranged at a distance of 1.2 m from each other. After each replication, the position of each coloured trap was interchanged. The bucket traps were filled with water (to a height of 7 cm) to keep the insects inside the trap. The water was renewed at the end of each repetition. Mean (± SE) temperature and RH during the trial were 19.5 ± 0.07 °C and 48.4 ± 0.36%, respectively. A total of 20 weevil adults per replication (10 males and 10 females), obtained from the field traps, were released simultaneously at a distance of 2 m from the traps. The traps were inspected between 48 and 72 hours after each release, and the number and sex of the captured adults were recorded.

### Spectral reflectance

The spectral reflectance of the coloured traps (black, red, and white) and the leaves and fibres of the upper external part of the trunk of *P. canariensis* was analysed by a Minolta CM-3500d spectrophotometer (Minolta, Tokyo, Japan). Sample reflectance was quantified using the CIE (International Commission on Illumination) standard illuminant C (average daylight) with a field-of-view of 10°. A wavelength range from 400 to 700 nm was measured at 10 nm sampling intervals, covering most of the insect spectral sensitivity range (Menzel and Backhaus, 1991).

### Female/male ratio of natural populations

*R. ferrugineus* cocoons were collected from infested palm trees in different sites near the town of Torrente, during summer and autumn of 2008. Collected cocoons (the number depending on availability) were held in individual sterilized 100 ml plastic containers with perforated lids and maintained in a climatic chamber at 25 ± 2 °C and 65 ± 5% RH. Adult emergence was checked once a day, determining the sex of the adult weevils.

### Statistical analysis

In order to detect the possible effect of trap colour on the number of captures and on the female/male ratio of captured adults, we used a Generalized linear mixed model (GLMM), assuming a Poisson error distribution and a log link function for the former and a binomial error distribution and an logit link function for the latter. The field test evaluated trap colour, date, and plot. In the semi-field test the factors analysed were: trap colour, date, and trap position. Trap colour was considered as a fixed factor, and date, plot, and trap position as random. The analyses were performed using the function 'glmer' of the R package 'lme4' (Bates *et al.*, 2014). The models were evaluated in terms of the Akaike's Information Criterion (AIC) (Akaike, 1987), comparing the ΔAIC of the null model with respect to the aforementioned model. Models with ΔAIC ≤ 10 were considered equivalent. In order to test for differences among the different levels of the fixed factor, a Tukey's post-hoc test was used by applying the 'glht' function of the R package 'multcomp' (Hothorn *et al.*, 2008). A paired *t*-test with a 95% confidence level was used to compare the internal trap climatic conditions of the different coloured traps. As the internal trap conditions values were not normally distributed, the data were transformed by ln (x) before the analysis. To study any differences in the percentages of males and females emerged from the cocoons collected in the field, we used a $\chi^2$ test with a 95% confidence level. All these analyses were performed using the statistical software 'R' (R Core Team, 2013).

## Results

### Test with olfactory attractants

A total of 935 *R. ferrugineus* adults (0.69 captures/trap/day) were captured during the field test; 589 in the black traps, 239 in the red, and 107 in the white. The trap colour influenced the level of adult weevil captures (ΔAIC = 390.1). The mean (± SE) number of captures/trap/day was 1.31 ± 0.23 in black traps, significantly greater than those captured by red (0.53 ± 0.09) and white traps (0.24 ± 0.04) (figure 1; Tukey's post-hoc test: black-red, $P < 0.0001$; black-white, $P < 0.0001$; red-white, $P < 0.0001$).

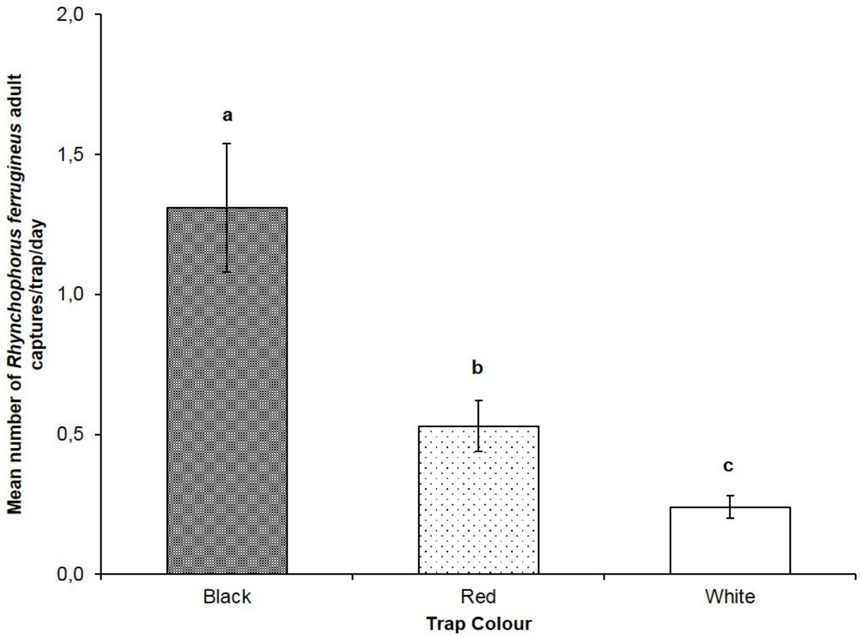


**Figure 1.** Mean (± SE) number of *R. ferrugineus* adults captured per trap and day in coloured traps, from beginning May to end of June 2009, in eight palm tree groves in Torrente (Eastern Spain). Different letters above the columns denote statistically significant differences at $P < 0.05$ (Tukey's post-hoc test).

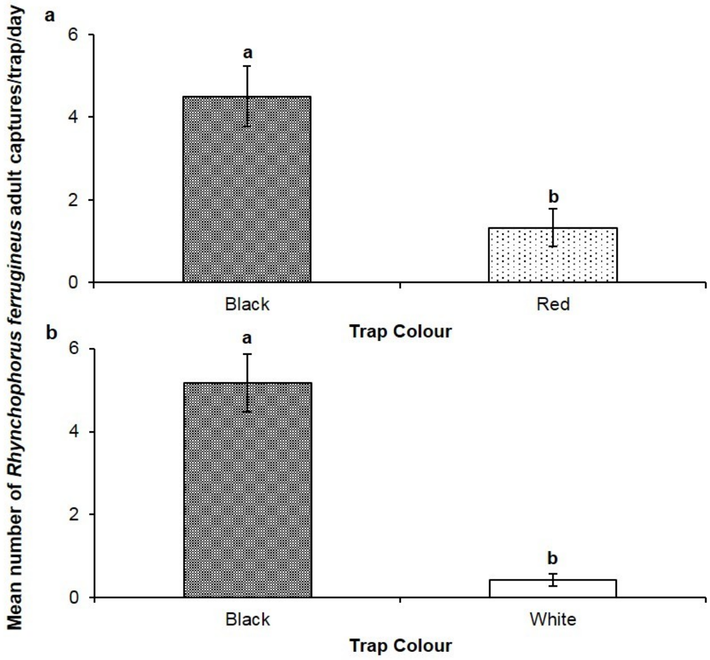


**Figure 2.** Mean (± SE) number of *R. ferrugineus* adults captured per trap and day in pairwise comparisons of coloured traps, in tests without olfactory attractants: (a) black *vs.* red, and (b) black *vs.* white. Different letters above the columns denote statistically significant differences at $P < 0.05$ (Tukey's post-hoc test).

### Internal trap climatic conditions

The mean (± SE) temperature in the black and white traps was 28.61 ± 0.38 °C and 28.19 ± 0.35 °C, respectively. The maximum temperature recorded in the black and white traps was 45.1 and 43.7 °C, respectively. Statistical analysis showed significant differences of temperature between black and white coloured traps, being greater in the first one ($t$ = 11.9521, df = 239, $P$ < 0.0001). The average (± SE) internal temperature difference was 0.42 ± 0.04 °C, and was higher in the black trap. For 23.8 % of the recording period there was either no temperature difference between the black and white traps or the temperature was higher in the latter. During the 73.3 % of the analysed time, temperature differences were between 0 and 2 °C, and were greater than 2 °C only during 2.9% of the test period, reaching a maximum of 2.9 °C. Mean (± SE) internal RH was 84.16 ± 1.4% in black traps and 84.05 ± 1.33% in the white ones. Maximum RH recorded was 100% in both coloured traps. The average (± SE) internal RH difference was 0.11 ± 0.48 °C, and was higher in the white trap. Statistical analysis indicated that RH did not differ significantly between black and white traps ($t$ = 0.2351, df = 239, $P$ = 0.8143).

### Test without olfactory attractants

In the first pairwise comparison, the black and red traps captured 54 and 16 of the 120 released adults, respectively. Mean (± SE) captures/trap/day was 4.50 ± 0.73 in the black traps and 1.33 ± 0.46 in the red ones. In the second pairwise comparison, the black and white traps captured 62 and 5 of the 120 released adults, respectively. Mean (± SE) captures/trap/day was 5.17 ± 0.70 in the black and 0.42 ± 0.15 in the white. As observed in the tests with no olfactory attractants, the colour of the trap influenced the number of captured adult weevils (black *vs*. red: ΔAIC = 19.8; black *vs*. white: ΔAIC = 55.3). Captures in black traps were significantly higher than in red (figure 2a; Tukey's post-hoc test: $P$ < 0.0001). The black traps also captured significantly more adult weevils than the white (figure 2b; Tukey's post-hoc test: $P$ < 0.0001).

### Female/male ratio

In the test with traps baited with olfactory attractants, the mean (± SE) female/male ratio (number of ♀: number of ♂) of the captured adults was always female-biased, ranging from 1.70 ± 0.2 to 2.42 ± 0.4 ♀ per trapped ♂. Trap colour did not influence this ratio (ΔAIC = −3.7).

In the tests with no olfactory attractants, the sex ratio of the captured adults was not always female-biased and, as under field conditions, was not influenced by trap colour (black *vs*. red: ΔAIC = −1.8; black *vs*. white: ΔAIC = −1.1).

With regard to the sex ratio of *R. ferrugineus* natural populations, the number of cocoons collected during summer and autumn were 165 and 225, respectively. In summer, 69 ♀ and 75 ♂ emerged, with 21 non-emerged cocoons. In autumn the number of emerged ♀ and ♂ was 104 for both sexes, and 17 cocoons did not emerge. The percentage of emerged ♀ and ♂ from the collected pupal cases was 50.9 and 49.1%, respectively, with a ratio of 1.03:1 ♀ per ♂. There were no significant differences between the female/male insect ratio of natural populations for both seasons ($\chi^2$ = 0.24; df = 3; $P$ = 0.9706).

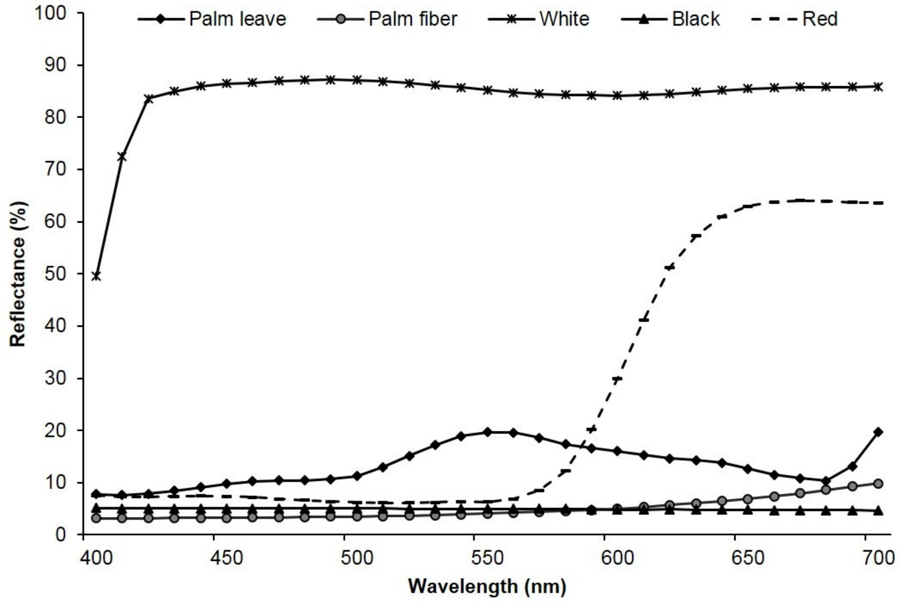


**Figure 3.** Spectral reflectance of palm leaves, palm trunk fibres, and coloured traps (black, red, and white). Reflectance values were obtained from a Minolta CM-3500d spectrophotometer.

### Spectral reflectance

The spectral reflectance values of the leaves and trunk fibres of *P. canariensis,* as well as of the coloured traps tested, are shown in figure 3. The values of the black traps were very similar to those of the palm trunk fibres across all the wavelengths measured, reflecting approximately only 5% of the light. Palm leaves and the remaining trap colours had more complex reflectance-wavelength curves. The palm leaves had a dominant peak from 540 to 580 nm and a second peak at 700 nm. Red traps had a peak from 660 to 700 nm. White had the highest reflectance, close to 90%, for all wavelengths above 430 nm.

## Discussion and conclusions

The tests both with and without olfactory attractants showed evidence of chromatic attraction in *R. ferrugineus*. There were significant differences in the captures of the different coloured traps. This is particularly important for improving the efficacy of weevil trapping systems, especially bearing in mind the highly variable results as regards the level of trap efficacy obtained in previous studies. Sansano *et al*. (2008) reported that reddish brown traps were more attractive than white ones or traps camouflaged with palm trunk fibres. On the other hand, Martínez *et al*. (2008) obtained more captures in yellow traps than in red or white ones. Kalleshwarswamy *et al*. (2006) and Faleiro (2005) compared different trap colours but did not obtain significant differences in captures, the latter work most likely because did not include red and black, which attracted most *R. ferrugineus* adults in this study. Similarly, Tapia *et al*. (2010) did not obtain significant differences when they compared white and yellow/orange traps. Black traps were only tested in comparison with white traps by Hallet *et al*. (1999), Abuagla and Al-Deeb (2012), Al-Saoud (2013), and Abdel-Azim *et al*. (2014), confirming the significantly higher attraction of *R. ferrugineus* adults to black. Vacas *et al*. (2013) compared black pyramidal traps with white bucket traps, analysing a combination of colour and shape, but without explaining if the greatest captures in the first one were due to the colour or the shape of the trap.

The second most efficient trap colour in our study was red, which captured significantly fewer adult *R. ferrugineus* than the black traps, but significantly more than the white. Abdallah and Al-Khatri (2005), Al-Saoud *et al*. (2010), Al-Saoud (2010; 2013), Abuagla and Al-Deeb (2012), and Abdel-Azim *et al*. (2014) reported similar results for red traps. The aforementioned studies generally combined the chromatic factor with other aspects, such as trap shape, trap location, type of pheromone dispenser or type of substance used as kairomone. There is a lack of knowledge about the effect of trap colour as the only factor influencing the captures of this weevil. Moreover, none of the current papers examines the possible reasons of the colour preferences of *R. ferrugineus*.

Hallet *et al*. (1999) suggested that the higher temperatures in black traps might cause greater pheromone release, which in turn would result in higher insect captures. In the present study, we measured the internal climatic conditions of differently coloured traps in order to determine possible effects on the release of olfactory attractants. The internal temperature values in the black and white traps were significantly different. Nevertheless, it is unlikely that an average difference of 0.42 °C in internal temperature would cause a greater pheromone release in the darker traps. According to the obtained results, we also consider that the time interval in which a temperature difference would cause a higher pheromone release in the darker traps is very short, between 2 and 3 °C during only 2.9% of the recorded time. Furthermore, in the test without olfactory attractants, black traps captured the highest percentage of *R. ferrugineus* adults, exactly as had occurred in the field trials. These results suggest that the efficiency of the black traps was mainly determined by chromatic attraction and not only by the possible higher emission of attractants. The observations in this study suggest that the attraction of *R. ferrugineus* adults to traps is the result of a two-step process: firstly, long-distance attraction to semiochemicals, and secondly, short-distance visual attraction to trap colour and semiochemicals. Both factors can have a synergic effect on the number of adults trapped. For example, Björklund *et al*. (2005) reported that traps with a combination of odour and visual stimuli captured higher numbers of pine weevils, *Hylobius abietis* (L.) (Coleoptera Curculionidae). Nevertheless, further studies are necessary to evaluate the exact effect of the internal trap climatic conditions on the emission of attractants and consequently on the number of *R. ferrugineus* captures at different temperatures and RH.

Entwistle (1963) and Timmons and Potter (1981) reported that some Coleoptera and Lepidoptera with wood-boring larvae are caught by red, brown and black traps, probably responding to dark shades and the contrast with the background rather than to any visual cue. Reddy *et al*. (2011; 2012) confirmed these results, capturing a significantly greater number of New Guinea sugarcane weevils, *R. obscurus*, and sweetpotato weevils, *C. formicarius*, in russet-brown and light-red colours, respectively. On the other hand, Leskey (2006) and Machial *et al*. (2012), did not obtain significant differences in the captures of the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera Curculionidae) and the warren root collar weevil, *Hylobius warreni* (Wood) (Coleoptera Curculionidae), for the colours tested. Entwistle (1963) speculated that the general attraction of scolytine and platypodid beetles to red-coloured traps was due to their resemblance to host bark. In the present study, the spectral reflectance of the tested colours and different palm tree components was measured. The spectral analyses revealed that black traps produced a spectral reflectance similar to the palm leaves and trunk fibres of the *P. canariensis* palm tree. The similarity between the spectral reflectance of the palm tree components, through which the adults enter their host or lay their eggs, and the colour black could explain the stronger attraction of *R. ferrugineus* to black traps. Moreover, the cryptic habits of the insect, which generally tends to hide (Viado and Bigornia, 1949) may also help to explain the chromatic affinity to this colour.

With regard to the sex ratio, the study of the natural populations of *R. ferrugineus* shows the existence of an equal number of males and females in the field. Hunsberger *et al*. (2000) pointed out the same ratio for other *Rhynchophorus* species, *R. cruentatus* (F.). On the other hand, in the study using olfactory attractants, a mean of 2.4 females per trapped male was obtained. These results are similar to previous studies reporting a sex ratio of 2 or 2.04 females per captured male (Faleiro, 2005; Al-Saoud, 2011). On the other hand, in the test without olfactory attractants, the sex ratio of the captured adults was not always female-biased. Therefore, although there is a 1:1 sex ratio in the field, the captures of females were higher when traps baited with olfactory attractants were used, regardless of trap colour. The female-biased sex ratio of the trapped *R. ferrugineus* in our field study is apparently due to the fact that females are more strongly attracted than males to the semiochemicals used to bait traps, whatever their colour. The female-biased sex ratio in traps with attractants has important practical implications for the mass trapping of *R. ferrugineus*. Higher female captures mean their numbers decrease in natural population, and therefore the biological potential of the pest is reduced (Oehlschlager, 2006).

In conclusion, the present study shows the importance of the colour of the traps used to capture insects. Commercial traps have been traditionally white or red (Hallett *et al*., 1999; Guarino *et al*., 2011), but black traps substantially increase insect captures, which are mostly female-biased. The upper temperature in black traps is not the only cause of its higher captures. It is also considered that the visual attraction of *R. ferrugineus* traps could be improved by the use of a colour with a spectral reflectance as close as possible to the host structures infested by the insects. The results obtained in this work could improve the monitoring and mass trapping of *R. ferrugineus* populations.

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**Authors' addresses:** Juan Antonio ÁVALOS (corresponding author, juavama@msn.com), Antonia SOTO (asoto@eaf.upv.es), Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camí de Vera s/n, 46022 València, Spain.

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