Acta Phytopathologica et Entomologica Hungarica 53 (2), pp. 233–240 (2018)
DOI: 10.1556/038.53.2018.013

The Effect of Date Palm Tissue and Aggregation Pheromone on Attraction and Trapping of Red Palm Weevil, *Rhynchophorus ferrugineus* Oliv. (Col.: Dryophthoridae)

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(Received: 3 May 2018; accepted: 4 July 2018)

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* Oliv. (Col.: Dryophthoridae), is a key pest of date palm in Asia, North Africa and Spain. A field study was conducted in south eastern Iran to find the effect of odor sources in traps on RPW behavior. This study consisted of four experiments; each included four treatments (odor sources) and eight replications. Each experiment was repeated 4 times (i.e. four different distances between odor sources (treatments) were considered: 0, 0.5, 2 and 5 meters). In each treatment, two traps were used. The treatments were as follows: 1) one trap included two pheromones and the other trap included two pieces of plant tissue, 2) both traps included one pheromone along with one piece of plant tissue, 3) both traps included one pheromone, and 4) both traps included one piece of plant tissue. Overall, much less RPWs were caught in the traps of treatments 3 and 4 (baited with only the pheromone or the plant bait) than in the traps of treatments 1 and 2 (baited with pheromone and plant tissue) evidencing the phenomenon of synergy, previously reported. The Results of analysis of variance of total catch of traps, at all between-trap distances indicated that there was significant difference among treatments. In all experiments, traps that contained just palm tissue had the least catch rate. The RPW distinguished pheromone from plant odor when both sources were separated by less than 5 m. At distances of 5 m, attractancy of plant odor was similar to pheromone attractancy. Our results suggest that each of the chemical cues probably induce a different behavioral process. Probably, pheromone would attract RPWs from a distance, making them fly towards the pheromone sources (traps) while the plant odor would arrest flight and induce landing and local search by walking for palm tissue, resulting in entry into traps.

Keywords: Red Palm Weevil, aggregation pheromone, capture, trap.

Date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae) is an important fruit tree cultivated mostly in arid areas of the world. Around 60% of date palm trees are in the North Africa and the Middle East, where it is important for life and culture of the people (Wakil et al., 2015). Iran has been the second date-producing country in the world during 1994–2014 (with a cultivated area of 184,000 ha and an annual production of 957,753 tons (FAOSTAT, 2017)). Palm trees have several key pests and diseases that stop the growth and fertility of palm trees, resulting in tree death. Therefore, it is important to implement correct IPM programs in palm fields.

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Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* Oliv. (Col.: Dryophthoridae), is a key pest of palm trees, which is distributed all over the world especially in Asia, Oceania, the Northern Africa, Europe and the Caribbean (EPPO, 2008, 2009). It was first described for attacking *Cocos nucifera* L. (Arecales: Arecaceae) in South and Southeast Asia and for damaging *P. dactylifera* in the Middle East (Fiaboe et al., 2012). In Iran, it was reported for the first time in 1990 at Saravan (Baluchestan region), where it was probably imported with infected offshoots from Pakistan (Avand-Faghih, 1996). However, in recent years, it has expanded its infestation range, and was observed in the Lar city (Fars province, Iran), hundreds of kilometers away from the Saravan (Mohammadpour, unpublished data).

Males of RPW produce an aggregation pheromone comprising 4-methyl-5-nonanol (ferrugineol, the most abundant compound), 4-methyl-5-nonanone (ferrugineone, the less abundant compound) (Hallett et al., 1993a) and 3-methyl-4-octanol (phoenicol, the minor compound) (Rochat et al., 1993). Ferrugineol attracts both sexes (Hallett et al., 1993b). Both virgin and mated individuals of both sexes are captured using pheromone traps, but in all field tests the majority of adults caught were mated females (Avand-Faghih, 1996; Faleiro et al., 2000; 2003; El-Sabay, 2003; Avand-Faghih et al., 2005). Pheromone-based trapping can be used to detect infestation spots and also as one of the methods to reduce population density in mass (Oehlschlager, 2007).

Like other weevil species, trapping efficiency is high when RPW aggregation pheromone is emitted in the presence of host plant volatiles; hence, RPW mass trapping with lures including the two odor sources is used in its management programs (Giblin-Davis et al., 1996; Hallet et al., 1999). It is proved that natural palm bait has poor attraction alone; however, strongly synergizes the effect of aggregation pheromone (Giblin-Davis et al., 1996). To maintain traps at an optimal efficiency, natural plant bait, that emits the synergistic odors, must be renewed once to twice per month. The labor cost of such operation is high (Guarino et al., 2012).

The volatile compounds emitted by host plant tissues have been studied using electrophysiological bioassays. Results showed that RPW antennae were responsive to palm tissue compounds, including the so-called ‘palm esters’ (Guarino et al., 2011; Vacas et al., 2014). Guarino et al. (2011) observed that a blend of the esters, i.e. ethyl acetate and ethyl propionate, improved RPM catch rate in field traps baited with pheromone and molasses, compared to traps baited with each ester alone. Vacas et al. (2014) found that catch rate of aggregation pheromone traps including ethyl acetate/ethanol blend (at a rate of 1:3) was twice of the catch rate of traps baited with aggregation pheromone alone. The catch efficacy was 76% of the trap composed of the kairomonal co-attractants including sugar molasses and stem of Canary Island date palm, *Phoenix canariensis* Chabaud. (Arecales: Arecaceae). Molasses, provided the ethanol needed for the kairomonal effect, whereas palm pieces provided fermenting odors, i.e. ethyl acetate and ethanol, which were the main compounds (Guarino et al., 2011; Vacas et al., 2014).

Study on the extraction of volatiles from date palm tissue, resulted in identification of 109 odor molecules (Avand-Faghih, 2004). Among these odor molecules, mixtures of ethanol and ethyl acetate (50:50), at a release rate of 300 mg per day, had a synergistic effect when accompanied by aggregation pheromone of RPW, which had similar catch rate with traps baited with 350–500 g of date palm core in the field (Avand-Faghih, 2004). At present, research has focused on identifying active co-attractant compounds that to be competitive with a natural plant odor. The goal of this study was to determine the role of odor sources, including pheromone and date palm tissue, in attraction of RPW.

Materials and Methods

This study consisted of four experiments; each included four treatments (odor sources) and eight replications. Each experiment was conducted at 4 different between-trap distances: 0, 0.5, 2 and 5 meters. In each treatment, two traps were used. The treatments were as follows: 1) one trap included two pheromone dispensers and the other trap included two pieces of plant tissue, 2) both traps included one pheromone dispenser along with one piece of plant tissue, 3) both traps included one pheromone dispenser, and 4) both traps included one piece of date palm tissue. Duration of each experiment was 4 weeks. All experiments were conducted based on a randomized complete block design.

Each odor source was placed in a trap. Each trap consisted of a 24-liter red plastic bucket. The lid of each bucket had eight radial openings (8×5 cm) for insect entrance. Soapy water was poured into the bucket to prevent the caught beetles from escaping. No insecticide was used.

Treatments that included date palm tissue consisted of one piece of date palm core (ca. 1 kg), freshly cut from a shoot, and was placed in a punctured plastic bag to reduce dehydration.

Aggregation pheromone dispenser was a polyethylene vial (20 mm in diameter and 35 mm in height) loaded with 0.7 g of ferrugineol (98% purity) (Agrisense Co., UK). The dispensers were weighed at the end of each assay to determine release rates of the chemicals. The dispenser and the date palm core were installed into the lid of the bucket.

All tests were carried out in Saravan (Sistan-and-Baloochestan province, Iran), where is the eastern limit of the area of date palm production in Iran, with a mean elevation of ca. 1200 m and cold winters. The area consists of scattered oases, with a traditional farming system of mixed vegetables, pasture, and fruit crops, with few or no fertilizer or pesticide application. Date palms were 'Mazafati' variety.

Traps were placed on the ground at the base of a date palm. The blocks were 500 m apart. The traps were checked at regular intervals (every 3 d) to collect trapped beetles. Date palm tissue was renewed and the treatments were re-randomized every 9 days.

Data were analyzed using procedures of SAS (SAS Institute Inc., 2002).Total catch of two adjacent traps were used in data analysis. Data were checked for normality and heteroscedasticity. In case needed, data were log-transformed. Trap catch data were subjected to a one-way analysis of variance. Means were compared by Tukey test ($\alpha = 0.05$).

Results

The ANOVA results of traps' total catch, at all between-trap distances, indicated that there was significant difference among treatments (Distance 0: $F_{3,28} = 84.29$, $P < 0.0001$; Distance 0.5: $F_{3,28} = 36.66$, $P < 0.0001$; Distance 2: $F_{3,28} = 41.41$, $P < 0.0001$; Distance 5: $F_{3,28} = 35.41$, $P < 0.0001$).

In experiment 1, when both traps of each treatment were close to each other (distance 0), the highest catch rate was observed when traps were baited with pheromone and date palm tissue, while the lowest catch rate was observed when traps were baited with only date palm tissue (Table 1).

In experiment 2, when traps were 0.5 m apart, similar result to experiment 1 was observed. However, the efficiency of traps with just pheromone was similar to the efficiency of traps with just date palm tissue (Table 1).

In experiment 3, when traps were 2 m apart, the highest catch rate was observed in traps that included one pheromone along with one piece of palm tissue, which caught significantly more RPW than treatment that included two pheromones in one trap and two palm tissues in the other trap (Table 1).

In experiment 4, when traps were 5 m apart, the highest catch rate was observed in traps that included one pheromone along with one piece of palm tissue. Similar to experiment 3, it caught significantly more RPW than treatment that included two pheromones in one trap and two palm tissues in the other trap (Table 1).

In treatment 1, that baits (odor sources) were different in each of the two traps, difference in catch rate between two odors sources were analyzed at each of the studied distances (Table 2). The results indicated that at between-trap distances of 0, 0.5 and 2 meters, the catch rate was higher (3 times more) in traps baited with palm tissue compared to traps baited with pheromone. However, when the distance between traps increased to 5 meters, the catch rate was not different between traps containing different types of odor sources (Table 2).

Table 1

Mean capture (± SE) rate of Red Palm Weevil (RPW), *Rhynchophorus ferrugineus,* by traps baited with different odor sources, when the traps were placed at four different distances apart

Treatments*	Mean capture ± SE** (different between-trap distances)			
	0 m	0.5 m	2 m	5 m
1	22.37 ± 3.07 a	30.87 ± 2.55 a	7.50 ± 1.18 b	4.62 ± 0.67 b
2	25.00 ± 2.17 a	35.50 ± 6.94 a	18.25 ± 2.25 a	20.62 ± 3.22 a
3	2.62 ± 0.88 b	5.25 ± 1.70 b	1.62 ± 0.73 c	3.75 ± 1.42 b
4	0.50 ± 0.18 c	3.12 ± 0.63 b	0.75 ± 0.31 c	0.50 ± 0.37 c

* Treatment 1 = two pheromones in one trap, two palm tissues in the other trap; Treatment 2 = one pheromone along with one palm tissue in each trap; Treatment 3 = one pheromone in each trap; Treatment 4 = one palm tissue in each trap. Refer to text for more detail.

** Means in a column followed by same letter were not significantly different based on Tukey test ($\alpha = 0.05$).

Table 2

Mean capture (± SE) rate of Red Palm Weevil (RPW), *Rhynchophorus ferrugineus,* by traps baited with different odor sources in treatment 1

Odour sources	Mean capture ± SE (different between-trap distances)			
	0 m	0.5 m	2 m	5 m
2 pieces of palm tissue	16.87 ± 2.27	23.75 ± 2.13	5.63 ± 1.08	2.13 ± 0.52
2 pheromones	5.50 ± 0.96	7.12 ± 1.27	1.87 ± 0.58	2.50 ± 0.50
F 1,14P	22.74*, 0.0003	35.96*, < 0.0001	11.5*, 0.0050	0.45, 0.5132

* Significant difference at $\alpha = 0.05$

Discussion

In our research, analyzing catch data of treatments (i.e. total catch of both traps of each treatment) indicated that RPW catch rate was significantly lower in treatment 3 (baited with only pheromone) and treatment 4 (baited with only palm tissue) compared to treatments 1 and 2 that contained the pheromone and palm tissue together (Table 1). Therefore, aggregation pheromone or the host plant tissue alone are weak attractants for adult RPW; however, when two sources were applied together in a trap, attraction and trapping efficacy considerably increased. Other research on chemical ecology of Rhynchophorinae showed that aggregation pheromone or kairomones alone are weak attractants, while their combination increases the attraction by 8- to 20-fold (Giblin-Davis et al., 1996).

An important point to find out is the role of host plant odor and aggregation pheromone of RPW in attraction and host-plant finding behavior and how they act together and co-influence RPW. Literature on effect of host–plant odor on aggregation pheromone and increase catch rate of traps shows that in some cases the ‘synergy’ term is used, while in some cases the ‘co-attraction’ term is preferred. Synergy is defined as increase or amplification of attraction, while co-attraction indicated that each of the odor sources, while is important and essential in host-plant finding alone, act together to complement each other role, resulting in successful host-plant finding. For example, Said et al. (2005) used ‘synergy’ term to indicate the relation between host-plant odor and aggregation pheromone of American Palm Weevil, *Rhynchophorus palmarum* L. (Col.: Curculionidae). Her laboratory studies indicated that acetoin (the main volatile component extracted from sugarcane and palm tissue) increased the antennal signals in the insect brain. Her field studies showed that the traps that contained acetoin and aggregation pheromone together, captured about 10 times more insects compared to traps that contained each of the odor sources alone. Vacas et al. (2016) used ‘co-attraction’ term to explain the relation between palm tissue odor and aggregation pheromone of RPW. His field studies on RPW showed that palm tissue fermenting odors acted as co-attractant on RPW host-plant finding behaviour. Hasni et al. (2017) mentioned that the main components of the odor from date palm core (include ethyl benzoate, 4-methylanisole and farnesol at a rate of 1:1:1) enhanced response of Date Palm Root Borer, *Oryctes agamemnon* Burmeister (Col.: Scarabaeidae), to the aggregation pheromone. Meanwhile, they mentioned that could not ascertain whether the mixture is a pheromone synergist or a simple co-attractant.

In our study, in treatments 2, 3 and 4 that each of the two nearby traps had similar odor sources, the catch rate was not different between the two traps. While, in treatment 1, that the odor source was different in two nearby traps, the catch rate was higher in the traps that contained host plant tissue compared to traps that contained aggregation pheromone (Table 2). Total catch rate of traps in treatment 1 decreased as distance between traps increased. When the between-trap distance reached 5 meters, the catch rate in treatment 1 was similar to catch rates of treatment 3 and treatment 4. Therefore, it is suggested that RPW can distinguish between palm tissue odor and aggregation pheromone at distances below 5 meters, preferring plam tissue odor over aggregation pheromone. Chemical ecology studies on *R. palmarum*, showed that weevils use a combination of odors to find the host plant. The odors that are emitted by host plant has been identified as ethanol, ethylacetate, and pentane, hexanal, isoamyl-acetate, and isopentanol, which makes the weevils to fly around the host plant at short distances. While, other odors such as rhynchophorol (the main component of *R. palmarum* aggregation pheromone) orient weevils at longer distances. Attraction of weevils at a far distance from the host plant is called ‘anemotaxis’ which is influenced by aggregation pheromone. In short distances from the host plant, the change in behaviour of the weevil and its attraction to the host plant is called ‘klinokinetic’ and is affected by host plant odors (Jaffé et al., 1993).

In conclusion, our field study suggested that each of the odor sources, palm tissue or aggregation pheromone, initiated one of the parts of host-plant finding behaviour process by RPW, and acted as co-attractants. Probably, aggregation pheromone acted at far distances from the host plant, while the plant tissue affected RPW host-finding behaviour at short distances from the host plant, i.e. less than 5 meters. The latter probably results in flight stop, landing and entering into the traps.

Literature

Avand-Faghih, A. (1996): The biology of red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) in Saravan region (Sistan and Balouchistan Province, Iran). Appl. Entomol. and Phytopathol. 63, 16–18.

Avand-Faghih, A. (2004): Identification et application agronomique de synergistes végétaux de la phéromone du charançon *Rhynchophorus ferrugineus* Olivier (1970). Mémoire présenté pour obtenir le titre de Docteure de l’INA-PG en Biologie et Agronomie ‘Protection des cultures’. pp. 105–130.

Avand-Faghih, A., Mohammadpour, K., Mohammadi, H., Khorshidi, H. R., Askari, M., Damghani, R., Rochat, D., Malosse, C., Letter, M. and Renou, M. (2005): Chemical ecology of coleopteran pests of date palm. In: Proc. of 1st International Festival and Symposium on Date Palm, Bandar Abbas, Iran. pp. 85–86.

El-Sebay, Y. (2003): Ecological studies on the red palm weevil *Rhynchophorus ferrugineus* Oliv., (Coleoptera: Curculionidae) in Egypt. Egyptian J. Agric. Res. 81, 523–529.

EPPO (2008): European and Mediterranean Plant Protection Organization. Data sheets on quarantine pests *Rhynchophorus ferrugineus*. EPPO Bulletin 38, 55–59.

EPPO (2009): *Rhynchophorus ferrugineus* found on *Howea forsteriana* in Sicilia, Italy. No. 32009/051. European and Mediterranean Plant protection Organization, Paris, France. Available at: https://archives.eppo.int/EPPOReporting/2009/Rse-0912.pdf. Accessed on: 12 June 2015.

FAOSTAT (2017): Food and agricultural commodities production. Available at: http://www.fao.org/faostat/en/#-data/QC/visualize . Last Update on 17 May 2017.

Faleiro, J. R., Abraham, V. A., Al-Shuaibi, M. A. and Kumar, T. P. (2000): Field evaluation of red palm weevil, *Rhynchophorus ferrugineus* Oliv. Pheromone (ferrugineol) lures. Indian J. Entomol. 62, 427–433.

Faleiro, J. R., Rangnekar, P. A. and Satarkar, V. R. (2003): Age and fecundity of female red palm weevils *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae) captured by pheromone traps in coconut plantations of India. Crop Protection 22, 999–1002.

Fiaboe, K. K. M., Peterson, A. T., Kairo, M. T. K. and Roda, A. L. (2012): Predicting the potential worldwide distribution of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) using ecological niche modeling. Florida Entomologist 95, 659–673.

Giblin-Davis, R. M., Oehlschlager, A. C., Perez, A., Gries, G., Gries, R., Weissling, T. J., Chinchilla, C. M., Pena, J. E., Hallett, R. H., Pierce, H. D. and Gonzalez, L. M. (1996): Chemical and behavioral ecology of palm weevils (Curculionidae: Rhynchophorinae). Fla. Entomol. 79, 153–167. doi: 10.2307/3495812.

Guarino, S., Lo Bue, P., Peri, E. and Colazza, S. (2011): Responses of *Rhynchophorus ferrugineus* adults to selected synthetic palm esters: Electroantennographic studies and trap catches in an urban environment. Pest Manag. Sci. 67, 77–81.

Guarino, S., Peri, E., Lo Bue, P., Germana, M. P., Colazza, S., Anshelevich, L., Ravid, U. and Soroker, V. (2012): Assessment of synthetic chemicals for disruption of *Rhynchophorus ferrugineus* response to attractant-baited traps in an urban environment. Phytoparasitica 41, pp. 79–88.

Hallett, R. H., Gries, G., Gries, R., Borden, J. H., Czyzewska, E., Oehlschlager, A. C., Pierce, H. D., Angerilli, N. P. D. and Rauf, A. (1993a): Aggregation pheromone of two Asian palm weevils, *Rhynchophorus ferrugineus* and *R. vulneratus*. Naturwissenschaften 80, 328–331.

Hallett, R. H., Oehlschlager, C. A., Gries, G., Angerilli, N. P. D., Al-Sharequi, R. K., Gassouma, M. S., Borden, J. H. (1993b): Field testing of aggregation pheromones of two Asian palm weevils. In: Proc. of International Palm Oil Congress, September 1993, Kuala Lumpur, Malaysia. pp. 661–668.

Hallett, R. H., Oehlschlager, A. C. and Borden, J. H. (1999): Pheromone trapping protocols for the Asian palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Int. J. Pest Manag. 45, 231–237.

Hasni, N., Pinier, C., Imed, C., Ouhichi, M., Couzi, P., Chermiti, B., Frérot, B., Saïd, I. and Rochat, D. (2017): Synthetic co-attractants of the aggregation pheromone of the date palm root borer *Oryctes agamemnon*. J. Chem. Ecol. 43, 631–643.

Jaffé, K., Sánchez, P., Cerda, H., Hernández, J. V., Jaffé, R., Urdaneta, N., Guerra, G., Martínez, R. and Miras, B. (1993): Chemical ecology of the palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): attraction to host plants and to a male-produced aggregation pheromone. J. Chem. Ecol. 19, 1703–1720.

Oehlschlager, A. C. (2007): Optimizing trapping for palm weevils and beetles. Actes de la 3e conférence internationale sur le Dattier EAU (2006). Acta Horticulturae 736, 347–36.

Rochat, D., Malosse, C., Lehere, M., Ramirez-Lucas, P., Einhorn, J. and Zagatti, P. (1993): Identification of new pheromone related compounds from volatiles produced by males of four Rhynchophorinae weevils (Coleoptera, Curculionidae). Comptes Rendus de l'Academie des Sciences, Serie II 316, 1737–1742.

Said, I., Renou, M., Morin, J. P., Ferreira, J. M. S. and Rochat, D. (2005): Interactions between acetoin, a plant volatile and pheromone in *Rhynchophorus palmarum*: Behavioral and olfactory neuron responses. J. Chem. Ecol. 31, 1789–1805. doi: 10.1007/s10886-005-5927-4.

SAS Institute Inc. (2002): SAS/STAT user's guide. Version 9.1. SAS Institute Inc., Cary, North Carolina.

Vacas, S., Abad-Pay, M., Primo, J. and Navarro-Llopis, V. (2014): Identification of pheromone synergists for *Rhynchophorus ferrugineus* trapping systems from *Phoenix canariensis* palm volatiles. J. Agric. Food Chem. 62, 6053–6064.

Vacas, S., Melita, O., Michaelakis, A., Milonas, P., Minuz, R., Riolo, P., Abbass, M. A., Lo Bue, P., Colazza, S., Peri, E., Soroker, V., Livne, Y., Primo, J. and Navarro-Llopis, V. (2016): Lures for red palm weevil trapping systems: aggregation pheromone and synthetic kairomone. Pest. Manag. Sci. 73, 223–231.

Wakil, W., Faleiro, J. R., Miller, T. A., Bedford, G. O. and Krueger, R. R. (2015): Date palm production and pest management challenges. In: W. Wakil, J. R. Faleiro and T. A. Miller (eds): Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges. Springer, pp. 1–11.