Screening Date Palm Cultivars for Resistance to Red Palm Weevil, Rhynchophorus ferrugineus (Coleoptera: Curculionidae)

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Source: Florida Entomologist, 97(4) : 1529-1536

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.097.0427

SCREENING DATE PALM CULTIVARS FOR RESISTANCE TO RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Date palm, *Phoenix dactylifera* L. (Arecales: Arecaeae) is the most important crop of the Arabian Peninsula. The Kingdom of Saudi Arabia is among the top 3 date producing countries of the world estimated to have over 400 date palm cultivars of which 25 are important and yield 1.3 million tons of dates annually. The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is a key pest of date palm in the Middle East. We studied the mechanisms of resistance against RPW in 7 major date palm cultivars of the Al-Ahsa oasis in Saudi Arabia *viz*. 'Khalas', 'Sheshi' , 'Reziz', 'Khasab', 'Hatmi', 'Shahal' and 'Gaar' by determining the extent of attraction of female RPW adults to fresh palm volatiles emitted from date palm frond tissue through four-arm choice olfactometer assays. Further, we assessed the degree of antixenosis and antibiotic effects if any by evaluating the number of eggs laid (oviposition), per cent egg hatch and larval tunnelling in these cultivars. Results revealed that the popular date palm cultivar 'Khalas' had the least antixenotic effect on female RPW adults where a high degree of attraction to palm tissue volatiles was recorded, which was statistically similar to the cultivars 'Reziz', 'Sheshi' and 'Hatmi'. The cultivars 'Khasab', 'Shahal' and 'Gaar' exhibited high degree of non-preference (antixenosis). Further, 'Reziz' registered the highest egg lay by RPW and was statistically onpar with the cultivars 'Khalas' and 'Sheshi'. Similar and non-significant values for egg hatch and larval tunnelling in the cultivars tested indicate no antibiotic effects against RPW in the 7 date palm cultivars. Since over 50% of the area in the Al-Ahsa oasis is under the cultivar 'Khalas' with several new plantations in the susceptible age of less than 20 years, RPW is likely to pose a major challenge to date farmers of the region in the years to come.

Key Words: Date palm, *Rhynchophorus ferrugineus*, antixenosis, olfactometer, palm volatiles

RESUMEN

La palma datilera, *Phoenix dactylifera* L. (Arecales: Arecaeae) es el cultivo más importante de la Península Arábiga. El Reino de Arabia Saudita es uno de los mejores tres países productoros de dátiles del mundo que se estima que tiene más de 400 cultivares de dátiles, 25 de ellos son importantes y producen 1.3 millones de toneladas de dátiles al año. El picudo rojo de la palma (PRP) *Rhynchophorus ferrugineus*(Olivier) (Coleoptera: Curculionidae) es una plaga clave de la palma datilera en el Medio Oriente. Estudiamos los mecanismos de resistencia contra PRP en 7 cultivares principales de palmeras datileras ('Khalas', 'Sheshi','Reziz', 'Khasab', 'Hatmi', 'Shahal' y 'Gaar') en el oasis de Al- Ahsa en Arabia Saudita al determinar el grado de atracción de las hembras adultas de RPW a los volátiles de la palma fresca emitidos de los tejidos de las frondas de las palmas en un ensayo de elección en un olfatómetro con cuatro ramas. Además, se evaluó el grado de antixenosis y los antibióticos, si habian, al evaluar el número de huevos puestos (oviposición), el porcentaje de los huevos eclosionados y los túneles hechos por las larvas en estos cultivares. Los resultados revelaron que el cultivar popular 'Khalas' de palma datilera tuvo el menor efecto antixenotico sobre las hembras adultas de RPW donde se registró un alto grado de atracción a los volátiles de los tejidos de las palmas, lo cual fue estadísticamente similar a los cultivares 'Reziz', 'Sheshi' y 'Hatmi'. Los cultivares 'Khasab', 'Shahal' y 'Gaar' exhibieron un alto grado de no preferencia (antixenosis). Además, 'Reziz' registró la puesta de huevos más alta por

PRP y fue estadísticamente paralelo a los cultivares 'Khalas' y 'Sheshi'. Valores similares y no significativos para la eclosión de huevos y túneles hechos por las larvas en los cultivares probados indican que no hay efectos antibióticos contra PRP en los 7 cultivares de la palma datilera. Puesto que más del 50% de la superficie en el oasis de Al-Ahsa se encuentra bajo el cultivar 'Khalas ' con varias plantaciones nuevas en la edad susceptible de menos de 20 años, RPW puede ser un desafío importante para los productores de dátiles de la región en el los próximos años.

Palabras Clave: palma datilera, *Rhynchophorus ferrugineus*, antixenosis, olfatómetro, volátiles palma

Date palm *Phoenix dactylifera* L. is the most important fruit crop of the Arabian Peninsula where it is closely associated with the life and culture of the people since pre-historic times. The kingdom of Saudi Arabia is among the top 3 date producing countries of the world where the crop is cultivated in over 172,000 ha accounting for nearly 17% of the global date production (FAOSTAT 2012). Saudi Arabia is reported to have over 400 date palm cultivars of which 25 are important (Anonymous 2006; Ashraf & Hamidi-Esfahani 2011). 'Khalas' is the most widely cultivated date palm cultivar in the Al-Ahsa oasis of Saudi Arabia where the cultivars 'Reziz' and 'Sheshi' are also important (Al-Abdoulhadi et al. 2011).

In 1985, the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) was first reported in the region from Rass-El-Khaima in the United Arab Emirates (Zaid et al. 2002).It has since spread rapidly to all countries of the Gulf region in the Middle East including Saudi Arabia mostly through infested planting material (Faleiro 2006; Al-Shawaf et al. 2013). The annual loss in the Gulf region due to eradication of severely infested palms has been estimated to range from US$1.74 million to US$8.69 million at 1 and 5% infestation, respectively (El-Sabea et al. 2009).

During the last three decades, the host range of RPW has significantly increased since the mid-1950s when Nirula (1956) reported the pest on only 4 palm species as compared to its present host range of 40 palm species worldwide (http://www.savealgarvepalms.com/en/weevil-facts/host-palmtrees). The increased host range is a result of its rapidly expanded geographical range in Asia, Africa, Australasia and the Americas as compared to other *Rhynchophorus* palm weevils (Wattanapongsiri 1966; Giblin-Davis et al. 2013). A recent study based on ecological niche modelling has predicted that RPW can expand its global range still farther (Fiaboe et al. 2012). It is important to detect infested palms in the early stage of attack so that these palms can be treated (stem injection) with insecticide (Abraham et al. 1998).

Saudi Arabia has a rich genetic pool of over 400 date palm cultivars (Anonymous 2006). Al-Ahsa in the eastern province of the kingdom is the most important date palm oasis in Saudi Arabia with an estimated 3,000,000 palms in which over 50% of the area under date palm is grown with the cultivar 'Khalas' (Sallam et al. 2012). Infestation reports from date plantations in Al-Ahsa indicate that the cultivar 'Khalas' is highly prone to attack by RPW (Sallamet al. 2012). RPW is managed in date palm using a pheromone (Ferrugineol) based Integrated Pest Management (IPM) strategy with varying degrees of success (Al-Shawaf et al. 2012; Hoddle et al. 2013) where the IPM component of host plant resistance has not been exploited.

Tolerance, antibiosis and antixenosis are conceptually recognized 3 modes of plant resistance to arthropods (Horber 1982; Smith 2005). Ideally, antibiosis and antixenosis may be mutually reinforcing modalities of resistance i.e.,antixenosis may deter antibiosis-resistance-breaking insect biotypes from colonizing a plant, and antibiosis may reduce the fitness of those individuals that colonized (Hesler & Dashiell 2011).Tolerance is a plant's ability to withstand or recover from arthropod damage. Antibiosis adversely affects arthropod development, reproduction, or survival, and antixenosis (non-preference) prevents arthropod colonization of a host plant. A plant may exhibit 2 or more modes of resistance and in some cases it may be difficult to differentiate between antixenosis and antibiosis as they may both affect arthropod populations (Smith 2005). Over the years, the present well known cultivars of date palm in Saudi Arabia and also in other date growing countries have evolved from seedlings selected by date palm farmers for good fruit qualities (mostly bigger fruit size). Seedling date palms are the original source of most of the present well established cultivars in several countries (Johnson et al. 2013). Genetic diversity is desirable for long-term crop improvement and reduction of vulnerability in plants to important crop diseases. Measurements of genetic diversity can be used in breeding programs to increase the genetic variation in base populations by crossing cultivars with a high level of genetic distance as well as for the introgression of exotic germplasm (Elmeer et al. 2011).

In Spain Barranco et al. (2000) and Dembilio et al. (2009) reported antibiotic and antixenotic

mechanisms in *Washingtonia filifera* (Lindl.) H.Wendl (Arecales: Arecaeae) and *Chamaeropshumilis* L.(Arecales: Arecaeae) against RPW, while *Phoenix canariensis* Chabaud (Arecales: Arecaeae) was highly preferred .Studies carried out in China to elucidate the development and reproduction of RPW on different host palms reveal that *P. canariensis* and *W. filifera* were the more suitable host plants, while *P. sylvestris* was the least suitable (Ju et al. 2011). Reports from Iran indicate that calcium inhibits RPW growth, while date palm varieties with high sugar levels enhance RPW oviposition and growth, but reduce mortality of RPW (Farazmand 2002).

RPW gains entry into a palm when female weevils are attracted to palm tissue volatiles to lay eggs. The latter hatch into damage inflicting grubs. Fresh wounds on frond bases (petioles) attract RPW females for oviposition, which results in infestation (Abraham et al. 1998; Faleiro 2006). In this study, we assessed the mechanism of resistance to RPW in 7 date palm cultivars viz., 'Khalas', 'Sheshi', 'Reziz', 'Khasab', 'Hatmi', 'Shahal' and 'Gaar' from Al-Ahsa in Saudi Arabia by determining (i) response by adult female weevils to fresh palm volatiles in olfactometer assays,(ii) extent of egg laying (oviposition) in the cultivar in choice test studies,(iii) hatching of RPW eggs in the above cultivars and (vi) semi-field assays to ascertain damage due to larval feeding in each of the 7 cultivars.

Materials and Methods

Test Insects

Adult weevils used in the olfactometer assays and oviposition trials were collected from the field using insecticide free food baited -pheromone (Ferrolure™) traps and reconditioned in the laboratory for 2 weeks by allowing the adults to feed on sugarcane in plastic cages (27 ± 1 °C, 76 ± 3% RH). Second to third instar larvae of RPW (average weight: 0.1g) used in the feeding trial were obtained from the laboratory culture maintained on the natural host (date palm trunk: 'Khalas' cv).

Attraction to Palm Tissue Volatiles

To study the extent of attraction (preference) to fresh palm tissue volatiles emitted from seven date palm cultivars viz.,'Khalas', 'Sheshi', 'Reziz', 'Khasab', 'Hatmi', 'Shahal' and 'Gaar', assays were carried out using a four arm-choice olfactometer® that was custom-made by Analytical Research Systems, Inc., Florida (ARS Inc., Florida) (www.ars-fla.com/mainpages/Bio-Assay/4&6-Choice/4&6-Choice.htm) and calibrated to specifications before the experiment as mentioned in Table 1.

In each of the Inlet Odor Source (IOS) adapters of the olfactometer freshly cut palm petiole pieces (5 × 1 × 1 cm) of a single cultivar was placed. Two experiments including 4 cultivars each were carried out. The first experiment (I) included the cultivars 'Khasab', 'Shahal', 'Gaar' and 'Khalas' and the second (II) 'Reziz', 'Sheshi", 'Hatami' and 'Khalas'. The later served as the control treatment in both experiments. Fifteen day-old field collected gravid adult female weevils were used in the assays. Five female weevils were placed in the insect release device of the olfactometer. After 5 min, the number of adult female weevils collected in the Insect Isolation Trap (IIT) was noted. The time that elapsed between the placement of petiole pieces in the IOS and the transfer of 5 female weevils was one min. Each experiment was replicated 8 times and the time that elapsed between 2 consecutive replications was 10 min. At the end of each assay (replication), palm tissue pieces and test insects used in the assay were discarded. New palm tissue pieces and test insects were used for every replication (assay). The IOS was moved sequentially to the next arm of the olfactometer at the end of each test replication so that every treatment was at the same arm of the olfactometer twice during each trial. This was done to eliminate bias if any in the instrument and environment.

Egg Laying (Oviposition) and Egg Hatch (Hatchability)

Petiole fibers from green fronds of the above cultivars prepared as a firm cylinder 5 cm long

Table 1. Calibration of the four arm choice olfactometer to test attraction of the red palm weevil (RPW) to date palm volatiles.

Inlet / outlet pressure and air flow rate of the olfactometer®	Test calibration
1. Olfactometer pressure (10 to 20 PSI)	15 PSI
2. Source inlet pressure (50 to 150 PSI)	60 PSI
3. Olfactometer vacuum : Central suction (-5" to -22" Hg)	Hg -10"
4. Vacuum pump pressure (60+PSI)	+60 PSI
5. Olfactometer air inlet flow (0 to 1.3 LPM)	0.9 LPM

®ARS Inc. Florida, USA
PSI = pounds/square inch, "Hg = inches of mercury, LPM = liters per minute.

× 2 cm diam was offered to 5 fertile and gravid adult RPW females. These were caged together with 2 active adult male weevils in humid plastic boxes(60 × 40 × 35 cm) for a week in choice test trials to assess the extent of eggs laid in the seven date palm cultivars. The number of eggs laid (oviposition) and larvae hatched in each cultivar were recorded and then - after 7 days - carefully extracted from the petiole fibers using a fine camel hail brush. The eggs were then placed on moist Whatman™ paper in a plastic dish (1.5 × 6.0 cm), which was closed and set aside for hatching. The number of eggs hatched was recorded every alternate day for a week. Each of the 3 oviposition trials was replicated 6 times while the single test on egg hatch was replicated 5 times.

Extent of Damage (Feeding) by RPW Larvae under Semi-Field Conditions

Second to third instar larvae of RPW (average weight: 0.1g) were inoculated at the frond base (petiole) of the above cultivars (3 years old) planted in a mesh house at the Date Palm Research Centre, King Faisal University, Al-Ahsa, to assess the extent of feeding by the grubs. One larva was inoculated at the base of the petiole by drilling a hole 1.0 cm deep. Upon inoculation, the hole was closed with a piece of the palm tissue. Three replications were maintained per cultivar on 3 different petioles of the same palm. After 7 days, the inoculated frond base was detached from the palm, brought to the laboratory, cut open and the length of the tunnel produced by larval feeding was measured.

Data on mean weevil attraction to volatiles of the test cultivars in olfactometer assays, oviposition, hatch and larval feeding in the semi-field feeding assay were square root transformed and subjected to statistical analysis (ANOVA, $P = 0.05$) where treatment F, treatment/error df and true P values were computed using the randomised block design through the web-based agricultural statistics software package (WASP.1) available at www.icargoa.res.in.

Results

Results pertaining to olfactometer assays presented in Fig. 1 on the attraction of female weevils to palm tissue volatiles of different date palm cultivars revealed that in Experiment-I, adult female weevils were significantly more ($F = 4.18$, df = 3/21, $P = 0.018$) attracted to tissue volatiles of the cultivar 'Khalas' as compared to the other 3 cultivars where similar and low levels of attraction (high degree of antixenosis) to the volatiles of the cultivars 'Khasab', 'Shahal' and 'Gaar' were observed. In Experiment-II adult weevils showed high levels of attraction (low level of antixenosis) to fresh tissue volatiles of all the 4 cultivars tested ('Khalas', 'Reziz', 'Sheshi' and 'Hatmi'), which were equally ($F = 0.51$, df = 3/21, $P = 0.682$) attractive to RPW. Tests on the extent of oviposition show that the cultivars 'Shahal' and 'Gaar' were least preferred for egg laying by RPW, indicating that these cultivars were not only least attractive to adult female weevils for tissue volatiles, but were also not preferred for egg laying in the choice tests, thereby exhibiting a high degree of oviposition antixenosis (non-preference). Cumulative analysis confirmed significant ($F = 0.7.05$, df = 6/30, $P = 0.0001$) differences among treatment means, where a high degree of antixenosis for oviposition by RPW was seen in the cultivars 'Shahal' and 'Gaar' followed by 'Hatmi', 'Khasab', 'Khalas' 'Sheshi' and 'Reziz'. The cultivar 'Khalas' besides being most preferred by RPW in olfactometer assays for tissue volatiles also registered a high level of oviposition (Fig. 2).

Cultivar-wise hatching of eggs was high, ranging from 72.0 on 'Hatmi' to 100% on 'Khalas' where treatment means (Fig. 2) and were statistically similar ($F = 2.42$, df = 6/24, $P = 0.057$), indicating no antibiotic effects of the host cultivars. Further, results on the length of tunnels due to larval feeding on frond petiole indicate non-significant ($F = 1.20$, df = 6/12, $P = 0.368$) differences ranging from 0.67 ('Reziz') to 5.50 cm ('Khalas') among the cultivars, which also discounts the possibility of any antibiotic effect in the cultivars tested.

Our findings indicate that antixenosis plays an important role in the initial attraction of RPW to date palm cultivars and the subsequent extent of oviposition. Tissue volatiles of the cultivar 'Khalas' recorded the highest attraction by RPW, and thereafter high oviposition, maximum egg hatch and also the largest feeding tunnels of the larvae. Absence of antibiotic mechanisms in 'Khalas' and other cultivars tested diminish the ability of these date palm cultivars to withstand and recover from damage due to RPW after ovipostion.

Discussion

In general, tissue volatiles of the cultivar 'Khalas' were most attractive to the weevil. Recent studies carried out in Qatar (Elmeer et al. 2011) using new microsatellite markers to assess the genetic diversity among 10 major date palm cultivars (including 5 from our study) revealed 2 distinct groups. Among the cultivars we studied for resistance to RPW, the report from Qatar placed 'Khalas', 'Sheshi' and 'Reziz' in one cluster of the 6 cultivars while the cultivars 'Khasab' and 'Shahal' in another cluster of 4 cultivars. Since seedling date palms are the original source of most of the present well established cultivars (Johnson et al. 2013), the RPW susceptible ('Khalas', 'Sheshi' and 'Reziz') and resistant cultivars ('Shahal' and 'Khasab') may have evolved from 2 separate seed-

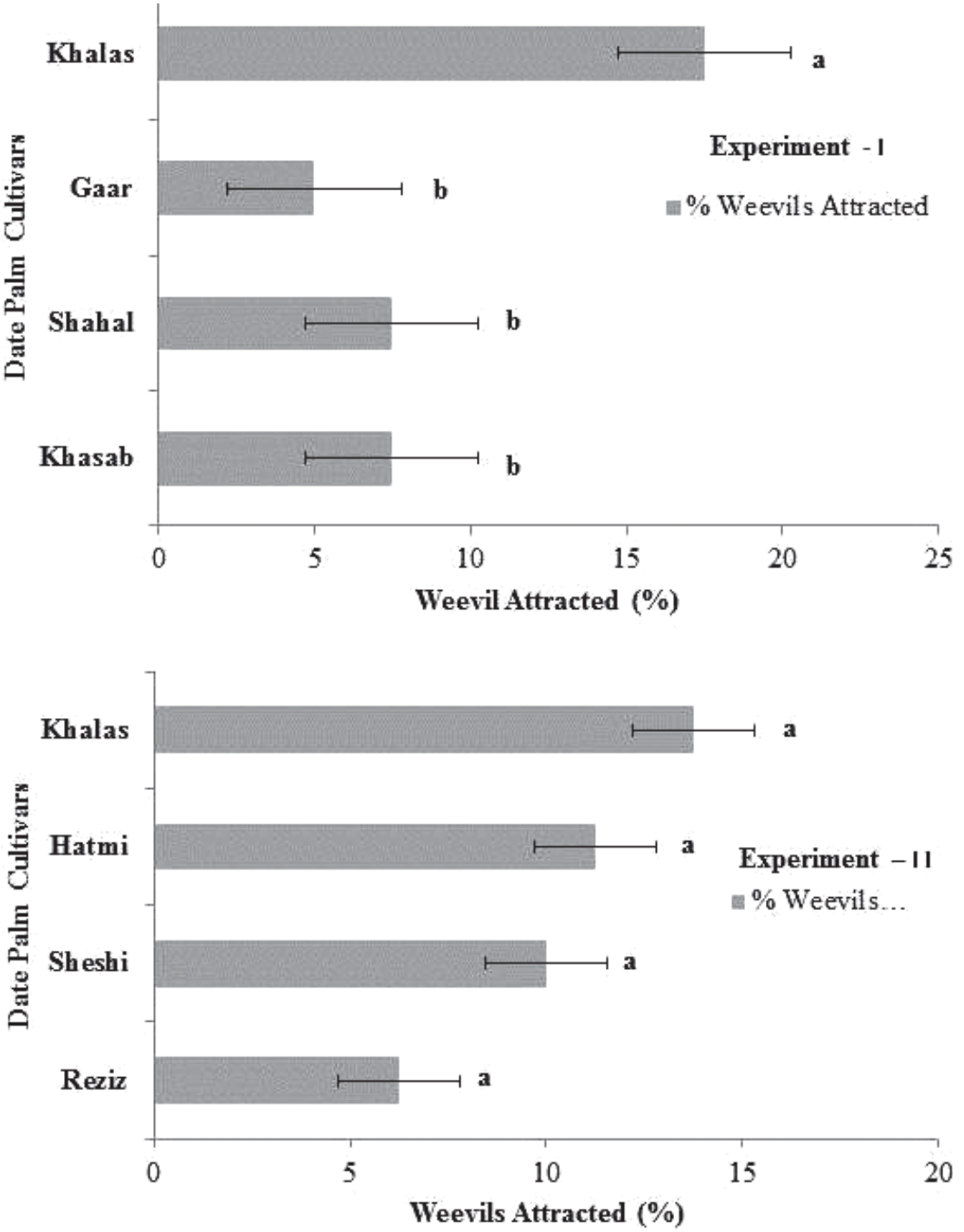

Fig. 1. Attraction of female red palm weevil (RPW) adults to date palm volatiles of various cultivars(Experiment I and II) in olfactometer assays. Bars denote standard error (SE).

ling date palm progenies with distinctly different genes for resistance to RPW.

Our findings are in agreement with previous reports from Saudi Arabia where the cultivar 'Shahal' was reported to be least preferred for egg laying by RPW among 25 date palm cultivars tested. The same study found Saudi Arabia's premier date palm cultivar 'Khalas' to be among the

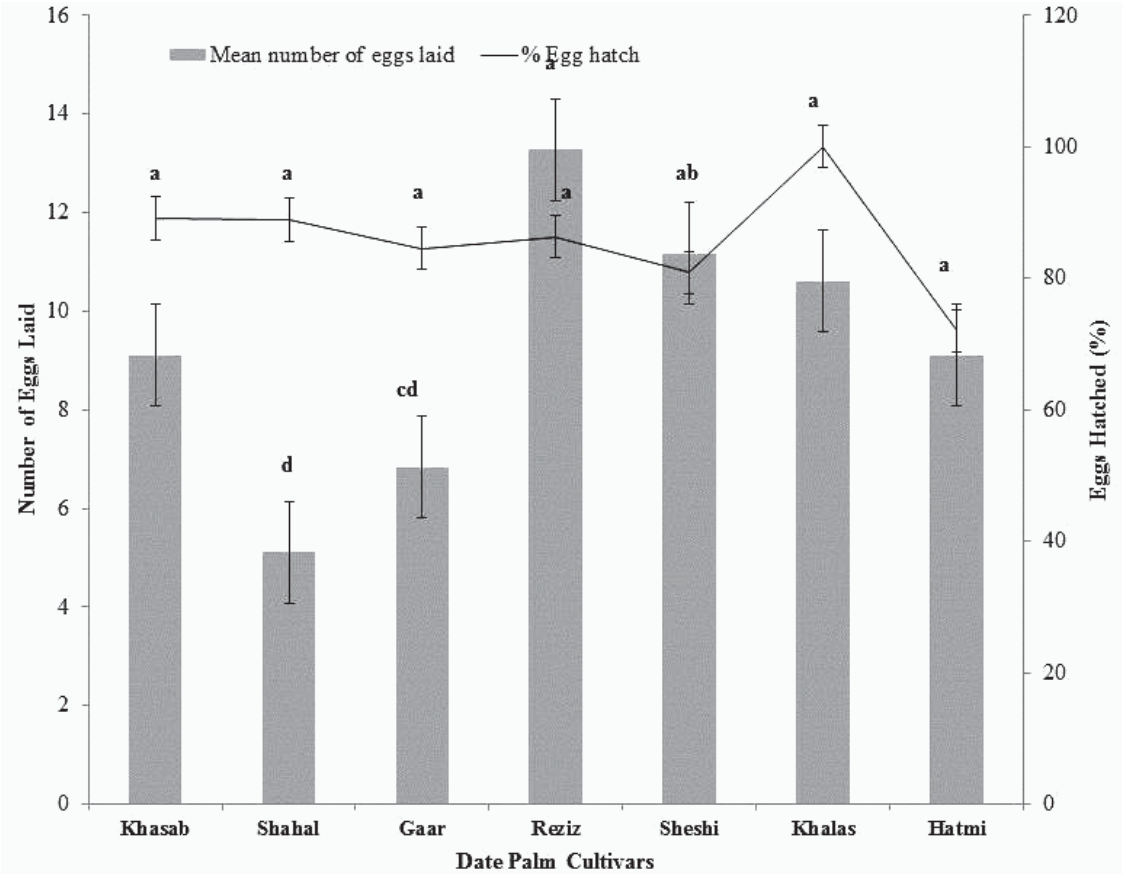

Fig. 2. Choice tests for oviposition and egg hatch by red palm weevil (RPW) in major date palm cultivars grown at the Al-Ahsa oasis in Saudi Arabia. Bars denote standard error (SE).

highly preferred cultivars for egg laying by RPW along with 'Anbara' in which the highest number of eggs were laid, and thereby exhibiting a low degree of oviposition antixenosis (Al-Bagshi et al. 2013). Oviposition antixenosis tests were carried out to identify resistant lines of sorghum against the spotted stem borer *Chilopartellus* (Sharma et al. 1992), and could serve as a protocol to determine resistance to a tissue borer like RPW in date palm.

Dembilio et al. (2009) studied the mechanisms of resistance to RPW in different palm species in Spain and found that *Washingtonia filifera* and *Chamaerops humilis* had antibiotic and antixenotic properties, respectively, against RPW, with *Phoenix canariensis* being highly preferred for the development of this pest. Similar studies in China showed that the development, survival and reproduction of RPW was better on *P. canariensis* and *W. filifera* as compared to *P. sylvestris,* which was the least suitable among the 5 palm species evaluated in the laboratory. On suitable host palms, RPW larvae may have fewer instars and thus, the developmental time can be shortened (Ju et al. 2011) resulting in higher frequency of adult emergence. In coconut and date palm, young palms less than 20 years old are mostly infested by RPW (Abraham et al 1998; Faleiro 2006) indicating that tissue hardness, which increases with the age of a palm, may deter RPW attack.

Laboratory rearing of RPW on different date palm cultivars in Saudi Arabia registered the longest lifespan of male weevils on the cultivar 'Khalas' followed by 'Sillaj', 'Sukary' and 'Khasab' (Al-Ayedh 2008). Though more cocoons were harvested from 'Khalas', frequency of adult emergence was better on Sukary (Al-Ayedh 2008). This study indicates low levels of antibiotic effects in the cultivar 'Khalas', and is in agreement with our findings.

The coconut cultivar, 'Chowghat dwarf green', was most preferred for egg laying by the RPW while 'Malayan dwarf' was least preferred (Faleiro & Rangnekar 2001). Reports from Iran suggest that calcium inhibits RPW growth, while date palm varieties with high sugar levels en-

hance oviposition and growth, while reducing mortality of RPW (Farazmand 2002).

Protecting wounds on palm tissue with insecticide immediately after frond shaving and offshoot removal in date palm from becoming oviposition sites for RPW is an important RPW-IPM practice (Abraham et al. 1998). Identifying the chemical components of tissue volatiles that trigger antixenosis in date palm to RPW will pave the way for future studies on chemical ecology of RPW and its interactions with date palm as a main host.

The perennial and heterozygous nature of date palm makes it difficult to identify genes for long lasting resistance to pests including RPW and to incorporate them into desirable cultivars through classical plant breeding programmes. Recently, the entire genome of the date palm cultivar 'Khalas' was sequenced (Al-Dous et al. 2011; Al-Mssallem et al. 2013).This could facilitate integration of genetic engineering techniques into date palm breeding programs that provide mechanisms to overcome the current constraints to conventional breeding in date palm and help to incorporate desirable traits of yield, quality, and resistance to abiotic and biotic stresses in date palm (El-Hadrami & Al-Khairy 2012). According to Al-Mssallem et al. (2013) stress resistance and sugar metabolism-related genes in date palm tend to be enriched in the chromosomal regions where the density of single-nucleotide polymorphisms is relatively low.

Over 50% of the area in the Al-Ahsa date palm oasis is planted to the cultivar 'Khalas' indicating its high preference among the farmers of Al-Ahsa with several new plantations in the susceptible age of less than 20 years (Sallam et al. 2012; El-Sabea et al. 2009). RPW is therefore likely to pose a major challenge to date farmers of Al-Ahsa and also in other date palm oasis within Saudi Arabia and in neighboring countries where the cultivar 'Khalas' is popular. Incorporating resistance to RPW in commercial date palm cultivars would significantly reinforce the current pheromone based IPM strategy against this lethal pest of date palm.

Acknowledgments

Support provided by the Date Palm Research Centre of Excellence, King Faisal University (KFU), Al-Ahsa, Saudi Arabia to carry out this study through the Saudi Aramco-KFU project on red palm weevil is gratefully acknowledged.

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