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REARING OPTIMIZATION OF RED PALM WEEVIL: *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE) ON DATE PALM: *PHOENIX DACTYLIFERA**

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ABSTRACT

Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (RPW), is the most deleterious pest of date palm *Phoenix dactylifera*, spreading rapidly in date palm growing countries around the globe. To facilitate behavioral and biological studies on this important pest, a study to optimize rearing procedures was conducted at King Saud University, Riyadh, Kingdom of Saudi Arabia. RPW pairs were introduced into date palm bolts and subsequent RPW populations were harvested at the pupal stage. The populations of RPW resulting from different numbers of introduced RPW pairs and the use of mated vs. virgin RPW pairs were compared for different date palm cultivars and sizes of the bolts. Results indicated that RPW can successfully be reared on date palm bolts. Subsequent population was increased with increases in numbers of introduced RPW pairs. Date palm cultivars significantly influenced the subsequent RPW population. Introducing mated vs. virgin RPW pairs did not show any significant effect on subsequent RPW population.

Key Words: Red Palm Weevil, Rhynchophorus ferrugineus, Rearing, Date palm cultivars

RESUMEN

El picudo rojo de las palmeras, Rhynchophorus ferrugineus Olivier (PRP), es la plaga más nociva de la palma dactilifera, Phoenix dactylifera, extendiéndose rápidamente en países donde sembran palmas dactiliferas alrededor del mundo. Para facilitar los estudios de comportamiento y biológia de esta importante plaga, un estudio para optimizar los procedimientos de crianza se realizó en la Unidad de Investigación de Entomología Económica (UIEE), Departamento de Protección Vegetal de la Facultad de Ciencias de Alimentación y Agricola, de la Universidad del Rey Saud, en Riad, Reino de Arabia Saudita. Se introducieron pares del PRP en pernos de los truncos de palmas dactiliferas y se cosecharon las subsiguientes poblaciones de PRP en el estadio de puparios. Se compararon las poblaciones de PRP resultantes de diferentes números de pares de PRP introducidos y el uso de pares de PRP con hembras acopladas versus las hembras virgenes para las diferentes variedades de palmeras dactiliferas y tamaños de los pernos. Los resultados indicaron que el PRP puede ser criado exitosamente en troncos de palmeras dactiliferas. Las poblaciones subsiguientes se incrementaron con el aumento en el número de pares de PRP. Los diferentes cultivares de palmeras dactiliferas influyeron significativamente la población de PRP subsiguiente. El grosor y la longitud del perno de palmera dactilifera se correlacionó positivamente con la población de PRP. La introducción de hembras acopladas versus hembras de PRP mostró ningún efecto significativo sobre la población PRP subsiguiente.

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* Olivier, is the most serious pest of the date palm, *Phoenix dactylifera*, and is spreading rapidly in date palm growing countries especially in the Persian Gulf region. The weevil has been reported from almost 50% of the date palm growing countries, where it is attacking at least 17 palm species (Faleiro 2006). The weevil was noticed for the first time in 1891 in India, and was reported as a pest of the coconut palm in 1906. Later in 1917, it also was reported on date palms (Mohan 1917). The weevil has established in every country of southeastern, southern and western Asia (EPPO 2005, 2008). In East Asia, RPW was recorded in Japan in 1975 in horticultural palms (Matsuura 1993), and China the weevil was detected in 2007 (Li et al. 2009). The weevil was also recorded in Australia (Li et al. 2009) and most recently from California USA (NAPPO 2010). In the Arabian Peninsula, the weevil was first detected on date palm in the mid 1980s (Gush 1997; Abraham et al. 1998; Murphy & Briscoe 1999). The weevil was reported from Saudi Arabia for the first time in 1986 in the Al-Katif Region (Al-Abdulmohsin 1987), from United Arab Emirates in 1986, and from the Republic of Iran in 1992. The weevil traveled to North Africa and was reported from Egypt in 1993 (Cox 1993).

Transport of date palm offshoots as a planting material has played a major role in rapid dispersal of the pest in the Middle East (Abraham et al. 1998). Azam et al. (2000) noted that 88-96% infestation resulted from offshoot removal and leaving the wounds without treatment. Agroclimatic conditions of the region, morphology of the date palm and modern farming systems have provided an environment conducive to the rapid establishment of red palm weevil in the Middle East (Abraham et al. 1998)

Damage to date palm is caused mainly by the feeding of larvae within the palm trunk. This cryptic feeding habit of the larvae makes it difficult to detect infestations at an early stage. The severe damage to the internal date palm tissues leads to the death of the date palm tree (Abraham et al. 1998). The weevil completes several generations per yr within the same host plant until the tree dies (Rajamanickam et al. 1995; Faghih 1996). Yield loss due to infestation can be mild to severe (Gush 1997).

Red palm weevil has been successfully reared on sugarcane (Rahalkar et al. 1972), and on artificial diet containing sugarcane bagasse, fresh coconut cake, brewer's yeast, sugarcane, potassium hydroxide, methyl parahydroxyl benzoate and sorbic acid (Rahalkar et al. 1978). Red palm weevil was reared on a semi-artificial diet in Egypt (Salama and Abdel-Razek 2002). Another artificial diet was developed using potato, carrot, casein, agar, cereals and vitamins B and D (El-Sebay et al. 2003). Al-Ayedh (2008) compared cv. 'Khalas', 'Khasab', 'Sillaj' and 'Sukkary' for the development of RPW and reported that the date palm cultivar significantly affects the growth and development of RPW. The objective of the present study was to optimize the red palm weevil rearing on date palm.

MATERIALS AND METHODS

Red palm weevil rearing on bolts of different date palm cultivars was studied at Economic Entomology Research Unit (EERU), Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. Pairs of RPW used to infest the bolts were collected as pupa from a colony established in the laboratory of EERU. The colony was maintained on 'Khasab', 'Khashram', 'Sillaj', 'Sufri', and 'Qanah' cultivars for 2 successive generations to eliminate the maternal diet effect. Each pupae were kept individually in a plastic container (7.0 cm diam. \times 8.5 cm ht) lined with a moist tissue paper to avoid cocoon drying, and kept under room temp (25 ± 2 °C) until adult eclosion. The pupal stage lasted in about 20 d. Newly emerged adults were paired in a mating container for 1 week and fed on sugarcane.

Date palm trees of 'Khasab', 'Khashram', 'Sillaj', 'Sufri', and 'Qanah' cultivars were obtained from the University Educational Farm, Diraab. The trees were about 3 to 4 yr old offshoots that had been taken from a date palm orchard maintained with normal farm practices. The trees were prepared for RPW rearing by removing all side and apical leaves, and the base. Originally, the trees were about 150-200 cm long with no offshoot. Each bolt, 100 ± 10 cm long and 20-40 cm thick, was cut longitudinally into 2 halves. A cavity (ca. 25 cm long × 10-cm wide × 10 cm deep) was carved for RPW introduction into the inner side of one-half of each bolt. After 2 to 20 mated or unmated RPW pairs were placed into the cavity, the 2 halves were joined together by steel wire and transferred to steel cages at ambient temp (25 ± 2) °C, 25-30% RH) to enable egg laying. The trunks were reopened after 7-days, the introduced pairs of RPW were removed, and the trunks were resealed and held in cages for egg hatching and subsequent larval growth. Each cage consisted of a steel frame covered with fine double steel mesh on each side, and the dimensions were 6 ft (183 cm) $tall \times 3$ ft (91.5 cm) wide $\times 3$ ft (91.5 cm) long. The environmental conditions in the cages were similar to ambient. The date palm trunks were reopened after 57 d.

In first experiment 3, 4, 5, 6, and 9 pairs of mated and unmated red palm weevil, respectively, were placed in 'Khasab,' 'Sillaj,' 'Qanah,' 'Sufri,' bolts with varying no. of replicates. Mated weevils were those that had already mated before they had been introduced into the date palm bolts, while unmated weevils were those that were virgin at the time of introduction and supposedly mated subsequently within the date palm bolts. The introduced weevil pairs were removed after 1 wk. The bolts were reopened after 57d to collect or harvest the resultant RPW population in order to compare the effect of introducing mated vs unmated RPW pairs.

In second experiment, 2, 3, 4, 6, 10 and 20 RPW pairs of mated adults, respectively, were placed in 'Khasab,' 'Sillaj,' 'Qanah,' 'Sufri,' bolts (with varying no. of replicates but not less than 3) and removed after 1 wk. The date palm bolts were reopened after 57d to collect or harvest the resultant RPW population in order to compare the effect of introducing varying number of RPW pairs. In third experiment, 4 pairs of mated adults were placed in 'Kashram,' 'Khasab,' 'Sillaj,' and 'Qanah,' bolts (replicated 3 time) and removed after 1 wk. The date palm bolts were reopened after 57d to collect or harvest the resultant RPW population to compare the effect of each cultivar on the size of the RPW population.

In fourth experiment, 2, 3, 4, and 50 RPW pairs of mated adults were placed in 'Khasab,' bolts (replicated 3 times) and removed after 1 wk. The date palm bolts were reopened after 57d to collect or harvest the resultant RPW population in order to correlate date palm bolt size and the size of the resultant RPW population.

The experiments were laid out in a completely randomized design with unequal no. of replications but not less than 3 per treatment. Date palm cultivars, bolt thickness (20-40 cm), and bolt length (100-150 cm), number of introduced RPW pairs, and reproductive status of introduced RPW pairs (mated vs. unmated) were the parameters tested for subsequent RPW population density per bolt. Al-Ayedh (2008) had already studied in detail the RPW growth and development related parameters, such as span of the larval stages, no. of instars, pupal span and adult span, fecundity and fertility. Data was analyzed using the oneway analysis of variance (ANOVA) PROC GLM procedure of SAS (SAS 9.2 2011). Means in different treatments were separated by least significant difference (LSD) at (P = 0.05).

RESULTS AND DISCUSSION

In these experiments, the introduction of virgin vs. mated RPW pairs into date palm trunks for laying eggs did not show any significant effect for 3 RPW pairs (F = 0.00; df = 1, 32; P = 0.98); 4 RPW pairs (F = 0.07; df = 1, 29; P = 0.79); 5 RPW pairs (F = 0.02; df = 1, 8; P = 0.90); 6 RPW pairs (F = 0.04; df = 1, 13; P = 0.84); 9 RPW pairs (F = 1.11; df = 1, 7; P = 0.33) (Table 1). Consequently, we pooled the data for virgin and mated pairs in the rest of the study.

The number of RPW pairs introduced per date palm trunk significantly affected the subsequent population size (total of larvae, pupae, and adults) in 'Khasab' (F = 17.35; df = 3, 34, P < 0.0001), 'Qanah' (F = 14.12; df = 4, 34; P < 0.0001), and 'Sillaj' (F = 44.45; df = 3, 14; P < 0.0001), but not 'Sufri' (F = 0.01, df = 1, 9; P = 0.92) (Table 2). There was no significant difference between populations of 2 to 4 pairs but an increasing trend was recorded with increases in no. of RPW pairs. Significantly higher populations of RPW were recorded with 6 and greater no. of pairs when compared with fewer RPW pairs.

The date palm cultivar had a significant impact (F = 4.99; df = 3, 11; P = 0.03) on the subsequent RPW population in tests when 4 RPW pairs were introduced. Significantly the largest RPW population was harvested from 'Khashram' followed by 'Khasab', 'Qanah' and 'Sillaj', respectively. Results indicated no significant differences among the rates of development on different date palm cultivars of larval (F = 0.99; df = 3, 11; P =0.44) and pupal (F = 1.37; df = 3, 11; P = 0.32) stages, but there were significant differences for adults (F = 8.15; df = 3, 11; P = 0.01). It appears that with 'Khashram' RPW development may have been faster, as there were more larvae/pupae that reached the adult stage. Possibly the large number of larvae produced in 'Khashram' bolts may have included some in the F2 generation of the introduced RPW pairs (Table 3).

Average RPW population sizes harvested from the date palm trunks were correlated with the bolt thickness and length in comparisons for the range of 2 to 5 introduced pairs of RPW. Results indicated a significant positive correlation with date palm bolt thickness but bolt length did not show any significant correlation (Table 4).

The differential effect of cultivar used as the bolt on the population growth of the RPW suggests that date palm tissue quality not only effects food consumption, survival, and development of the larvae but also weight, size, reproductive ability, and longevity of adults (Al-Ayedh 2008; Leather 1990; Tammaru 1998). In the present study no significant difference has been observed on rate of development, as no differences have been observed between populations of immature stages harvested from date palm trunks.RPW population growth was positively

TABLE 1. EFFECT ON AVERAGE RPW POPULATION SIZE OF INTRODUCING VIRGIN VS MATED RPW PAIRS INTO DATE PALM BOLTS.

	No. of RPW Pairs Introduced				
Mating Status	$\begin{array}{c} 3\\ (\text{Mean} \pm \text{SE}, N) \end{array}$	$\begin{array}{c} 4\\ (\text{Mean} \pm \text{SE}, N) \end{array}$	$5 (\text{Mean} \pm \text{SE}, N)$	$\begin{array}{c} 6\\ (\text{Mean} \pm \text{SE}, N) \end{array}$	9 (Mean \pm SE, N)
Virgin Pairs Mated Pairs	,	· · · ·	,	91.25 ± 18.09 a, 4 86.30 ± 13.70 a, 10	· · · · ·

Means within a column followed by the same letter are not significantly different $\alpha = 0.05$.

	Khasab	Qanah	Sillaj	Sufri
RPW Pairs Introduced	$(Mean \pm SE, N)$	(Mean \pm SE, N)	$(Mean \pm SE, N)$	$(Mean \pm SE, N)$
2	39.00 ± 7.88 b, 7	15.00 ± 2.52 d, 3	36.67 ± 4.06 b, 3	_
3	27.25 ± 5.57 b, 8	36.33 ± 5.50 cd, 6	44.00 ± 4.03 b, 6	_
4	41.93 ± 7.15 b, 14	45.40 ± 6.29 bc, 10	31.33 ± 11.29 b, 3	_
6	118.67 ± 14.93 a, 6	64.50 ± 8.60 b, 8	_	_
10		_	153.00 ± 14.43 a, 3	169.29 ± 19.40 a, 3
20	_	99.00 ± 8.42 a, 8		174.00 ± 62.22 a, 7

TABLE 2. EFFECT OF NUMBER OF RPW PAIRS INTRODUCED INTO DATE PALM BOLTS ON MEAN RPW POPULATION SIZE IN DIFFERENT DATE PALM VARIETIES.

Means within a column followed by the same letter are not significantly different $\alpha = 0.05$.

TABLE 3. NUMBERS OF RPW OF DIFFERENT DEVELOPMENTAL STAGES HARVESTED FROM VARIOUS DATE PALM CULTI-VARS AFTER INTRODUCTION OF FOUR MATED PAIRS.

	Average pooled and various stages of RPW population collected from different date palm cultivars			
Date Palm Cultivars	Larvae (Mean ± SE, <i>N</i>)	Pupae (Mean \pm SE, N)	$\begin{array}{c} \text{Adults} \\ (\text{Mean } \pm \text{SE}, N) \end{array}$	Pooled Population (Mean \pm SE, N)
Khashram	24.67 ± 17.22 a, 3	32.67 ± 14.77 a, 3	35.33 ± 11.67 a,3	92.67 ± 15.93 a, 3
Khasab	7.00 ± 2.88 a, 3	53.00 ± 7.51 a, 3	0.67 ± 0.67 b, 3	60.67 ± 9.68 ab, 3
Sillaj	5.00 ± 2.08 a, 3	25.00 ± 11.14 a, 3	1.33 ± 1.33 b, 3	31.33 ± 11.29 b, 3
Qanah	10.67 ± 2.67 a, 3	$41.00 \pm 4.62 \text{ a}, 3$	3.33 ± 0.67 b, 3	55.00 ± 6.00 ab, 3

Means within a column followed by the same letter are not significantly different $\alpha = 0.05$.

correlated with the thickness of the date palm trunk, probably because thick date palm trunk provides more space and food to accommodate and support the development of the RPW population.

RPW can be reared on both natural and synthetic (semi-artificial) diets (Salama & Abdul-Razek 2002). Among natural diets, RPW has been successfully cultured on cut petiole or stem tissue of coconut palms (Krishnakumar & Maheswari 2004; Rananavare et al. 1975), sugarcane (Rahalkar et al. 1972; Rananavare et al. 1975), and date palm trunks (Al-Ayedh 2008). RPW has also

TABLE 4. PEARSON CORRELATION COEFFICIENTS (R) BE-TWEEN HARVESTED RPW POPULATION AND THE LENGTHS AND THICKNESSES OF DATE PALM BOLTS, BASED ON NUMBER OF RPW PAIR INTRO-DUCED.

RPW Population/ No. of RPW Pairs	Date palm length	Date palm thickness
2	-0.06	0.40
3	0.42	0.48^{*}
4	0.40	0.75^{*}
5	0.20	0.79^{*}

*Indicating significance at P < 0.05.

been successfully reared on artificial diet containing sugarcane bagasse (fibre), coconut cake, yeast, sucrose, minerals, vitamins and preservatives (Rahalkar et al. 1978, 1985) and on another recently developed semi artificial diet containing wheat flour, corn flour, shredded date palm fronds and water (Al-Ayedh 2011).

A literature review revealed that various natural and synthetic diets have been used for the rearing of RPW. However, rearing on artificial diet is more difficult and labor intensive because it requires diet preparation and replacement of the diet on a regular basis, and the handling of delicate larvae. Most of the artificial diets are susceptible to microbial contamination, which sometimes resulted in complete colony failure. Therefore diet preservatives are needed that might have negative impacts on insect health. Rearing of the RPW on date palm bolts has the advantages that it is less labor intensive, less costly, and unlikely to involve microbial contamination. Moreover when reared in bolts, the larvae are exposed to an environment that is nearly natural for them, and they grow more rapidly and without any interruption than on an artificial diet.

In conclusion, RPW can be easily and efficiently reared on date palm bolts on a large scale. The date palm cultivar, 'Khashram', was found to be the best among tested date palm cultivars in terms supporting reproduction and most prolific growth of the RPW population. RPW populations reared in bolts increased with increasing number of RPW pairs and date palm bolt diam.

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