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A study on a trap for autodissemination of the entomopathogenic fungus *Beauveria bassiana* by red palm weevil adults in date palm plantations

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Abstract: A trap was designed to allow red palm weevil adults to pass through it so that they come out contaminated with high density of the fungus conidia for spreading them amongst the red palm weevil population in date palm plantations. A fungus inoculum containing 10% conidia was prepared and used in the trap. An adult was contaminated with $9.53x10^7$ conidia per trap visit with a lethal time of 8.25 days. Field trials were carried out using 20 traps in 3 date palm plantations in the period from April 2006 to May 2007 in the Northern Region of the United Arab Emirates. Efficiency of the trap was evaluated by assessing the monthly mortality caused by the fungus in the insect population. In the last two months, mortality of adults caused by the fungus in the field population ranged from 41.2-51.3% compared to 4.8– 4.9% in the control. Results showed that the trap is effective for spread of the fungus *Beauveria bassiana* in *Rhynchophorus ferrugineus* population.

Key words: transmission, fungus conidia, adult population, date palm plantations.

Introduction

The red palm weevil (RPW) *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) is a serious insect pest infesting date palm, *Phoenix dactylifera* in Arabian Gulf Region and other different countries including Egypt (Nirula, 1956; Saleh, 1992; El Ezaby *et al.* 1998; Faleiro, 2006). In date palm plantations, adults occur in the basal buried part of the trunk and in the soil where they aggregate, feed, mate and lay their eggs in the succulent tissues of the tree leaf axils. The larvae occur inside complicated tunnels in the tree trunk, where they feed on the soft tissues, till the trunk is completely hollowed out and the tree dies. The Biological Control Project (BCP) of Red Palm Weevil (3rd phase) was conducted and directed by Arab Organization for Agricultural Development (AOAD) in 2004 with the objective of developing integrated biological control technology against the insect using local microbial control agents. Laboratory and field investigations using a local strain of the entomopathogenic fungus, *Beauveria bassiana* in United Arab Emirates indicated that the foliar applications of the fungus are efficient for the control of RPW (El-Sufty *et al*. 2007; 2009). However, more advantage is essential to increase the control effciency of the fungus in date palm plantations to achieve long term management of this destructive insect. In the last few years, the use of a proportion of the target insect population to facilitate the pathogen dispersal in an insect population has received considerable attention (Vega *et al.* 2000, Furlong & Pell 2001, Vail *et al*. 2006).

The present paper reports on a control method that manipulates a proportion of the field adult population of the red palm weevil to disperse the fungus in the wider insect population.

Materials and Methods

Fungus inoculum

The fungus used was a local strain of *Beauveria bassiana* (UAE-B2), which has been isolated for the first time by BCP and was found to be potentially effective for RPW control. The fungus was identified by CABI Bioscience, UK and samples were also dispatched to Brook University, Canada for finger printing. The fungus was maintained on Sabouraud dextrose yeast agar (SDAY) as described by Goettel and Inglis (1997) with regular passages through RPW-adults. For mass production of conidia, the fungus was cultured on a medium composed of 40 g granulated rice grains, 8 g peptone, 10 g agar and 2g yeast extract per 1L water as described by El-Sufty *et al*. (2007). Dried conidia were used to prepare a powder formulation composed of 55g sand, 35g talkum and 10 g conidia. One g of the powder contained $5x10^9$ conidia. The powder bio-preparation was freshly prepared and used as inoculum in the autodissemination trap.

The trap

The trap was designed as one way road. It allows passing of RPW adults (males and females) inside it so that they come out with their bodies contaminated with a large quantity of conidia to spread them in the field population by passive transfer from the contaminated adults to the healthy ones and by indirect contamination by conidia deposited in the habitat. The trap consists of two chambers; an attractive chamber with two entrance openings, baited with RPW aggregation pheromone bag and a bottle containing 15 ml palm tree kairomone and a contamination chamber with one exit, which was furnished with a Petri dish cover (15 cm diameter) containing 15g of the fungus inoculum. When the

adult weevil is attracted to the trap and enters the attractive chamber, it falls down to the contamination chamber. The trap was partially buried in the ground up to the openings of its attractive chamber and the exit opening of the contamination chamber was free so that the contaminated adults can leave the trap.

Bioassays

Adults of RPW used in all bioassays were collected from date palm plantations using insecticide free pheromone-kairomone traps developed by BCP and maintained under room conditions for a week in plastic containers provided with food (moistened pieces of palm wood). Dead and injured insects were discarded and healthy ones were used in the experiments. A powder formulation composed of simple inert substances mixed with dry *B. bassiana* conidia was prepared and used in dose-mortality assays against RPW adults. Conidia were added at ratios of 5%, 10% and 20% to the carrier substances and evaluated for their virulence as described by Lacey *et al*. (1994). Five adult weevils were introduced into a plastic container closed with a cap. One g of the preparation was added to the container. For each dosage, 4 replicates were used. The containers were periodically rotated end-over-end for 2 min. Insects of a replicate were removed and maintained inside a plastic container provided with palm wood pieces as food. Control insects were treated with inert substances without conidia. Containers were kept under room conditions. Treated and control insects were daily examined, dead individuals removed and recorded. Mortality and lethal time were calculated.

To determine the conidial density contaminating a RPW adult after a trap visit, 10 RPW adults were allowed to enter a trap supplied with 15 g fungus preparation of 10% conidia one after the other. Time response for an adult to leave the trap was recorded and then each adult was introduced into a screw neck vial containing 10 ml water and 0.1% tween 80, vigorously agitated and number of conidia per cadaver was determined by direct enumeration using haemocytometer (Goettel and Inglis, 1997).

To determine mortality and lethal time for the contaminated adults leaving the trap, 20 RPW adults were allowed to enter a trap supplied with 15 g of the fungus inoculum (10% conidia) one after the other. Each 5 adults were maintained inside a plastic container provided with palm wood pieces as food. Control insects (20 adults) were treated with inert substances without conidia. Containers were kept inside screen cages (50X50X50 cm) placed in a date palm plantation. Insects were daily examined, dead individuals were recorded and mortality as well as lethal time were calculated.

Trap visiting rate and other insect visitors

To evaluate visiting activity of RPW adults to the trap in the field and identify the other insect species visiting it, five traps free of fungus inoculum and without exit of contamination chamber were installed at equal distances of 100 m apart in a date palm plantation for 10 weeks starting on the 4th of March 2005. Readings were weekly taken, number of RPW adults was recorded and other insect species were identified.

Survival of the fungus inoculum in traps

To assess survival of the fungus inoculum in the trap, 4 traps (4 replicates) were installed in a date palm plantation at equal distances of 100 m apart. Each trap was furnished with 15 g of a fungus powder formulation containing 10% *B. bassiana* conidia. Other four quantities of the fungus formulation inside Petri dishes were kept in the laboratory and served as control. Samples, each of 0.5 g of the fungus formulation were weekly taken from a trap as well as from a Petri dish and conidia viability were determined by germinating propagules on a translucent SDAY medium on slide (Goettel and Inglis (1997). Percentage of germination was assessed for 6 weeks.

Field experiments

Twenty autodissemination traps were installed in 3 date palm plantations (DPP) at Ras Al Khaima, United Arab Emirates; DPP-1, 8 ; DPP-2, 8 and DPP-3, 4 at equal distances of 100 m apart. Traps were experimented from April 2006 to may 2007 and each one was monthly supplied with a slow release bag containing 700 mg of the aggregation pheromone (methyl-5 nonanol and 4-methyl-5-nonanone) which releases an average of 10-20 mg /day (Chemaica International S.A., Costa Rica) and 25 ml bottle containing 20 ml of a kairomone mixture extracted locally from date with 2 mm hole in its cover allows the release 0f 0.3 ml kairomone/day (AOAD, Progress report, 2001). At 4 weeks intervals, each trap was supplied with 15 g of the fungus inoculum in a Petri dish cover fixed in the center of its contamination chamber. A plantation located about 5 km far from the experimental plantations (DPP-4) served as control.

To evaluate the distribution of the fungus in the RPW adult populations of the 4 plantations, terrestrial food-baited aggregation traps were used and installed at equal distances of 200 m apart (2 traps for DPPs 1, 2, and 4 and 1 trap for DPP 3). The food-baited traps were weekly examined and RPW adults were collected in the period extended from March 2005 to May 2007. For each weekly trap sample, cadavers of dead weevils were disinfected by immersing in 1% sodium hypochlorite for 30 seconds, washed in sterile water and exposed to mycosis test (Lacey and Brooks, 1997). The cadavers were individually placed on moistened filter papers inside Petri dishes. The dishes were maintained at room temperature for 10 days and the fungus growth was observed. The alive adults were individually

maintained inside plastic boxes provided with moistened pieces of palm wood for 7 days and the dead ones were recorded and tested for mycosis as previously described. Cadavers showing external growth of *Beauveria bassiana* were considered killed by the fungus.

Statistical Analysis

Mortalities were corrected according to Abbot's formula (1925) and analysis of variance was used to evaluate the impact in bioassays.

Results and Discussion

Fungus inoculum and visit duration of an adult in inoculation chamber

In order to obtain a suitable and effective fungus inoculum for the trap, three powder formulations with conidial concentrations of 5, 10 and 20% were bioassayed for their virulence against RPW adults. The results showed that the three formulations caused 90, 100 and 100% mortality with lethal times of 10.3, 7.6 and 6.8 days with significant differences between the first and the other two concentrations. Therefore the formulation of 10% conidia was used in the following bioassays and in the trap.

Assessment of conidial density contaminating a RPW adult after a visit of a trap supplied with 15 g fungus inoculum containing 10% *B. bassiana* conidia indicated that an adult was contaminated with $9.53X10^7$ conidia. The adult visit duration to a trap ranged from 1-6 min (average 2.3 min). Similar results were obtained by Furlong *et al*. (1995) for an autodissemination trap of the entomopathogenic fungus *Zoophthora radicans* by adults of *Plutella xylostella* (Lepidoptera: Yponomeutidae), who reported that the moth remained in the contamination chamber for 1.47 min. to be contaminated with an amount of conidia enough to cause satisfied mortality.

Mortality response of adults

All the contaminated RPW adults visited the trap showed *B. bassiana* mycosis symptoms and killed by the fungus after 5-11 days with a mean of 8.25 days (Fig. 1). This means that the contaminated adults could carry and transfer the fungus conidia among its population for a suitable period before their death.

RPW adult visiting rate and other insect visitors

Under date palm plantation conditions, the RPW adult visiting rate to a trap was 2.84 adults/week. The rate appears to be low and the trap may need more improvement to facilitate its attraction power. On the other hand, the trap attracted other different insects which included species from Order Orthoptera (grasshoppers and crickets), Hymenoptera (ants), Dictyoptera (roaches) and Coleoptera (soil beetles and chafers). These insects have the ability to increase the fungus dessimination by transferring conidia from site to another and spread them in RPW habitat during their activity. In a similar study, Dowd and Vega (2003) reported that the sap weevil was able to transfer *B. bassiana* conidia from an inoculative device to the overwintering sites of the insects.

Inoculum viability in inoculation chamber

Data illustrated in Fig. 2 indicate that the fungus conidia remained viable inside the inoculation chamber of the trap for 4 weeks with germination percentages ranging from 89.2-96.5%. Conidial viability significantly decreased to 66.7% during the 5^{th} week. Therefore, the fungus inoculum was replaced by a fresh one at 4 weeks interval.

Efficiency of the trap in date palm plantations

Figures 3, 4 and 5 demonstrate that the trap was efficient as autoinoculative device to spread the entomopathogenic fungus *B. bassiana* amongst RPW adult population in date palm plantations. In each of the three experimental plantations, the fungus killed a proportion of the population during two successive seasons which extended from April 2006 to May 2007. In the three plantations, the fungus caused monthly mortality ranging from 3.6-16.7% during the first five months, then the mortality steadily increased to reach 14.3-34.4% during the following 5 months with some fluctuations often due to the natural population fluctuation of the insect. In the last four months, mortality caused by the fungus

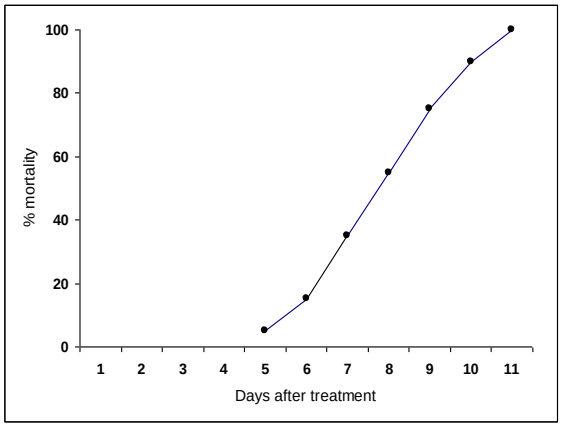

Fig 1: Mortality curve of *Rhynchophorus ferrugineus* adults attracted to the trap and fell into the inoculation chamber provided with 15 g of a powder formulation containing 10% *Beauveria bassiana* conidia.

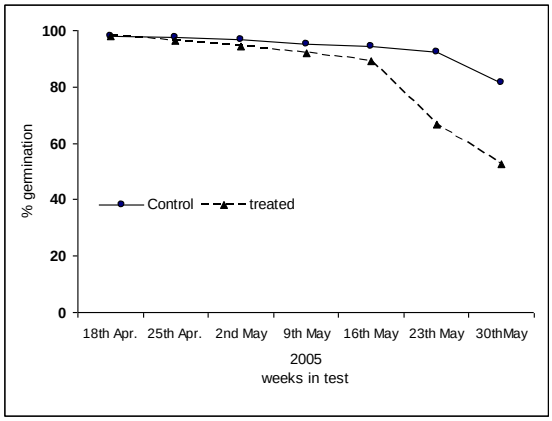

Fig 2: Survival of *Beauveria bassiana* conidia in the inoculation chamber of the trap under field conditions compared with conidia maintained under laboratory conditions

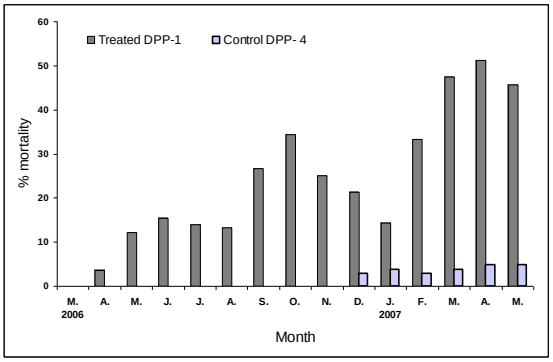

Fig 3: Mortality of red palm weevil adults as a result of autodissemination of the fungus *Beauveria bassiana* in its population in the date palm plantation-1 in Ras Al khaima, United Arab Emirates.

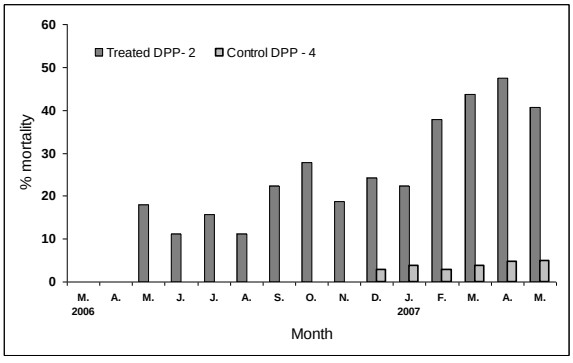

Fig. 4: Mortality of red palm weevil adults as a result of autodissemination of the fungus *Beauveria bassiana* in its population in the date palm plantation-2 in Ras Al khaima, United Arab Emirates.

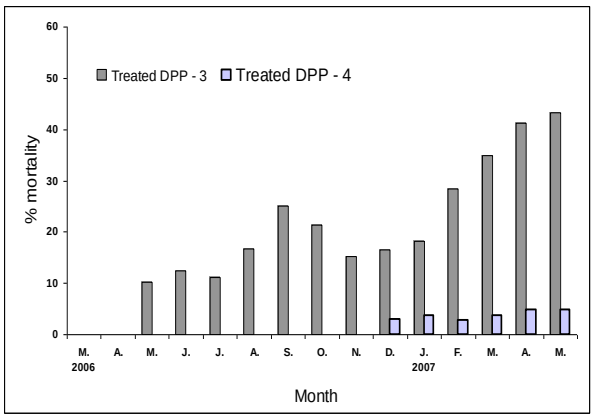

Fig 5: Mortality of red palm weevil adults as a result of autodissemination of the fungus *Beauveria bassiana* in its population in the date palm plantation-3 in Ras Al khaima, United Arab Emirates.

sharply increased to reach maximum values (45.7-51.3, 40.7-47.5 and 41.2-43.3% of the adult populations in DPP-1, DPP-2 and DPP-3 respectively) compared to 4.8-4.9% in the control. In the control plantation (DPP-4), which was about 5 km far from the treated ones, RPW adults killed by the fungus were detected 7 months after starting the experiment. At the same time (February – May 2007) mycosed RPW adults infected with *B. bassiana* were collected in some other plantations, where the fungus was not applied. These results indicate that the trap was designed to be effective in spreading the fungus by RPW adults in the wider population. It is clear that the adult weevils themselves function as very efficient carriers and disseminators of the fungus conidia. Because RPW adults are susceptible to *B. bassiana* infection (El-Sufty, 2009), routes of infection of the population individuals were direct contamination by passive transfer and indirect transmission of conidia from the sporulating mycosed cadavers killed by the fungus in the habitat. Furlong and Pell (2001) found that adults of diamondback moth, could effectively transport the two entomopathgenic fungi, *B. bassiana* and *Zoophthora radicans* from the source of inoculation to the field population. Huger (2005) referred to a successful release of the *Oryctes* virus in the population of the coconut palm rhinoceros, *Oryctes rhinoceros* (Col.: Scarabaeidae) by the adult beetles and considered this method as a classical biological control, which can bring the target insect pest under control. These results represent the first report on the trap autodissemination of *B. bassiana* by RPW. Additional work is still needed to improve its effectiveness and to increase the mortality rates.

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