REDIA, XCVI, 2013: 57-67

VALERIA FRANCARDI (*) - CLAUDIA BENVENUTI (*) - GIAN PAOLO BARZANTI (*)
PIO FEDERICO ROVERSI (*)

AUTOCONTAMINATION TRAP WITH ENTOMOPATHOGENIC FUNGI: A POSSIBLE STRATEGY IN THE CONTROL OF *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER) (COLEOPTERA CURCULIONIDAE)

(*) *Consiglio per la ricerca e la sperimentazione in agricoltura - Research Centre for Agrobiology and Pedology, via Lanciola 12/A, 50125 Firenze, Italy;* e-mail: valeria.francardi@entecra.it

Francardi V., Benvenuti C., Barzanti G., Roversi P.F. – Autocontamination trap with entomopathogenic fungi: a possible strategy in the control of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Curculionidae).

An experimental autocontamination trap was devised to infect *Rhynchophorus ferrugineus* (Olivier), the Red Palm Weevil, adults with entomopathogenic fungi. The aim was to develop an autocontamination device to support integrated *R. ferrugineus* control programs. In laboratory bioassays, the delivery system successfully attracted, infected and released weevil adults after they contacted cereal substrata inoculated with indigenous strains of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin. Tests carried out with the experimental traps showed that *M. anisopliae* was the more virulent pathogen, causing 75% cumulative mortality in adults, while *B. bassiana* gave a 45% cumulative mortality. Infectivity of *M. anisopliae* was not affected by different cereal substrata, *i.e.* wheat and rice, since curculionid cumulative mortality (95%) and treatment efficiency (95% Abbott) were very high on both of them and Red Palm Weevil LT_{50} was reached within the same time (15 days). Conidial persistence and germinability of *M. anisopliae* grown on the rice substratum were examined in field conditions inside traps located in sunny and shady positions in spring, summer and autumn. The results showed that the traps preserved fungal inoculum stability longer in spring and summer than in autumn. No significant difference in *M. anisopliae* conidial persistence was found between sunny and shady traps during the various seasons.

KEY WORDS: Red Palm Weevil, microbiological control, *Beauveria bassiana*, *Metarhizium anisopliae*, autocontamination trap.

INTRODUCTION

Increasing efforts are being made to devise a valid strategy for management of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier), the most harmful pest of several species of palms in the Mediterranean area (EPPO, 2008). These efforts involve chemical treatments and sanitation cuttings, alone or in combination, to save the palms and to limit the insect's spread in the environment. Chemical insecticides are efficient in Red Palm Weevil control but, as they are short-lived, they need to be applied periodically, with possible negative consequences for human health and the emergence of resistance in the insect (FERRY & GOMEZ, 2002; FALEIRO, 2006; LLÁCER *et al.,* 2012a). Microbiological treatments with the entomopathogenic fungi *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin offer an alternative and ecologically compatible pest management strategy (INGLIS *et. al.*, 2001). Their effectiveness and persistence in the field may be increased by auto-dissemination devices, which have been proved effective in the control of various groups of insects (VEGA *et al.,* 1995). The success of the autoinfection system depends on the virulence and persistence of the entomopathogen strain, and the efficacy of the autodisseminator in attracting the target insect and transmitting inoculum to it (FURLONG & PELL, 2001; KREUTZ *et al.,* 2004; QUESADA-MORAGA *et al.*, 2004, 2008).

The recent identification of strains of *B. bassiana* and *M. anisopliae* with high virulence against the Red Palm Weevil has increased the possibility of a more efficient microbiological control of the curculionid. Laboratory studies have been conducted to determine efficacious methods to infect Red Palm Weevil adults *via* different contaminated substrata, such as a natural diet (date pulp) and cereals (rice and wheat) (DEADMAN *et al,.* 2001; GINDIN *et al*., 2006; FRANCARDI *et al.,* 2012). Field studies have examined inoculum transfer *via* specimens treated with conidial suspensions or contaminated with a fungus inoculum in an attractive trap (EL-SUFTY *et al.,* 2011; SEWIFY *et al.,* 2009, DEMBILIO *et al.,* 2010). Recently LLÁCER *et al.* (2012b) advanced the possibility of using sterile irradiated males as a vector of *B. bassiana* for microbiological control of *R. ferrugineus.*

Various types of "attract-infect and release" devices have been set up to control insects of agricultural importance via entomopathogenic fungi and they have been tested in laboratory and field assays with promising results (VEGA *et al.,* 1995; KLEIN & LACEY ,1999; MANIANIA, 2002; DOWD &VEGA, 2003). Several studies used various delivery systems baited with substrata contaminated with entomopathogenic fungi for the control of different insect groups, such as aphids, coleopterans and lepidopterans (FURLONG *et al.,* 1995; VEGA *et al.,* 1995, 2007; KLEIN & LACEY, 1999; HARTFIELD *et al.,* 2001; MANIANIA, 2002; DOWD & VEGA, 2003). Autocontamination methods have generally used traps adapted for the different behaviours of the targeted species, which attract insects to entomopathogen infection sites in response to environmental, semiochemical or food stimuli; laboratory and field tests of these methods are summarized in VEGA *et al.* (2007).

In the control of Red Palm Weevil, EL-SUFTY *et al.*

– Received 24 October 2013 Accepted 25 November 2013 Published 6 December 2013

(2011) recently tested a trap designed as one way road and consisted of two chambers: an "attractive chamber" baited with Red Palm Weevil aggregation pheromone plus palm tree kairomone and a "contaminating chamber" containing a Petri dish with a dried formulation of *B. Bassiana* conidia. The Authors didn't obtain satisfactory results in the mortality levels of Red Palm Weevil inside curculionid population in field as monthly mortality varied from 3.6 to 51.3% during 14 months of study. Furthermore the trap visiting rate by adults resulted low (2.84 adults per week).

In the present study, we tested the efficacy of an experimental "attract-infect and release" trap to infect Red Palm Weevil adults with indigenous *B. bassiana* and *M. anisopliae* strains isolated from *R. ferrugineus*. The efficacy of the more virulent strain in transferring infective inoculum to adults was tested on different cereal-based growth substrata in laboratory bioassays, while its conidial persistence and both persistence and germinability were evaluated in laboratory and in field conditions respectively. The aim was to develop an autocontamination device which attracts and infects adult weevils and which contains a substrate that can mantains long-term infectivity of the pathogen in order to succesfully and both persistence infect *R. ferrugineus* beetles in support of integrated control management**.**

MATERIALS AND METHODS

R. FERRUGINEUS REARING

Red Palm weevil adults used in the present research were provided in 2012 by Sustainable Management of Agro-ecosystems Laboratory UTAGRI ECO ENEA CR Casaccia, Rome (Italy). Before the bioassays, the adults were maintained for two weeks on pieces of Golden apples in plastic boxes, closed with a hermetic cover in which a circular hole was plugged with a fire-glued metallic fine-knit mesh. Insect breeding was carried out in a climatic room at 24±2°C, 70±5% RH, with a photoperiod of 12:12 h (L:D).

M. ANISOPLIAE AND *B. BASSIANA* REARING

For the bioassays, we used *M. anisopliae* (M.08/I05) and *B. bassiana* (B.09/I01) strains obtained from infected Red Palm Weevil adults collected on attacked palms in Sicily and Lazio respectively; these strains have shown high virulence towards *R. ferrugineus* in previous laboratory investigations (FRANCARDI *et al.,* 2012). These two isolates were cultured on quarter-strength Sabouraud Dextrose Agar plus 0.25% (w/v) yeast extract (SDAY) in Petri dishes and maintained in a climatic chamber at 25°C.

The *M. anisopliae* (M.08/I05) and *B. bassiana* (B.09/I01) strains are stored as frozen dried cultures in the entomopathogenic fungi collection of C.R.A. – Research Centre for Agrobiology and Pedology, Florence (Italy).

EXPERIMENTAL TRAP

The trap consists of an adapted 3.0-litre polypropylene (PP) watering trough for chicks, width 22.5 cm, height 21.0 cm, with a truncated cone-shaped receptacle (River Systems, Italy). Rectangular holes (4x9 cm) were made with an incandescent knife on the two opposite sides of the receptacle to allow insects to enter/exit. The external surface of the receptacle was painted with an acrylic spray paint in black (Del Bono Aerosol, Assago, Milan, Italy) as several studies on the effect of colour on Red Palm Weevil trapping have shown that black or dark-coloured traps are generally more effective in catching *R. ferrugineus* in the field (AL-SAOUD *et al.*, 2010; ABUAGLA & AL-DEEB, 2012). The lower screw-on plate was instead left the original orange colour**.** A screw-on cup (150 ml) with two hand-cut ovoid lateral holes (3x3.5 cm) and closed with a fire-glued metallic fine-knit mesh was suspended inside the receptacle by a metallic thread hooked on a small hole made in the top of the receptacle. The screw-on cup contained a blend of attractive compounds consisting of 10 grains of Rhyfer 220 aggregation pheromone (Intrachem Bio, Italy) left in the plastic bag of the pheromone package, an Eppendorf tube with a perforated cap and with 1 ml of ethyl acetate (10%), and a piece of Golden apple to simulate an available food source (Fig. I, 1, 2 and 3). The experimental traps were placed separately inside wooden frame cages described below.

CAGES

Four cages (1x1x1 m) were made with wooden panels, one at the base and one at the side opposite the open one;

Fig. I – Experimental trap components: 1. Watering trough for chicks; 2. The screw on cap containing attractive compounds inside; 3. The complete trap with the plate containing the infected substratum.

Fig. II – The laboratory cage containing the autocontamination trap and Red Palm Weevil adults.

the other sides were closed with a fine-knit mesh nailed to the wooden frame**.** The open side, completely removable, was latched hermetically to the cage (Fig. II). Different tests were carried out separately with the experimental autocontamination traps in order to: (A) compare the virulence of *M. anisopliae* (M.08/I05) and *B. bassiana* (B.09/I01) strains grown on wheat against Red Palm Weevil adults and to select the more virulent one; (B) assess the infectivity of the more entomopathogenic fungus grown on different cereal substrata (wheat and rice) against Red Palm Weevil adults; (C) evaluate its conidial persistence (numerical levels) in laboratory conditions; and (D) evaluate its conidial persistence and germinability in the field.

Laboratory Bioassays

(A). *Virulence of* M. anisopliae *and* B. bassiana

300 g of wheat were prepared according to the procedure of Gindin *et al.* (2006) for a solid rice-based medium; 100 g of sterilized wheat were transferred into each of 3 sterilized 200-ml conical glass Erlenmeyer flasks: in one flask the wheat medium was inoculated with an agar plug (about 4 x 4 x 4 mm) of sporulated mycelium of *B. bassiana* (B.09/I01) and in another flask with the same quantity of sporulated mycelium of *M. anisopliae* (M.08/I05) grown on SDAY cultures in Petri dishes; in a third flask the wheat was not inoculated (control). The flasks were then plugged with sterilized cotton and placed in a climatic chamber at 27°C. After 20-25 days, *B. bassiana* (B.09/I01) and *M. anisopliae* (M.08/I05) had sporulated abundantly on the wheat; inoculated and the uninoculated wheat (control) were then put, separately, on the plates of the three experimental traps. Traps were placed in separate cages in which 20 Red Palm Weevil adults (10 male and 10 female) were set free to move or fly for 24 h**.** Laboratory room conditions were kept at 27°C, about 60% RH and 12:12 h (L:D). After 24 h, the adults were removed and placed individually in plastic food containers (250 ml) in which 18 holes of about 2 mm diameter were made. The individuals were fed on Golden apple pieces changed weekly and were maintained in the same laboratory room conditions. The assay ended after four weeks.

(B). *Infectivity of the entomopathogenic fungus* M. anisopliae *on different substrata.*

On the basis of the (A) test results, only the *M. anisopliae* (M.08/I05) strain was employed. 200 g of wheat (100 g inoculated with *M. anisopliae* (M.08/I05) and 100 g uninoculated as the control) and 200 g of brown rice (100 g inoculated with *M. anisopliae* (M.08/I05) and 100 g uninoculated as the control) were prepared according to the above-mentioned procedure. Fungus-sporulated substrata and control ones were transferred onto the plates of four experimental traps which were then placed inside four separated cages. 40 Red Palm Weevil adults (20 male and 20 female) were put inside each cage for 24 h. For the controls with no contaminated wheat and rice, 40 Red Palm Weevil adults (20 male and 20 female) were released in each of the two cages for the same time period. Laboratory room rearing conditions were kept at 27°C, 60% RH and 12:12 h (L:D). A video camera was positioned behind the traps to monitor trap acceptance by Red Palm Weevil in the first 6 h of the tests. After 24 h the adults were removed and placed individually into plastic food containers (250 ml) in which 18 holes of about 2 mm diameter were made; they were reared as reported in (A). As in (A), the assay ended four weeks after the treatments. *R. ferrugineus* adult mortality was controlled daily. Similarity dead specimens were placed individually on moistened filter paper inside Petri dishes at room temperature (20-25°C); only cadavers showing external growth of the entomopathogenic fungi were considered in the analysis.

(C). *Conidial persistence on contaminated substrata in the laboratory*

24 h after the (B) test, the wheat and rice substrata inoculated with *M. anisopliae* (M.08/I05) were maintained in the traps inside the cages for a further four weeks in the above-mentioned conditions. Conidial numbers were checked immediately after the test on 0.2 g of sporulated wheat and rice randomly sampled from the substrata. Each grain in that amount was put into 1 ml of distilled and sterile water supplemented with 0.1 ml of polysorbate detergent TWEEN 80 (0.1%) in a glass tube; the tube was shaken for 1 minute in a vortex mixer. The conidial concentration was estimated with a hemocytometer (Thoma-Zeiss counting chamber) and expressed as the mean value of 6 counts per grain contained in the 0.2 g of infected substrata. At the beginning of the assay the mean *M. anisopliae* (M.08/I05) conidia concentrations were 7.1×10^6 conidia per ml on rice and 7.5×10^6 conidia per ml on wheat.

(D). *Field Bioassay*

M. anisopliae (M.08/I05) grown on rice was used in the field test for the greater compactness of the substratum, which could limit accidental loss of grains from the trap. 300 g of brown rice were prepared and inoculated with *M. anisopliae* (M.08/I05) according to the above-mentioned procedure. 100 g of well sporulated rice were transferred onto the plates of two experimental traps hung on broad-

leaved trees, one in a sunny position and the other in the shade, in the park outside our Centre at Cascine del Riccio, province of Florence (Italy). For the control, the remaining 100 g of sporulated rice were maintained in the flask and placed in a climatic cell at 26°C, about 60% RH and 12:12 (L:D) h.

The field tests were carried out in periods chosen randomly during the seasons of highest adult Red Palm Weevil abundance reported in Sicily (Italy), i.e. from April to October (CALDARELLA *et al.,* 2008). The investigated periods were: in spring from 13 May to 16 June 2011; in summer from 11 July to 15 August 2011; in autumn from 10 October to 14 November 2011. Local climatic data were taken from the 3Bmeteo web site (http://www.3bmeteo.com/meteo_regione-toscana.htm).

The mean conidial concentration was evaluated on 0.2 g of sporulated rice randomly sampled weekly from each field trap and from the control according to the above-mentioned procedure. For assessment of conidial germinability, the same conidial suspensions used to evaluate conidial concentrations were diluted 1:10 with sterile distilled water. 40 µl of the diluted conidial suspension were plated on SDAY in 60 mm Petri dishes. Each plate was divided into four quadrants and, after incubation at room temperature (23°-25°C) for 20/22 h, the rate of conidia germination was determined by counting 100 conidia in each quadrant (400 conidia per plate). A conidia with a germ tube longer than its width was considered germinated. The mean value of the four readings was considered to be the actual germination rate of the plate. This germinability test was performed on three different plates per experimental condition (adapted from LIU *et al.*, 2003).

At the beginning of the assay, the conidial numbers on the inoculated rice were $1x10^7$ conidia per ml in spring, $3x10^7$ conidia per ml in summer and $1x10^7$ conidia per ml in autumn with a germinability of 95.28%, 59.2% and 58.50% respectively. Afterwards the conidial concentration on the inoculated rice were recorded once a week for 4 weeks.

STATISTICAL ANALYSIS

B. bassiana (B.09/I01) and *M. anisopliae* (M.08/I05) virulence was expressed by cumulative mortality (%), treatment efficacy (Abbott's formula) (ABBOTT, 1925) and mean lethal time (LT_{50}) after 28 days from treatment. Survival analyses were performed separately for each substratum with the Wilcoxon (Gehan) test using SPSS 15.0.

Data on *M. anisopliae* (M.08/I05) conidial concentration and germinability were analysed with ANOVA and Tukey HSD test (P=0.05). The percentage values were analysed after transformation into angular coefficients.

RESULTS

LABORATORY BIOASSAYS

(A). *Virulence of* M. anisopliae *and* B. bassiana

M. anisopliae (M.08/I05) resulted more virulent than *B. bassiana* (B.09/I01); 28 days after contact, the cumulative mortality of *R. ferrugineus* adults contaminated on wheat inoculated with *M. anisopliae* (M.08/I05) was higher (75%) than that of adults contaminated on the same substratum with *B. bassiana* (B.09/I01) (45%). The cumulative mortality of curculionid adults put in conctat with uninoculated wheat (control) was 25%. Survival analysis (Wilcoxon -Gehan test) showed that the mortality of *R. ferrugineus* infected with *M. anisopliae* (M.08/I05) was significantly higher than that of adults infected with *B. bassiana* (B.09/I01) and than that of the control while the last two values were not significantly different from each other. The *M. anisopliae* (M.08/I05) treatment efficiency (Abbott) was the highest (67%) and LT_{50} of contaminated Red Palm Weevil was reached in 3 days. In contrast, LT_{50} of adults contaminated with *B. bassiana* (B.09/I01) and that of the control could not be determined after 28 days (Table 1).

(B). *Infectivity of the entomopathogenic fungus* M. anisopliae *on different substrata.*

The video camera observations of *R. ferrugineus* behaviour in the traps showed that the baited experimental trap was suitable to attract the adults inside, to keep them on the inoculated substrata and to release infected specimens. No repellent effect on Red Palm Weevil adults due to conidia abundance of *B. bassiana* (B.09/I01) and *M. anisopliae* (M.08/I05) grown on wheat and rice substrata was observed during the tests. The first curculionid adults attracted into the traps were observed walking on or tunnelling in the grains after about 1 h from their release in the cages. At the end of the tests (after 24 h), most of the adults were found outside the traps and their bodies were coated with fungal conidia.

The virulence of *M. anisopliae* (M.08/I05) was not influenced by the growth substrata. Curculionid cumulative mortality was very high and similar (95%) on both the inoculated wheat and rice, as was the treatment efficiency (Abbott) (95%). Moreover, Red Palm Weevil LT_{50} was reached at about the same time (in 12 days on wheat and in 15 days on rice). The cumulative mortality of the control was 5%. Survival analysis (Wilcoxon Gehan test) did not show a significant difference between mortality levels of *R. ferrugineus* adults contaminated on the two substrata, while the mortality levels on both these substrata were significantly different from that of the control after 28 days (Table 2).

(C). *Conidial persistence on contaminated substrata in the laboratory* M. anisopliae

(M.08/I05) conidial persistence in laboratory conditions did not vary appreciably on the inoculated wheat in the first three weeks (from $7x10^6$ conidia per ml to $1x10^7$ conidia per ml) but then decreased rapidly to $5x10^6$ conidia per ml in the last week. Similarly, on the contaminated rice substratum, *M. anisopliae* (M.08/I05) conidial numerical levels was constant in the first three weeks ($7-8x10^6$ conidia per ml) and then crashed to $3x10^6$ conidia per ml in the fourth week. ANOVA did not show a significant difference between the number of *M. anisopliae* (M.08/I05) conidia on inoculated wheat while on rice the values of the second and fourth week were significantly different one from the others (Table 3).

(D). *Field Bioassays*

M. anisopliae (M.08/I05) conidial persistence under field conditions showed similar fluctuations on the inoculated rice medium in both the sunny and shady trap within the considered seasonal periods (Table 4).

In spring, from 13 May to 16 June 2011, when the local temperature was between 20 and 25°C and RH was 50%, *M. anisopliae* (M.08/I05) conidial presence on inoculated rice persisted till the third week in both the sunny and

Table 1 – Bioassay (A). Virulence of *B. bassiana* (B.09/I01) and *M. anisopliae* (M.08/I05) against the Red Palm Weevil adults after 28 d from contact with entomopathogenic fungi-sporulated wheat.

R. ferrugineus adults (no.)	Fungal strains	Cereal substratum	Cumulative mortality %	Treatment efficacy (Abbott%)	LT_{50} (days)	Survival analysis (Wilcoxon-Gehan test)
20	*B. bassiana* (B.09/I01)	wheat	45	27	n.d.	a
20	*M. anisopliae* (M.08/I05)	wheat	75	67	3	b
20	control	wheat	25	n.d.	n.d.	a

Survival analysis (Wilcoxon-Gehan test): values with the same letter are not significantly different. (P = 0.05).
n.d.= not determined**.**

Table 2- Bioassay (B). Virulence of *M. anisopliae* (M.08/I05) against the Red Palm Weevil adults after 28 d from contact with entomopathogenic fungus-sporulated wheat and rice.

R. ferrugineus adults (no.)	Fungal strains	Cereal substratum	Cumulative mortality %	Treatment efficacy (Abbott%)	LT_{50} (days)	Survival analysis (Wilcoxon-Gehan test)
40	*M. anisopliae* (M.08/I05)	wheat	95	95	15	a
40	*M. anisopliae* (M.08/I05)	rice	95	95	12	a
40	control	wheat	5	n.d.	n.d.	b
40	control	rice	0	n.d.	n.d.	b

Survival analysis (Wilcoxon-Gehan test): values with the same letter are not significantly different. (P = 0.05).
n.d.= not determined.

Table 3 – Bioassay (C). Weekly persistence of *M. anisopliae* (M.08/I05) conidial concentration on inoculated wheat and rice within 28 d at laboratory room conditions (Temp. 27°C, about 60% RH and 12:12 (L:D) h.

Week	Substratum	no. of conidia per ml (mean)	ANOVA values
1	wheat	$7x10^6$ a	$F_{(3.20)} = 2.201$ *P = 0.125*
2	wheat	$1x10^7$ a	
3	wheat	$8x10^6$ a	
4	wheat	$5x10^6$ a	
1	rice	$7x10^6$ ab	$F_{(3.21)} = 3.893$ *P = 0.026*
2	rice	$8x10^6$ b	
3	rice	$7x10^6$ ab	
4	rice	$3x10^6$ a	

Means followed by the same letter are not significantly different (ANOVA; Tukey-HSD test, $P<0.05$).

shady trap with a concentration varying from the initial 1-$3x10^6$ conidia per ml to $1.55x10^7$ conidia per ml in the sunny trap and to $2.6x10^7$ conidia per ml in the shady one, (Table 4; Figs. III, IV). In the summer, from 11 July to 15 August 2011, the local temperature was about 25°C and air RH was 60% and, in this period, *M. anisopliae* (M.08/I05) conidia persisted into the fourth week in both field traps, varying from the initial 7-$8x10^6$ conidia per ml to $3.1x10^7$ conidia per ml in the fourth week in both traps (Table 4, Figs. V, VI). In the autumn, from 10 October to 14 November 2011, *M. anisopliae* conidia persisted during the first week in both traps, with a peak of $2.1x10^7$ conidia per ml in the sunny trap when the local temperature was about 15°C and the air RH 55% but in the second week with a temperature below 15°C, conidial numerical levels crashed rapidly to lower values (1.6-$2.7x10^6$) in both traps (Table 4, Figs. VII, VIII).

In the control climatic chamber, *M. anisopliae* (M.08/I05) conidia numerical levels on the rice was always higher than the values recorded in the field traps during the spring and summer tests. ANOVA showed that conidial persistence in the control was significantly higher than the values recorded in both the shady and sunny trap in spring and summer, while the values for the shady and

Table 4 – Bioassay (D). Weekly persistence of *M. anisopliae* (M.08/I05) conidial concentration on inoculated rice in the field during the study periods. Temperature and relative humidity (RH%) values in field are reported.

Seasons	Trap position	no. conidia per ml			ANOVA	Temp. °C			RH%
		mean	Min.	Max.	values	mean	Min.	Max.	
Spring: from 2011/05/13 to 2011/06/16	sunny	8.4 x10^6 a	1 x10^6	1.55 x10^7	$F_{(2,15)} = 6.77$ $P = 0.008$	21	14	28	52
	shady	1.2 x10^7 a	3 x10^6	2.6 x10^7					
	control	2.5 x10^7 b	1.1 x10^7	3.7 x10^7					
Summer: from 2011/07/11 to 2011/08/15	sunny	1.5 x10^7 a	7.1 x10^6	3.1 x10^7	$F_{(2,15)} = 7.755$ $P = 0.005$	24	18	30	60
	shady	1.6 x10^7 a	8 x10^6	3.1 x10^7					
	control	3.5 x10^7 b	2.4 x10^7	6 x10^7					
Autumn: from 2011/10/10 to 2011/11/14	sunny	6.2 x10^6 a	1.6 x10^6	2.1 x10^7	$F_{(2,15)} = 0.487$ $P = 0.524$	14	9	19	70
	shady	4.2 x10^6 a	2.7 x10^6	7.6 x10^6					
	control	3.6 x10^6 a	1.4 x10^6	7.6 x10^6					

Means followed by the same letter are not significantly different (ANOVA; Tukey-HSD test, $P<0.05$).

sunny trap were not significantly different. In the autumn test, there was no significant difference in conidia number between the control and the field traps (Table 4).

No correlation was found between conidial numerical levels in the traps and field RH conditions (R^2= 0.19), while a higher correlation was observed between conidial numerical values and field temperature (R^2= 0.43).

M. anisopliae (M.08/I05) conidial germinability in the field showed a similar trend in both the sunny and shady trap during the spring and summer tests. In particular, in spring**,** *M. anisopliae* (M.08/I05) conidial germinability in the sunny trap decreased from the initial 95.28% to 81.17% during the first 3 weeks and then crashed rapidly below 40% in the fourth week. In the shady trap, the conidial germinability decreased from 87.92 to 78% in the same period but maintained a higher level in the last week (70.42%). In the control, *M. anisopliae* (M.08/I05) conidial germinability decreased progressively from 93.67 to 70% during the four weeks (Table 5, Fig. IX, 1).

In summer, *M. anisopliae* (M.08/I05) conidia germinability decreased from the initial 59.25 to 43% in the sunny trap and to 48.33% in the shady one during the first 3 weeks but increased to 59.58 and 60.67% respectively during the fourth week. In the control, *M. anisopliae* (M.08/I05) conidial germinability varied from the initial 59.25 to 73.83% in the fourth week (Table 6, Fig. IX, 2).

In autumn, *M. anisopliae* (M.08/I05) conidial germinability in the shady trap decreased progressively during the four weeks from the initial 58.50 to 25.83%, while in the sunny trap the value increased to 67.75% during the second week and then crashed to 21.58% in the last week. In the control, *M. anisopliae* (M.08/I05) conidia germinability increased over 70% in the second week and remained high till the end of the second one, after which the value collapsed to around 4% (Table 7, Fig. IX, 3).

ANOVA showed that, in general, there was no significant difference between *M. anisopliae* (M.08/I05) conidial germinability in the sunny and shady traps in each week of the considered periods. In particular, no significant difference in *M. anisopliae* (M.08/I05) conidial germinability emerged between the field traps and the control in the first week of each seasonal test or in the third week of the spring and summer trials.

DISCUSSION

A new approach in the microbiological control of insect pests is the use of various attractive devices acting as a "focus of entomopathogenic" micro-organisms to infect the target insects (VEGA *et al.*, 2007). This technique offers the advantages of a selected infectivity in the field, the possibility to contaminate many individuals, and the prospect of dispersing inoculum within the pest population by direct contact among adults. With regard to entomopathogenic fungi important factors in reaching these goals are, *intra alia*, the availability of an isolate highly virulent towards the target insect, suitability for mass spore production on an appropriate contamination substratum, an efficient delivery system and inoculum stability and germinability in field conditions (IBRAHIM *et al,.* 1999; ZHANG *et al,.* 2011).

As regards the first factor, several authors have isolated strains of *M. anisopliae* and *B. bassiana* obtained from different sources that are virulent against *R. ferrugineus*. In particular, a *B. bassiana* strain isolated from dead Red Palm Weevil in Egypt proved highly virulent towards the curculionid and it was patented (EL-SUFTY *et al.,* 2007, 2009; SEWIFY, 2007; SEWIFY *et al.*, 2009). This suggests that the identification of indigenous entomopathogenic fungi already active on the weevil may offer better prospects for its biological control. However, our study demonstrated different levels of virulence towards adults of the curculionid by the indigenous entomopathogenic fungi isolated from mycosed *R. ferrugineus*. In particular, it highlighted the virulence of *M. anisopliae* (M.08/I05) which produced higher Red Palm Weevil cumulative mortality (75%) and treatment efficacy (67%) within 28 days and caused LT_{50} in only 3 days from treatment.

These finding confirmed our previous studies on the

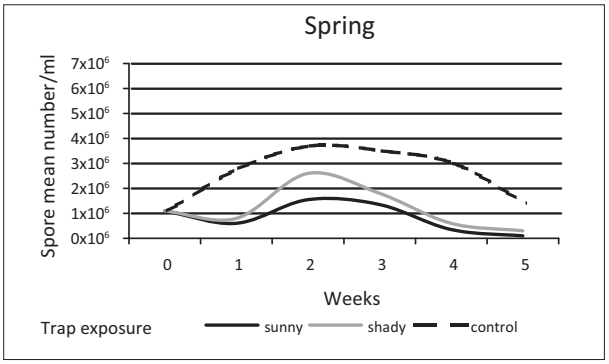

Fig. III – Spring: *M. anisopliae* conidia persistence on inoculated rice in the sunny and shady trap in the field.

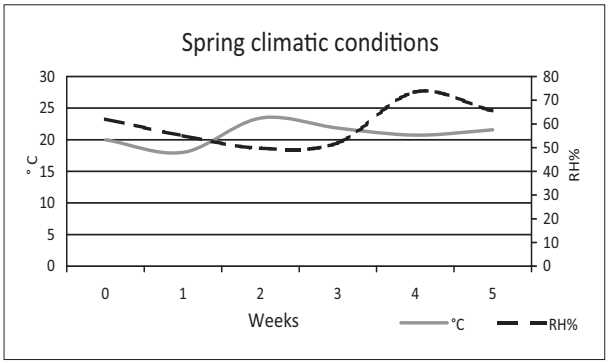

Fig. IV -Climatic conditions in spring 2011.

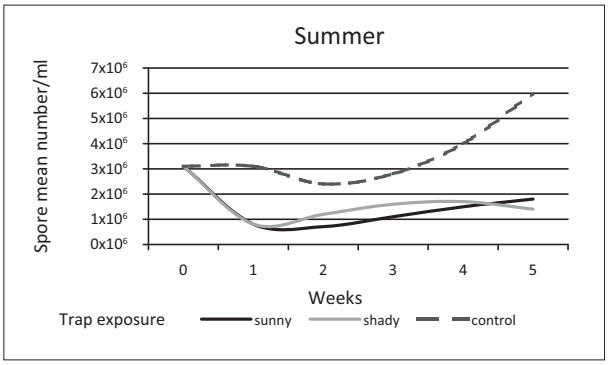

Fig. V – Summer: *M. anisopliae* conidia persistence on inoculated rice in the sunny and shady trap in the field.

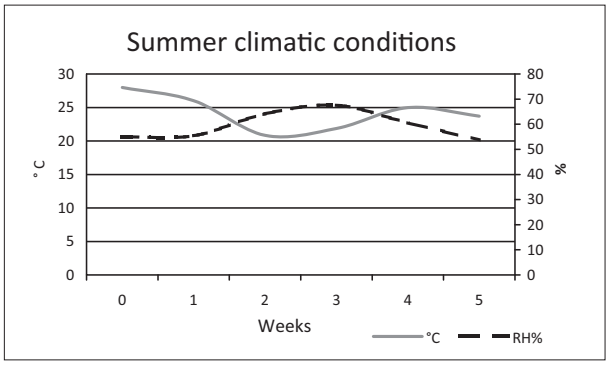

Fig. VI - Climatic conditions in summer 2011.

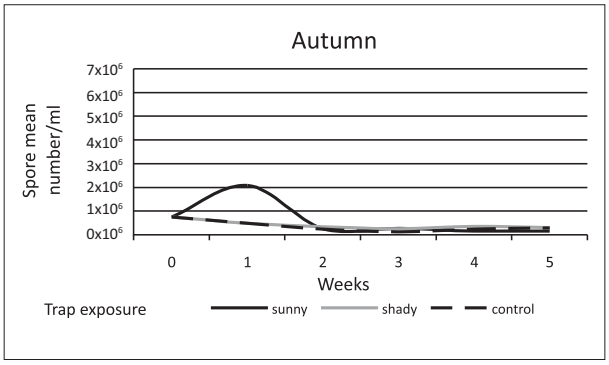

Fig.VII – Autumn: *M. anisopliae* conidia persistence on inoculated rice in the sunny and shady trap in the field.

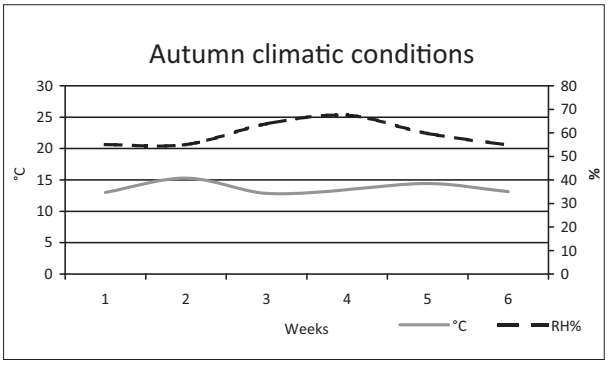

Fig. VIII – Climatic conditions in autumn 2011.

Table 5 – Spring: conidia germinability in the sunny and shady trap.

Trap position	26/May/2011	02/June/2011	09/June/2011	16/June/2011
sunny	86.25% A	83.67% AB	81.17% A	36.5% A
shady	87.92% A	88.5% B	78% A	70.42% B
control	93.67% A	82.58% A	75.83% A	70% B

Table 6 – Summer: conidia germinability in the sunny and shady trap.

Trap position	11/July/2011	18/July/2011	25/July/2011	15/August/2011
sunny	59.25% A	50.25% A	43% A	59.58% A
shady	59.25% A	54.58% A	48.33% A	60.67% A
control	59.25% A	65.8% B	48.08% A	73.83% B

Table 7 – Autumn: conidia germinability in the sunny and shady trap.

Trap position	11/October/2011	18/October/2011	25/October/2011	01/November /2011
sunny	58.5% A	67.75% A	23.33% A	21.58% A
shady	58.5% A	49.42% B	28.67% A	25.83% A
control	58.5% A	71.42% A	59.42% B	4% B

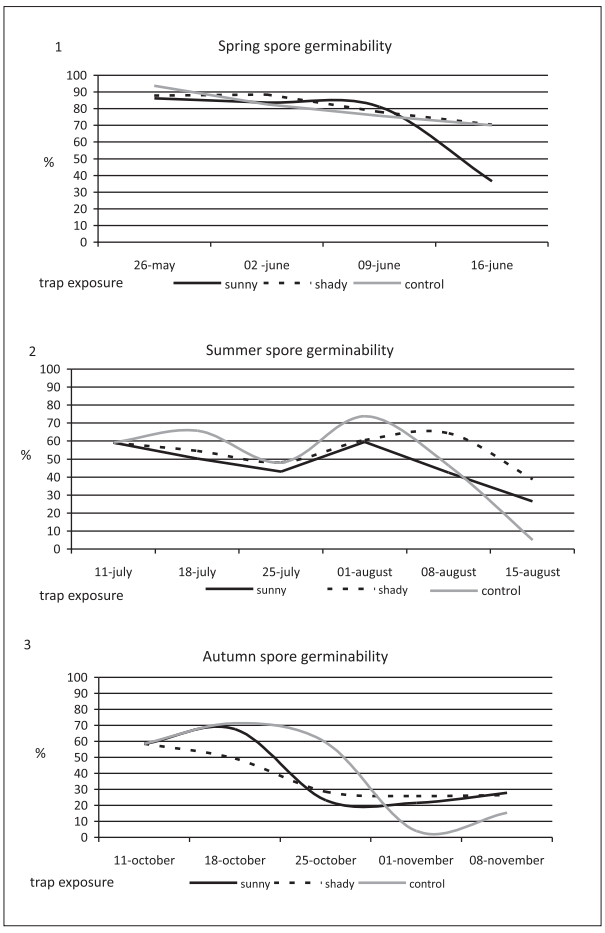

Fig. IX – *M. anisopliae* viability (germinability) in the field: 1. Spring; 2. Summer; 3. Autumn.

entomopathogenicity of the same *B. bassiana* (B.09/I01) and *M. anisopliae* (M.08/I05) strains carried out against larvae and adults of the curculionid infected on different contaminated substrata (Sabouraud Dextrose Agar (SDA) and wheat) (FRANCARDI *et al,.* 2012). In those bioassays, the *M. anisopliae* (M.08/I05) strain showed higher values of cumulative mortality against adults (90%) treated on inoculated wheat and caused LT_{50} in 13.1 days than on SDA (cumulative mortality of 53%; LT_{50} in 26.8 days). Diversely, the adult mortality caused by *B. bassiana* (B.09/I01) in both the substrata was lower (20%) and not significantly different from the control. GINDIN *et al.* (2006) also observed high mortality (84.6%) of *R. ferrugineus* adults treated with dry spores of *M. anisopliae* from a grown culture after 2 weeks from contact.

Infecting substrata so play an important role in the success of any autocontamination device, as they may determine the quantity of spores put in contact with the host and the persistence and germinability of the inoculum over time. In this regard, wheat and rice have just proved to successfully support the growth and sporulation of the entomopathogenic fungi (IBRAHIM & LOW, 1993, SOUNDARAPANDIAN & CHANDRA, 2007; SAHAYARAJ & NAMASIVAYAM, 2008). The present study confirms that both theese cereals are good sporulating and infecting substrata for *M. anisopliae* towards Red Palm Weevil adults in laboratory assays, as also observed by GINDIN *et al.* (2006) and FRANCARDI *et al.* (2012). In other studies DEADMAN *et al.* (2001) successfully infected Red Palm Weevil adults with 20 ml of pulped dates with 25% by volume molasses amended with 10 g of *B. bassiana* spores inside a plastic bowl above which was suspended a pheromone lure; the contaminated *R. ferrugineus* adults showed 90-100% mortality within 9-20 days from contact. Those authors observed burrowing of the adults inside the contaminated date pulp mixture, suggesting that this behaviour may ensure a more successful infection of the curculionid and inoculum transmission to the beetles. More recently EL SUFTY *et al.* (2011), in laboratory bioassays, recorded the mortality of all the Red Palm Weevil adults let in a trap provided with a *B. bassiana* dried conidia (10%) powder formulation in a mean of 8.25 days from the contact.

Our experimental e trap proved to be effective in attracting and infecting Red Palm Weevil beetles in the laboratory. The attractive blend, consisting of the curculionid aggregation pheromone plus volatile food compounds and ethyl acetate, is a valid formula to increase attractiveness to Red Palm Weevil and to all palm weevils (GIBLIN-DAVIS *et al.*,1996; ABDALLAH & AL-KHATRI, 2005; FALEIRO & SATARKAR, 2005; OEHLSCHLAGER, 2005; GUARINO *et al.,* 2010). In the present study, it probably also played a role in prolonging contact with the pathogenic conidia by stimulating tunnelling activity inside the contaminated substrata in search of a food source and/or by favouring cryptic behaviour of adults which usually take refuge between petioles and offshoot bases on palms, as observed by GINDIN *et al.* (2006). This behaviour also showed the absence of any repellent effect on *R. ferrugineus* adults by *M. anisopliae*-sporulated wheat and rice. In all cases, the final outcome was adults exiting the experimental contamination trap with the body dusted with spores (Fig. X). EL-SUFTY *et al.*(2011) in an autodissemination trap baited with only Red Palm Weevil aggregation pheromone plus a data palm kairomone recorded, instead, a low attractiveness toward Red Palm Weevil (2.84 adults per week) in field.

Environmental conditions, in particular temperature, relatively humidity and UV light, can negatively influence entomopathogenic spore germinability, persistence and virulence, eliminating or greatly reducing the effectiveness of isolates as biocontrol agents (ZIMMERMANN, 1982; MOORE *et al.,* 1996; BUTT & GOETTEL, 2000; INGLIS *et al.,* 2001; ZHANG *et al.*, 2011).

The results of the present study show that the shape of the proposed experimental trap maintained *M. anisopliae*

Fig. X – Adult of *R. ferrugineus* with the body dusted with *M. anisopliae* (M.08/I05) conidia in the autocontamination trap.

(M.08/I05) inoculum and spore germinability quite constant in the considered seasonal periods, during which there was no significant difference in spore numbers or germinability between the traps located in sunny and shady positions. Relative humidity in the field did not seem to affect conidial levels and temperature also appeared to have little influence. This is an important result showing the ability of *M. anisopliae* (M.08/I05) to persist in the field with propagules that may cause disease in the target insect.

Although fungal isolates exhibit a wide range of tolerance to environmental factors, the present study indicated that the optimal temperature range for conidial persistence and germinability in the field was 21-25°C, while temperatures lower than 20°C or higher than 28°C were detrimental to conidial germinability. In contrast KLEIN & LACEY (1999) carried out field tests during August, with a mean daily temperature of 26°C and humidity of 82%, and found that after one week there was a decrease from about 50 to 34.5% in the germinability of *M. anisopliae* conidia diluted with bran flour and placed in autodissemination traps for the biocontrol of the Japanese beetle *Popillia japonica* Newman. Our results are in agreement with those of other authors obtained in laboratory assays: EKESI *et al.* (1999) reported that the optimal temperature for germinability, growth and virulence of four strains of *M. anisopliae* was 25° (range 25°-30°C) while germinability crashed at 15° and 35°C. Other studies have reported good growth temperatures for most entomopathogenic Hyphomycetes originating from Europe between 8° and 30°C, with optimal temperatures between 20° and 25°C and inhibited growth above 30°C (Inglis et al. 2001 and references therein). A negative influence of high temperature on *M. anisopliae* conidial germinability has also been reported by other authors who indicated limiting effects between 30°C and 40°C (MORLEY-DAVIS *et al.*, 1995).

In the United Arab Emirates EL-SUFTY *et al.* (2011) observed that the survival of a fungus powder formulation containing 10% of *B. Bassiana* conidia, inside an auto dissemination trap, remained high for 4 weeks (89.2-96.5%) in a date palm plantation, then decreased to 66.7% during the 5th. No data on the local climatic conditions are reported. The conidial germinability of the control, maintained under laboratory conditions (not reported), remained instead higher until the 5th week then it decreased.

In summary, our study has identified the indigenous *M. anisopliae* (M.08/I05) as the most virulent strain against *R. ferrugineus* adults and wheat and rice as good fungus-growing and infective substrata. The data on *M. anisopliae* (M.08/I05) virulence (Red Palm Weevil cumulative mortality, LT_{50}, treatment efficacy) in laboratory bioassays and on fungal conidial persistence and germinability in field conditions provide useful information to predict the potential efficacy of the considered *M. anisopliae* strain in the biocontrol of *R. ferrugineus*. Furthermore, the autocontamination trap appears suitable to attract, infect and release Red Palm Weevil adults and to preserve fungal inoculum stability in the field for at least three-four weeks in spring and summer**.** Nevertheless further investigations are necessary to verify the transmission of *M. anisopliae* (M.08/I05) inoculum within the Red Palm Weevil population in field in order to provide a valid means of *R. ferrugineus* control within IPM programs.

ACKNOWLEDGMENTS

The Authors are grateful to Dr. Silvia Arnone of the Sustainable Management of the Agro-ecosystems Laboratory UTAGRI ECO ENEA CR Casaccia, Rome (Italy) for the periodic supply of *R. ferrugineus* adults.

The research was supported by a grant from Ministry of Agricultural, Food and Forestry (MiPAAF) national project "Protection of ornamental and indigenous palms from the biological invasion of the Red Palm Weevil"– PROPALMA" (D.M. 25618/7301/11, 2012/12/01)

RIASSUNTO

TRAPPOLA CONTAMINANTE CON FUNGHI ENTOMOPATOGENI: UNA POSSIBILE STRATEGIA NEL CONTROLLO DI RHYNCHOPHORUS FERRUGINEUS *(OLIVIER) (COLEOPTERA CURCULIONIDAE)*

Una trappola sperimentale "attract, infect and release trap" è stata messa a punto per infettare gli adulti del Punteruolo Rosso, *Rhynchophorus ferrugineus* (Olivier) con funghi entomopatogeni allo scopo di essere impiegata nell'ambito di programmi di lotta integrata. Come isolati fungini sono stati utilizzati ceppi indigeni di *Metarhizium anisopliae* (Metchnikoff) Sorokin e *Beauveria bassiana* (Balsamo - Crivelli) Vuillemin, ottenuti da adulti di *R. ferrugineus* raccolti in campo. Nei test di laboratorio il tipo di trappola e il blend attrattivo utilizzato sono risultati efficaci nell'attrarre gli adulti del Punteruolo rosso e a trattenerli temporaneamente su substrati di riso e grano inoculati con gli isolati di *B.bassiana* e *M. anisopliae* posti all'interno della trappola. Prove di confronto sulla entomopatogenicità dei due isolati fungini, hanno evidenziato la maggiore virulenza dell'isolato di *M. anisopliae* nei confronti degli adulti del curculionide con una mortalità cumulativa percentualmente superiore (75%) a quella osservata per la *B. bassiana* (45%).

La virulenza del *M. anisopliae* non è risultata influenzata dai diversi substrati di crescita utilizzati, grano e riso , in quanto la mortalità cumulativa degli adulti (95%) e l'efficienza del trattamento (Abbott = 95%) sono stati, in entrambi i casi, molto alti e con un LT_{50} raggiunto entro i 15 giorni dall'inizio delle prove. La persistenza e la germinabilità dei conidi dell'isolato di *M. anisopliae* su substrato di riso è stata infine esaminata in condizioni di campo all'interno di trappole situate in posizione soleggiata ed in ombra nel corso di quattro settimane in primavera, estate ed autunno . I risultati hanno mostrato che all'interno delle trappole sperimentali l'inoculo fungino è rimasto più stabile in primavera e in estate che in autunno. Nessuna differenza significativa nella concentrazione dei conidi è stata rilevata fra il substrato di riso inoculato con *M. anisopliae* nella trappola al sole e all'ombra durante i periodi di osservazione in campo.

REFERENCES

ABBOTT W. S., 1925 – *A method of computing the effectivenes of an insecticide.* - J. Econom. Entomol. 18: 265-267.

ABDALLAH F. F., AL-KHATRI S. A., 2005 – *The effect of pheromone, kairomone and food bait on attracting males and females of red palm weevil.* - Egypt. J. Agric. Res., 83:169-177.

ABUAGLA A. M., AL-DEEB M. A., 2012 – *Effect of bait quantity and trap color on the trapping efficacy of the pheromone trap for the red palm weevil,* Rhynchophorus ferrugineus. - J. Insect Sci., 12.120. Available online: http://www.insectscience.org/12.120.

AL-SAOUD A. H., AL-DEEB M. A., MURCHIE A. K., 2010 – *Effect of color on the trapping effectiveness of Red Palm Weevil pheromone traps.* - J. Entomol., 7 (1): 54-59.

BUTT T. M., GOETTEL M. S., 2000 – *Bioassays of entomogenous fungi*. In: A. Navon and K. R. S. Ascher Eds., Bioassays of entomopathogenic microbes and nematodes. CABI Publishing, UK, pp.141-191.

CALDARELLA C. G., GRECO C., LO VERDE G., 2008 – *Monitoraggio del Punteruolo rosso nella città di Palermo con l'uso di trappole.* In: La ricerca scientifica sul punteruolo rosso e gli altri fitofagi delle palme in Sicilia. Report 2008 Regione Siciliana. Assessorato Agricoltura e Foreste. Dipartimento interventi infrastrutturali. 1: 65-68.

DEADMAN M. L., AZAM K. M., RAZVI S. A., KAAKEH W., 2001 – *Preliminary investigations into the biological control of the red palm weevil using* Beauveria bassiana. In: The Second International Conference on Date Palms held at the United Arab Emirates University, 2001 March 25-27. UAE Press, Al-Ain, UAE (http://www.pubhort.org/datepalm/datepalm2/datepalm2_31.pdf).

DEMBILIO Ó., QUESADA-MORAGA E., SANTIAGO-ÁLVAREZ C., JACAS J. A., 2010 – *Potential of an indigenous strain of the entomopathogenic fungus* Beauveria bassiana *as a biological control agent against the Red Palm Weevil,* Rhynchophorus ferrugineus. - J. Invertebr. Pathol., 104: 214-221.

INGLIS D.G., GOETTEL M.S., BUTT T. M.,STRASSER H., 2001 – *Use of Hyphomycetous Fungi for managing Insect Pests.*In: Fungi as Biocontrol Agents, Progress, Problems and Potential, eds T.M. Butt, C. Jackson & N. Magan, CAB International, pp.23-69.

DOWD P. F.. VEGA F. E., 2003 – *Autodissemination of* Beauveria bassiana *by sap beetles (Coleoptera: Nitidulidae) to overwinter sites.* - Biocontrol Sci. Techn., 13: 65-75.

EKESI S., MANIANA N. K., AMPONG-NYARKO K., 1999 – *Effect of temperature on germination, radial growth and virulence of* Metarhizium anisopliae *and* Beauveria bassiana *on* Megalurothrips sjostedti. - Biocontrol Sci. Techn., 9: 177-185.

EL-SUFTY, R., AL-AWASH S. A., AL ALMIRI A., SHAHDAD A., AL BATHRA A., MUSA S. A., 2007 – *Biological control of the Red Palm Weevil,* Rhynchophorus ferrugineus *(Col.: Curculionidae) by the entomopathogenic fungus* Beauveria bassiana. - Proc. 3rd International Date Palm Conference, Abu Dhabi, UAE, ISHS Acta Hort. 736: 399-404.

EL-SUFTY R., AL-AWASH S. A., AL BGHAM S., SHAHDAD A. S., AL. BATHRA A. H., 2009 – *Pathogenicity of the fungus* Beauveria bassiana *(Bals.) Vuill to the Red Palm Weevil* Rhynchophorus ferrugineus *(Oliv.) (Col.: Curculionidae) under laboratory and field conditions.* - Egypt. J. Biol. Pest Co., 1: 81-85.

EL-SUFTY R., AL BGHAM S., AL-AWASH S. A., SHAHDAD A., AL BATHRA A., 2011 – *A trap for autodissemination of the entomopathogenic fungus* Beauveria bassiana *by Red Palm Weevil adults in date palm plantations.* - Egypt. J. Biol. Pest Co., 21(2): 271-276.

EPPO,. 2008 – Rhynchophorus ferrugineus. - EPPO Bull., 38: 55-59.

FALEIRO, J. R. , SATARKAR V. R., 2005 – *Attraction of food baits for use in Red Palm Weevil* Rhynchophorus ferrugineus *(Olivier) pheromone trap.* - Indian J. Plant Protection., 33 (1): 23-25.

FERRY M. , S. GOMEZ., 2002 – *The red palm weevil in the Mediterranean area.* - Palms, 46: 172-178.

FRANCARDI V., BENVENUTI C., ROVERSI P. F., RUMINE P., BARZANTI G., 2012 – *Entomopathogenicity of* Beauveria bassiana *(Bals.) Vuill. and* Metarhizium anisopliae *(Metsch.) Sorokin isolated from different sources in the control of* Rhynchophorus ferrugineus *(Olivier) (Coleoptera Curculionidae).* – Redia, 95: 49-55.

FURLONG, M. J., PELL J. K., ONG P. C. , SYED A. R., 1995 – *Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen* Zoophthora radicans *(Entomophthorales) by the diamondback moth,* Plutella xylostella *(Lepidoptera: Yponomeutidae).* - B. Entomol. Res., 85: 331-337.

FURLONG, M. J., PELL J. K., 2001 – *Horizontal transmission of entomopathogenic fungi by the diamondback moth.* - Biol. Control., 22: 288-299.

GIBLIN-DAVIS R. M., OEHLSCHLAGER A. C., PEREZ A., GRIES G., GRIES R., WEISSLING T. J., CHINCHILLA C. M., PEÑA J. E., HALLETT R. H., PIERCE H. D. JR. , GONZALEZ L. M., 1996 – *Chemical and behavioral ecology of palm weevils (Curculionidae: Rhynchophorinae).* - Fla. Entomol., 79 (2) :153-167.

GINDIN G., LEVSKI S., GLAZER I., SOROKER V., 2006 – *Evaluation of the entomopathogenic fungi* Metarhizium anisopliae *and* Beauveria bassiana *against the Red Palm Weevil* Rhynchophorus ferrugineus. - Phytoparasitica, 34 (4): 370-379.

GUARINO S., LO BUE P., PERI E., 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.

HARTFIELD C. M., CAMPBELL C. A. M., HARDIE J., PICKETT J. A., WADHAMS L. J., 2001 – *Pheromone traps for the*

dissemination of an entomopathogen by the damson-hop aphid Phorodon humili. - Biocontrol Sci Techn., 11: 401-410.

IBRAHIM L., BUTT T. M., BECKETT A., CLARK S. J., 1999 – *The germination of oil-formulated conidia of the insect pathogen,* Metarhizum anisopliae. - Mycol. Res., 103: 901-907.

IBRAHIM J. B., LOW W., 1993 – *Potential of mass production and field efficacy of isolates of the entomopathogenic fungi* Beauveria bassiana *and* Paecilomyces fumosoroseus *against* Plutella xylostella. - Int. J. Pest Manag., 39(3): 288-292.

INGLIS G. D., GOETTEL M. S., BUTT T. M., STRASSER H.., 2001 – *Use of* Hyphomycetes *fungi for managing insect pests.* In: Butt T. M., Jackson C., Magan N. Eds. Fungi as biocontrol agents: progress, problems and potential. CABI Publishing, Wallingford, Oxon, U.K., pp. 23-69.

KLEIN M. G., LACEY A. L., 1999 – *An attractant trap for autodissemination of entomopathogenic fungi into populations of the Japanese beetle* Popillia japonica *(Coleoptera: Scarabaeidae).* - Biocontrol Sci. Techn., 9: 151-158.

KREUTZ J., ZIMMERMANN G., VAUPEL O., 2004 – *Horizontal transmission of entomopathogenic fungus* Beauveria bassiana *among the Spruce Bark Beetle,* Ips typographus *(Col., Scolytidae) in the laboratory and under field conditions.* - Biocontrol Sci. Techn., 14 (8): 837-848.

LIU H., SKINNER M., BROWNBRIDGE M., PARKER B. L., 2003 – *Characterization of* Beauveria bassiana *and* Metarhizium anisopliae *isolates for management of tarnished plant bug,* Lygus lineolaris *(Hemiptera: Miridae).* - J. Invertebr. Pathol., 82: 139-147.

LLÁCER E., NEGRE M., JACAS J. A., 2012a – *Evaluation of an oil dispersion formulation of imidacloprid as a drench against* Rhynchophorus ferrugineus *(Coleoptera, Curculionidae) in young palm trees.* - Pest Manag. Sci,. 68: 878-882.

LLÁCER E., SANTIAGO-ALVAREZ C. , JACAS J. A., 2012b – *Could sterile males be used to vector a microbiological control agent? The case of* Rhynchophorus ferrugineus *and* Beauveria bassiana. - B. Entomol. Res., 103 (2): 241-250.

MANIANIA N. K., 2002 – *A low-cost contamination device for infecting adult tsetse flies,* Glossina *spp., with the entomopathogenic fungus* Metarhizium anisopliae *in the field.* - Biocontrol Sci. Techn., 12: 59-66.

MOORE D., HIGGINGS P. M. , LOMER C. J., 1996 – *Effect of simulated and natural sunlight on the germination of conidia of* Metarhizium flavoviride *Gam and Rozsypal and interaction with temperature.* - Biocontrol Sci. Techn., 6: 63-76.

MORLEY-DAVIS J., MOORE D., PRIOR C., 1995 – *Screening of* Metarhizium *and* Beauveria *spp. conidia with exposure to simulated sunlight and range of temperatures.* - Mycol. Res., 100 (1): 31-38.

OEHLSCHLAGER C., 2005 – *Current status of trapping palm weevils and beetles.* - Planters. , 81 (947): 123-143.

QUESADA-MORAGA E., SANTOS-QUIRÓS R., VALVERDE-GARCÍA P., SANTIAGO-ÁLVAREZ C., 2004 – *Virulence, horizontal transmission, and sublethal reproductive effects of* Metarhizium anisopliae *(Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae).* - J. Invertebr. Pathol., 87: 51-58.

QUESADA-MORAGA E., MARTIN-CARBALLO I., GARRIDO-JURADO I., SANTIAGO-ÁLVAREZ C., 2008 – *Horizontal transmission of* Metarhizium anisopliae *among laboratory populations of* Ceratitis capitata *(Wiedemann) (Diptera: Tephritidae).* - Biol. Control., 47: 115-124.

SAHAYARAJ K., NAMASIVAYAM K. R., 2008 – *Mass production of entomopathogenic fungi using agricultural products and by products.* - Afr. J. Biotechnol. ,7 (12): 1907-1910.

SEWIFY G. H., 2007 – *Egyptian strain of entomopathogenic fungus* Beauveria bassiana. (WO/2007/087813). WIPO Service, World Intellectual Property Organization, 2007 August 9. International publication number WO2007/087813 A2 http://www.sumobrain.com/patents/wipo/Egyptian-strain-entomopathogenic-fungus-Beauveria/WO2007087813A2.pdf

SEWIFY G. H., BELAL M. H., AL-AWASH S. A., 2009 – *Use of entomopathogenic fungus,* Beauveria bassiana *for the biological control of the red palm weevil,* Rhynchophorus ferrugineus *(Olivier).* - Egypt. J. Biol. Pest Co., 19 (2): 157-163.

SOUNDARAPANDIAN P., CHANDRA R., 2007 – *Mass production of endomopathogenic fungus* Metarhizium anisopliae *(Deuteromycota; Hyphomycetes) in the laboratory.* - Res. J. Microbiol., 2: 690-695.

VEGA F. E., DOWD P. F., BARTELT R. J., 1995 – *Dissemination of microbial agents using an autoinoculating device and several insect species as vectors.* - Biol. Control., 5: 545-552.

VEGA F. E., DOWD P. F., LACEY L. A., PELL J. K., JACKSON D. M., KLEIN M. G., 2007 – *Dissemination of beneficial microbial agents by insects.* In: Lacey L. A. and Kaya H. K., Eds., Field Manual of Techniques in Invertebrate Pathology, Springer, Dordrect, The Netherlands, pp. 127-146.

ZHANG L.-W., LIU Y.-J., YAO J., WANG B., HUANG B., LI Z.-Z., FAN M.-Z., SUN J.-H., 2011 – *Evaluation of* Beauveria bassiana *(Hyphomycetes) isolates as potential agents for control of* Dendroctonus valens. - Insect Science, 18: 209-216.

ZIMMERMANN G., 1982 – *Effect of high temperatures and artificial sunlight on the germinability of conidia of* Metarhizium anisopliae. - J. Invertebr. Pathol., 41: 36-40.