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PRELIMINARY INVESTIGATIONS INTO THE BIOLOGICAL CONTROL OF RED PALM WEEVIL USING *BEAUVERIA BASSIANA*

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ABSTRACT

A series of experiments were conducted using the biological agent *Beauveria bassiana*. The objectives were to determine the optimum bait mixture for attracting adult insects, the residence time of adult insects within bait "traps", the mortality of adult insects following treatment with *B. bassiana* mixed with bait and the extent of horizontal transmission of *B. bassiana* infection from the treated insects to exposed, healthy insects. The results showed that male and female insects visit pheromone traps and showed burrowing behaviour traits into date pulp. When B. bassiana spores were mixed with the date pulp effective mortalities could be achieved after a treatment time of just 15 minutes. The results also showed significant subsequent levels of horizontal infection transfer from treated insects to healthy insects.

INTRODUCTION

The use of biological control in the management of insect pests has increased in recent years. This is not only because of concerns about the extent of pesticide use in developed and less developed countries, but also because of increased levels of sophistication in the delivery of these agents. The use of biological control against insect pests might include direct application of an entomopathogenic fungus, such as *Beauveria*, or the use of toxins derived from these fungi (Amiri *et al*., 2000; Bandani *et al*., 2000).

The technique of using naturally infected, live insects as a means of dispersing biocontrol agents is long established. For example, work reported from Rothamsted Experimental Station, UK, by Butt *et al.* (1998) showed the use of honey bees for the effective delivery of the biocontrol agent *Metarhizium anisopliae* to the site of plant infestation by pollen beetle. Indeed, time and again experience has shown that even when an extremely potent biological control agent is available, it is very often far from effective

in the field unless there is an efficient and effective delivery system (Ibrahim *et al*., 1999).

Recent research has shown that delivery of inoculum is most effective, and mortality is correspondingly highest, when the infection is transferred from a dead insect to a live insect, that is, via horizontal transfer between individuals. Mortality is lower when the inoculum is applied to soil, or directly to plant parts. Research using the banana weevil has shown that the most effective means of delivering biological control inoculum to live insects was through the use of bait material such as maize or rice meal into which the fungus had been incorporated. In this way there was effective horizontal transfer of inoculum between adults, as well as transfer of infection from females to eggs and larvae (Nankinga, 1999).

MATERIALS AND METHODS

Insect visits to bait traps

The current study was devised to develop an effective delivery system for biocontrol agents. The results obtained were from experiments conducted under controlled conditions, using insects caughted from within infested date palm gardens. The bait used for all experiments consisted of 20ml of pulped dates with 25% by volume molasses. The container used was a plastic bowl with a diameter of 30cm. Its outside walls were covered with hessian material to assist insect access to the inside. A pheromone lure, Rhynchopherol, as Ferrolure+ (Chem Tica International SA, Costa Rica) was suspended 10cm above the container. A video camera was positioned above the bait such that a view was obtained of the bowl and a the small surrounding area.

For each experimental run, 20 adult insects (10 male and 10 female, all marked with numbers for subsequent identification) were released into the room. The distance from the site of release to the bait was 2m. Video data was collected for the entire period of each experimental run. Video data was analysed for information relating to insect residence time within the traps, frequency of insect revisits to traps and for differences between male and female insects in their behaviour towards the traps.

Testing Insect Mortality

The effectiveness of the *B. bassiana* inoculum against red palm weevil insects, were obtained by amended date pulp / molasses bait material with *B. bassiana* at the rate of 10g spores per 20ml bait. Ten adult insects (treated individuals) were placed in contact with the amended bait for fixed period. After removal, and to test the horizontal transfer of infection, the treated insects were placed in a clean container and 10 marked, healthy insects (exposed individuals) were added. The duration of the treatment time and the expsoure time were varied for different experiments (Table 1).

Table 1. Corresponding times of adult red palm weevil treatment with, and exposure to, *Beauveria bassiana* conidia

Treatment time (hours)	Exposure time (hours)
0.25	0.50
0.50	1.00
1.00	2.00
2.00	4.00
4.00	8.00

After fixed exposure times the two sets of insects were separated and cultured in insect rearing containers. They were supplied with food (sugar cane pieces) and liquid (sugar/water mix on cotton). Periodic assessments of insect mortality were made.

RESULTS

Insect visits to bait traps

The results suggest that the average residence time within the traps was approximately 22 minutes (Figure 1). However, the mean residence time for male insects was significantly longer (30.1 minutes) than for female insects (11.5 minutes). Although the average duration of each visit was 22 minutes, there was a significant variation in the length of each visit. Although 37.5% of visits lasted longer than 15 minutes, in some cases up to 4 hours, many visits lasted only a few seconds. Indeed, 25% of visits lasted for less than 1 minute (Figure 2). The longer duration visits usually involved the insect burrowing into the date pulp and showing little activity for extended periods. Both shorter and longer duration visits to the bait involved

matings or near matings. These occurred at a frequency of approximately 1 per hour; individual males often coupling with several females.

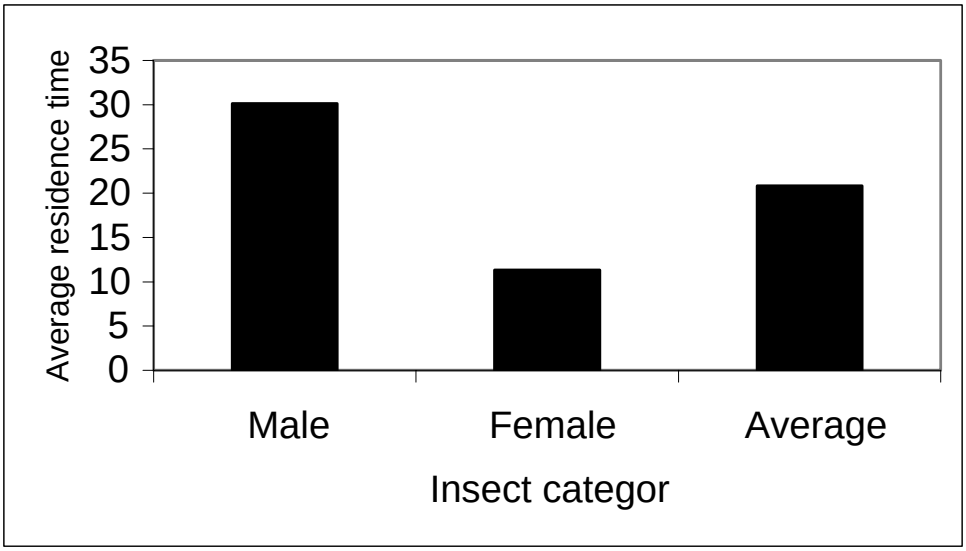

Figure 1. Average residence time for male and female adult red palm Weevils in date pulp traps

Of the total number of visits to the bait traps, 80% were made by male insects and 20% by female insects. Revisits by indentified individuals were often made within short periods of time.

Insect mortality due to infection by B. bassiana

The progression of mortality was linear over time (Table 2). There was no significant difference with increasing treatment and exposure times. Indeed 100% mortality was achieved most quickly following a treatment time of 0.25h; the corresponding exposure time of 0.l5h caused 90% mortality after 7 days.

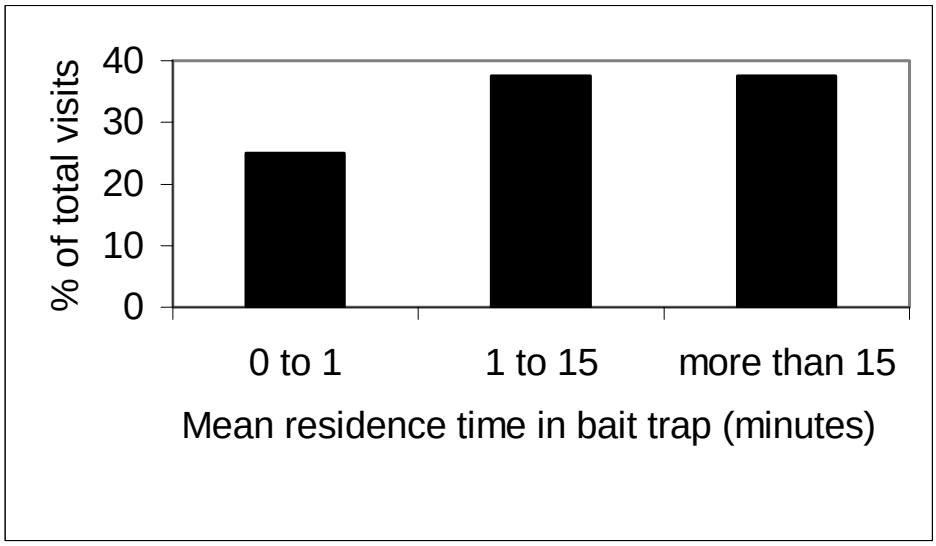

Figure 2. Duration of individual visits by adult red palm weevils to date pulp traps

Table 2. Adult red palm weevil mortality (%) after various periods of treatment and exposure to date pulp bait containing *Beauveria bassiana*

Days after treatment/exposure

	0	1	2	4	5	6	7	9	10	11	13	15	17	19	20
Treatment 0.25h	0	10	30	60	70		90	100							
Exposure 0.5h	0	10	20	50	70	80	90								
Treatment 0.5h	0	20	40					90	100						
Exposure 1.0h	0		10				20	70	80	90					
Treatment 1.0h	0									50	60	70		100	
Exposure 2.0h	0				10									60	70
Treatment 2.0h	0				10		20	30			80	90			100
Exposure 4.0h	0				20						60	70	80		
Treatment 4.0h	0					50		70			80				100
Exposure 8.0h	0							20			40				100

The corresponding rates for insect mortality, time to 50% mortality and the correlation coefficients for the linear component of insect mortality are shown in Table 3.

Table 3. Rate of insect mortality and time to 50% mortality of adult red palm weevils treated or exposed to date pulp bait containing *Beauveria bassiana*

Exposure time/ Treatment time (hours)	Rate of insect mortality (day-1)	Time to 50% mortality (days)	Correlation coefficient (r^2) for linear mortality0
Treatment 0.25h	12.4	4.0	.9615
Exposure 0.5h	13.1	3.8	.9899
Treatment 0.5h	10.2	4.9	.9365
Exposure 1.0h	7.2	6.9	.8733
Treatment 1.0h	4.0	12.6	.9315
Exposure 2.0h	3.8	13.3	.9383
Treatment 2.0h	4.6	11.0	.8788
Exposure 4.0h	4.2	12.0	.9886
Treatment 4.0h	5.8	8.7	.9025
Exposure 8.0h	4.1	12.1	.8835

DISCUSSION

The results showed that during their residence in the trap, each adult has high probability of receiving sufficient *Beauveria* to cause death in approximately 4 days. There was also a significant probability of horizontal transfer from infected individuals to other insects coming into contact with treated individual, but away from the bait site.

The observations made using the video data have other important implications for future research. For successful infection to occur, a lethal dose needs to be accumulated by the adult insect within a time of approximately 30 minutes for males and 12 minutes for females. With a male residence time of 30 minutes this suggests the potential for effective horizontal transfer of infection to females through mating events or to males and females through the normal congregation events of adult insects.

The video data showed that adult insects frequently burrowed into date pulp mixture. This is an important observation if an effective delivery is to be achieved. Previous studies with *Cosmopolites sordidus* (Nankinga, 1998) have shown that immersion in a spore suspension of *Beauveria* is more successful in causing infection than insects moving over a surface impregnated with spores. The observation that frequent visits to the bait traps were made by females, suggests the potential for horizontal transfer of infection from adult to eggs and thence to larvae.

Spores of *B. bassiana* can be inactivated by prolonged exposure to direct sunlight. This is a potentially seriuous handicap to a system of delivering spores in a bait trap. However, by mixing the inoculum with date pulp, exposure to sunlight will be limited. This exposure can be further limited by trap design. Furthermore, the moisture in the mixture should maintain the temperature of the bait at a level lower than the ambient air temperature.

Further research is required to examine the effect of short duration exposure of adults to biocontrol agents mixed with date pulp. It is also necessary to test other biocontrol agents, as previous results have shown the differential sensitivity of insects to particular isolates of fungi such as *B. bassiana* (Nankinga, 1998).

In conclusion, the preliminary evidence suggests that the trap design and bait material used in the current study could provide an effective

delivery system for a biological control agent. Further research is required to refine trap design and to assess the performance in the field. The potential is there, the future will show how promising these results truly are.

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