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STUDIES ON THE USE OF *METARHIZIUM ANISOPLIAE* TO CONTROL *ORYCTES RHINOCEROS*

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Selected strains of *Metarhizium anisopliae* (METSCH.) SOR. were grown on oat grain and tested in field experiments against *Oryctes rhinoceros* L. It was found that *M. anisopliae* survived in breeding site materials for at least 24 months, with survival not greatly affected by the type of material or by seasonal factors. Naturally occurring breeding sites were examined 3 months after they had been surface treated with oat grain inoculum of *M. anisopliae*. In most sites all the larvae had been killed by the fungus while some contained both diseased and apparently healthy larvae. Recommendations are made for the use of *M. anisopliae* as part of a general control programme for this coconut pest.

The coconut rhinoceros beetle (*Oryctes rhinoceros* L.) is of great importance in many countries because of the damage the adult insect causes by feeding on coconut palms (CATLEY, 1969). The entomogenous fungus *Metarhizium anisopliae* (METSCH.) SOR. attacks the insect and symptoms have been well described by NIRULA *et al.* (1955). Attempts to control *O. rhinoceros* by inducing epizootics with this fungus have met with little success (BRYCE, 1923; YOUNG, 1971). However, promising results have been obtained when it has been used against localized insect populations (NIRULA *et al.*, 1955; KURIAN, 1969; MARSCHALL, 1969). MARSCHALL (1969) grew the fungus on Sabouraud's agar and sprayed the spores on to both natural and artificial breeding sites of *O. rhinoceros*. YOUNG (1970) examined some of these sites in the Kingdom of Tonga. He found that the majority of areas inoculated 3 months previously contained larvae infected with *M. anisopliae* but areas sprayed 5-6 months previously contained many healthy larvae and there was no trace of the fungus. He also found little spread of the fungus from inoculated areas to adjacent breeding sites. Apart from these observations there is little information on the persistence of *M. anisopliae* when it has been sprayed as a spore suspension on to *O. rhinoceros* breeding areas.

LATCH (1976) screened a number of entomogenous fungi to determine their pathogenicity to *O. rhinoceros*. Several isolates of *M. anisopliae* were selected for further study in field trials. An inexpensive and simple method of growing large quantities of the fungus on oat grain was devised for these trials.

This paper reports the results of field experiments in the Kingdom of Tonga to determine the longevity of *M. anisopliae* in breeding material utilized by *O. rhinoceros*. The feasibility of using *M. anisopliae* as a "microbial insecticide" against naturally occurring populations of *O. rhinoceros* was also ascertained.

MATERIALS AND METHODS

INOCULUM

Three isolates of *M. anisopliae* (table 1) were used in these experiments. They were selected after screening a large number of isolates of entomogenous fungi (LATCH, 1976).

Cultures 10P and 2A, originally isolated from *O. rhinoceros*, were highly pathogenic to *O. rhinoceros* in these tests, while the short-spored culture WT10 isolated from *Conoderus* sp. was less virulent.

TABLE 1

Sources and conidial dimensions of M. anisopliae *isolates*

Isolate Number	Source		Conidium dimensions (a) μ	
	Host	Location	Length	Width
10P	*O. rhinoceros*	Kerala, India	11.9 (11.0 — 13.0)	3.2 (3.0 — 3.5)
2A	*O. rhinoceros*	Western Samoa	11.9 (10.0 — 14.0)	3.1 (2.8 — 3.5)
WT10	*Conoderus* sp.	North Carolina, U.S.A.	6.5 (5.5 — 7.5)	2.5 (2.0 — 2.8)

(a) Mean of 25 conidia from 14 day, 25°C PDA cultures. Figures in parentheses indicate range.

The fungi were grown on oat grain (*Avena sativa* L.) in Roux bottles or large Erlenmeyer flasks. Tap water was mixed with the oats in a ratio grain to water of 1: 1.4. Flasks were almost half filled with oats and autoclaved at 121°C for 30 minutes. They were then inoculated with spore suspensions of *M. anisopliae* which had been grown on Buttermilk agar (LATCH, 1976), and incubated at 25°C. After 2-3 weeks growth the oat inoculum was dried at 35-40°C for two days. Spore yield was generally 9 g of spores (2×10^{10}) per 100 g dry weight of oats. In some cases wet inoculum, straight from culture flasks, was used in field trials.

LONGEVITY EXPERIMENT

A total of 151 pits, each measuring 0.6 m × 0.6 m and 0.5 m deep were dug in soil at the Coconut Replanting Scheme Headquarters, Tokomololo, Tongatapu. The pits were spaced 0.6 m apart in rows and the distance between rows was 1.5 m. They were filled with 1 of 3 materials utilized by *O. rhinoceros* for breeding; partially decomposed sawdust of mixed wood types (sawdust), a mixture of domestic rubbish and compost obtained by clearing horticultural land with bulldozers (compost), and a mixture of oxidized sewage and coral sand (sewage). At later stages in the experiment domestic rubbish was discarded and field compost was used alone. Each pit was inoculated when it was filled, by mixing grain inoculum with the top 10 cm of material. A few pits had the inoculum mixed with the top 25 cm of material. Pits were covered with 1.5 cm wire netting to keep out beetles. Treatments shown in table 2 were applied four times over a 12 month period with each treatment being replicated twice each time it was applied. Control pits were inoculated with freshly boiled oat grain.

At 3 month intervals, 7 samples were taken from each pit with a garden trowel. Three samples were taken from the top 10 cm of material, 2 from 10-20 cm down, and 2 from 20-30 cm. They were taken from areas undisturbed by previous samplings and the trowel was surface sterilized between each pit. Each sample approximately half filled a 300 ml can and to each a healthy third instar *O. rhinoceros* larva was added. These larvae were obtained from the FAO insect rearing unit in Apia, Western Samoa. Cans containing samples and larvae were stored indoors at ambient temperature for 15 days, at which time larvae were inspected and the degree of attack by *M. anisopliae* was assessed. Infection was considered positive if characteristic lesions were present

or, if the larvae were dead, they showed signs and/or symptoms of fungal infection such as dehydration of the cadavers and the development of mycelium and spores. Survival of *M. anisopliae* in experimental pits was determined by calculating percentage infection for each treatment.

Rainfall and temperature records for the trial period were obtained from the Meteorological Office, Nuku'alofa, Tongatapu situated about 4.5 km from the experimental site.

TABLE 2

Treatments applied to longevity experiment pits

Treatment number	Breeding site material	*M. anisopliae* isolate	Dry weight of inoculum added (g)	Times treatments applied (a)			
				April 1973	July 1973	Oct. 1973	Jan. 1974
1	Sawdust	10P	50	+	+	+	+
2	—	2A	50	+	+	+	+
3	—	WT10	50	+	+	+	+
4	—	Control	50	+	+	+	+
5	Sewage	10P	50	+	+		
6	—	2A	50	+	+		
7	—	WT10	50	+	+		
8	—	Control	50	+	+		
9	Compost	10P	50	+	+	+	+
10	—	2A	50	+	+	+	+
11	—	WT10	50	+	+	+	+
12	—	Control	50	+	+	+	+
13	—	10P (b)	50	+			
14	—	2A (b)	50	+			
15	—	WT10 (b)	50	+			
16	—	Control (b)	50	+			
17	—	10P	5	+			
18	—	10P	20	+			
19	—	10P	500	+			
20	—	10P	5	+	+	+	+
21	—	10P	20	+	+	+	+
22	—	10P	500	+	+	+	+
23	—	WT10 (wet) (b)	70	+			
24	—	WT10 (wet)	70	+			
25	—	10P mixed in top 25 cm	50	+			
26	—	10P mixed in (b) top 25 cm	50	+			
27	—	10P (wet)	70		+	+	+
28	—	2A (wet)	70		+	+	+
29	—	WT10 (wet)	70		+	+	+
30	—	Control	50		+	+	+

(a) Treatments applied at dates marked +

(b) Pits had 6 healthy 3rd instar *O. rhinoceros* larvae added

FIELD INOCULATION TRIALS

On 4 occasions during the 12-month period of trial work sites were selected for field releases of *M. anisopliae*. Sites used were rural compost heaps, some of which resulted from bulldozer clearance of horticultural land, coconut log sawdust traps used previously to release the Rhabdionvirus pathogen of *O. rhinoceros* and then abandoned, and sawmill sawdust dumps. In all these sites healthy populations of larvae at all stages of development were found prior to inoculation.

Oat inoculum of the *M. anisopliae* isolates was spread over the surface and lightly covered with breeding site material. Three months after inoculation these sites were dug over and the condition and size of *O. rhinoceros* populations present were determined.

RESULTS

Longevity experiment

Although only the top 10 cm of substrate was inoculated in most pits, test larvae in substrate taken from all 3 levels in pits (0-10 cm, 10-20 cm, 20-30 cm) were equally infected. Survival of *M. anisopliae* isolates over the 12-month trial period is indicated in figure 1. Between 9 and 12 months after inoculation the amount of inoculum surviving was reduced, but at 12 months the long spored isolates 10P and 2A still achieved between 50-70 % infection of test larvae.

The amount of infection in samples from pits inoculated 3 months previously indicated seasonal effects on survival of fungus isolates. Figure 2 shows these effects to be slight with only the less virulent isolate WT10 giving small reductions in infection during the warmer, wetter period from October to April (fig. 3).

The effect of inoculum level on survival of isolate 10P, the only isolate for which this was investigated, is shown in figure 4. An initial application of 14 g of oat inoculum/m^2 resulted in 43 % of test larvae becoming infected with the fungus after 12 months. A 100 fold increase in the level of inoculum only doubled this amount of infection at the end of 12 months. The 13 % infection in the control pits indicates the degree of contamination from neighbouring treatments.

Different breeding site materials affected the survival of *M. anisopliae* (fig. 5). Compost and sewage allowed a better survival of the fungus than did sawdust. Severe fungus contamination occurred in both compost filled control pits set up in July 1973. This accounts for the high average figure for contamination even though compost filled control pits set up at other times of the year had little fungus contamination. No difference in inoculum survival was detected when dried inoculum was compared with wet inoculum straight from culture flasks.

The effects of the 3 *M. anisopliae* isolates on test larvae are summarized in table 3. Treatments such as type of substrate, level of inoculum, and time have been ignored. The 2 long spored isolates 10P and 2A gave similar levels of infection. The short spored isolate WT10 was less virulent over the 15 day test period, far fewer larvae were killed but the incidence of cuticular lesioning was higher than with the other 2 isolates.

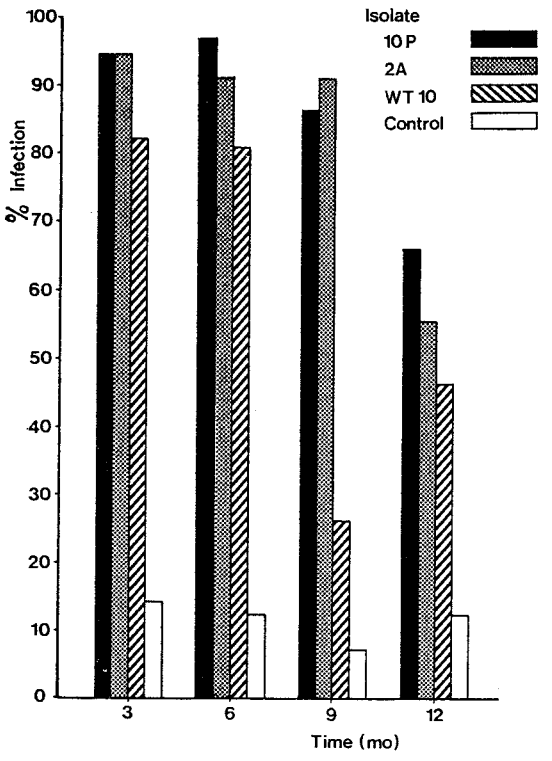
FIG. 1. Effects of time on survival of *M. anisopliae* isolates in all 3 breeding site materials. Dry inoculum added at rate 56 g/m^2 at time O.

FIG. 2. Seasonal effects on survival of *M. anisopliae* isolates in all 3 breeding site materials. Dry inoculum added at 56 g/m^2 in all cases.

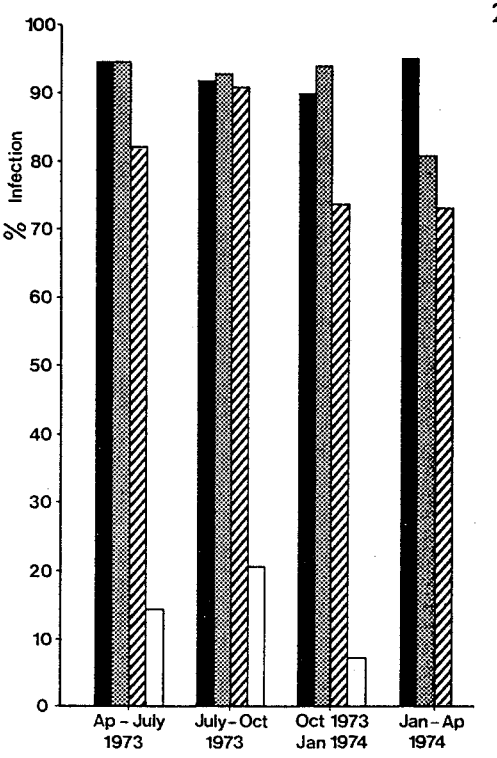
FIG. 3. Rainfall (●——● 25 year mean) and mean monthly temperatures (■——■ maximum, ——□ 22 year mean maximum, ▲——▲ minimum, Δ——Δ 22 year mean minimum) recorded at Nuku'alofa, Tonga for period of trials.

FIG. 4. Survival after 12 months of *M. anisopliae* (isolate 10P) as affected by quantity of dry inoculum·

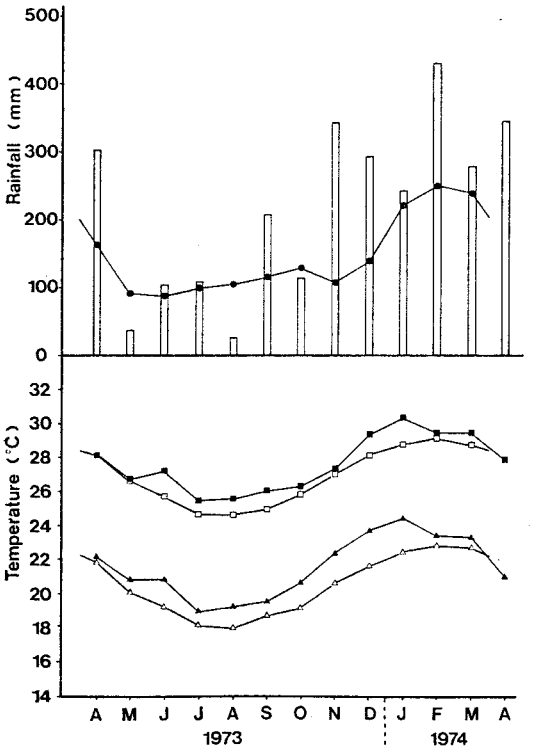
1


Isolate
10 P
2A
WT 10
Control
% Infection
Time (mo)
3
6
9
12

2


% Infection
Ap - July 1973
July - Oct 1973
Oct 1973 Jan 1974
Jan - Ap 1974

3


Rainfall (mm)
Temperature (°C)
A M J J A S O N D J F M A
1973
1974

4

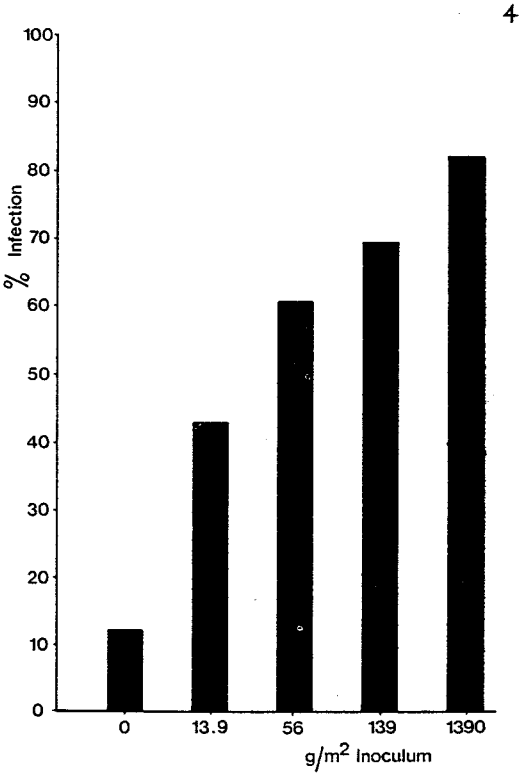

% Infection
0
13.9
56
139
1390
g/m² Inoculum

TABLE 3

Percentages of infected and healthy larvae after 15 days' exposure to different isolates of M. anisopliae

Isolate number	Infected by *M. anisopliae* (%)	Lesioned (%)	Dead (%)	Dead from unknown causes (%)	Apparently healthy (%)
—	—	—	—	—	—
10P	86.10	16.99	69.11	4.47	9.43
2A	82.11	17.30	64.81	3.78	14.12
WT10	60.70	47.44	13.26	5.27	34.03
Control	15.27	4.50	10.80	5.03	79.70

FIELD INOCULATION TRIALS

Table 4 summarizes results obtained from inoculated breeding sites. Long spored isolates of *M. anisopliae* brought about complete annihilation of *O. rhinoceros* populations in 5 of the 9 undisturbed sites. In 3 of the remaining 4 undisturbed sites over 40 % of larvae present were killed by or infected with the fungus. Many of these apparently healthy larvae were at the first or second instar stage. The remaining site was a compost heap which had rotted away and contained 2 apparently healthy larvae.

TABLE 4

Results of field breeding site inoculation trials

Treatment	Total sites treated	Sites disturbed or destroyed	Sites where *O. rhinoceros* population annihilated	Condition of *O. rhinoceros* populations in remaining undisturbed sites		
				Infected with *M. anisopliae* (%)	Dead unknown causes (%)	Apparently healthy (%)
—	—	—	—	—	—	—
10P	8	2	2	44.9	4.2	50.9
2A	4	1	3	—	—	—
WT10	4	3	0	0	4.0	96.0
Control	11	5	1	43.3	0	56.7

The short spored isolate was used to treat 4 sites. When these sites were examined 3 months after they had been inoculated 2 were found to be destroyed and 1 site was disturbed by pigs and contained no larvae. The 1 undisturbed site contained only apparently healthy larvae.

Infection in untreated control sites gave some indication of the movement of *M. anisopliae* in the environment. Where control sites contained infected populations the infection had occurred comparatively recently. Several control areas situated 300-400 m from the nearest inoculated site contained infected larvae. Otherwise infected control sites were adjacent to or within a few metres of inoculated sites.

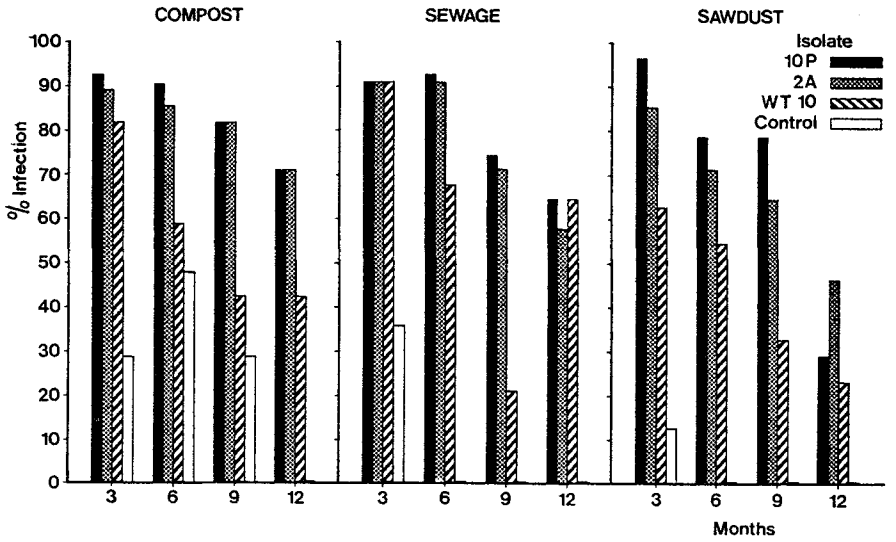


FIG. 5. Effect of different *O. rhinoceros* breeding media on survival of *M. anisopliae* isolates. Dry inoculum added at rate 56 g/m² at time O.

DISCUSSION

In assessing the efficacy of a biological insecticide, it is necessary to determine the length of survival of the agent in the environment to which it is to be introduced. Field compost heaps created by bulldozer operations, or coconut plantation refuse heaps, are the breeding sites most commonly utilized by *O. rhinoceros* in the Kingdom of Tonga. It takes 6-9 months for material in these sites to decompose completely and become unattractive to adult insects. Other breeding sites where fresh material is added regularly are more permanent. Sawdust heaps at sawmills and domestic rubbish heaps fall into this category.

This study has shown that the 2 long spored isolates of *M. anisopliae* can survive in 3 of the main *O. rhinoceros* breeding substrates for at least 12 months. It is possible that under natural field conditions fungus survival would be prolonged even further by the presence of spores on diseased cadavers. Larvae would also move through the fungus contaminated substrate and be exposed to a greater quantity of inoculum for a longer period of time than the 15 days each test larva spent in 150 cc of substrate. Hence, survival of the fungus in nature would probably be longer than that shown in the Tokomololo longevity experiment and larval mortality would be higher. Survival was little affected by the time of the year when fungus inoculum was applied. It was also not greatly affected by breeding site material, although compost and sewage gave slightly better fungus survival than did sawdust. The fungus can therefore be used successfully for controlling larvae growing in a wide range of substrates at any time of the year. Although the findings of YOUNG (1970) and our results are not strictly comparable, the longevity of the fungus is considerably greater in our trials. This may be due to the type of inoculum we used. Some of the oat grains containing viable mycelium were still recognisable in the substrate 3 months after they had been scattered there.

Of great practical importance is the quantity of oat inoculum required to provide satisfactory biological control. The longevity experiment indicated that 14 g dry weight of inoculum/m^2 was sufficient to infect over 40 % of test larvae some 12 months after the compost was inoculated. The quantity of inoculum applied to the substrate had to be increased considerably to achieve greater infection of test larvae. Information from the completed field trials shows that good control of larvae in natural breeding grounds was attained by scattering 10-30 g dry weight of oat inoculum/m^2. Thus, it would seem that a figure in the region of 25 g dry weight of inoculum/m^2 should be adequate to give control.

In the longevity experiment *M. anisopliae* was present in material taken 20-30 cm from the surface. For the majority of pits inoculum was only mixed in with the top 10 cm so presumably spores were washed down the profile by rain. Movement of spores in the substrate probably also occurs through the activities of small animals such as ants, millipedes and cockroaches which infest the soil. It is therefore unnecessary to incorporate fungus inoculum to a depth of more than a few cm. A light covering of substrate is desirable because oat inoculum left on the surface is eaten by birds.

Both wet and dry inoculum were equally effective in trials. Each form has its advantages and disadvantages. Wet inoculum can be used immediately, spore viability is very high and larvae may become infected as soon as the inoculum is distributed in the breeding sites. On the other hand it must be used within a few hours of removal from the culture vessels as it heats up if left in bulk. It can however be stored at 4° or —15° for many months if left in the original culture vessels (LATCH, 1976). Dry inoculum has to be dried at 30-40° for several days and so ovens are necessary. Once scattered in breeding sites it takes about a week for a fresh crop of spores to form on the oats. The main advantage of dry oat inoculum is that it can be stored at room temperature for several months before use.

Our field trials indicated that the spread of *M. anisopliae* from inoculated areas did occur but that most of this spread was only to areas several metres distant. A few experimental control sites became infected even though they were 300-400 m away from fungus inoculated heaps. Such spread would probably be from infected adults although only 2 adults killed by *M. anisopliae* were found in our trials. Thus, although use of the fungus in the classical role of a biological control agent is unsatisfactory it may be useful as a "biological insecticide" where repeated applications of the fungus are made on breeding grounds.

The 2 long spored isolates of *M. anisopliae* controlled *O. rhinoceros* effectively in field trials but some sites contained apparently healthy larvae as well as those killed by the fungus. Many of these larvae were first or second instar stages and therefore had only been in the inoculated sites for a few weeks. Probably they too would succumb to the fungus eventually. It is likely that many of these larvae were diseased at the time they were examined but that they showed no symptoms, for it was noticed that only half the test larvae killed by the fungus in the Tokomololo longevity experiment had brown spots on the integument.

Laboratory screening experiments by LATCH (1976) had shown that larvae died over a much longer period of time when inoculated with the short spored isolate of *M. anisopliae* than did larvae inoculated with long spored cultures originally isolated from *Oryctes* species. This character was considered desirable for it may allow the isolate to survive longer in breeding sites than more virulent isolates. Only 1 of the field trial sites inoculated with this isolate was not destroyed or disturbed. The fungus was ineffectual at this site but it would be premature to dismiss the short spored isolate on the basis of 1 trial.

Our experiments indicate that either of the 2 long spored isolates of *M. anisopliae* could be used successfully as a biological insecticide against *O. rhinoceros* in the Kingdom of Tonga. We recommend that the fungus is grown on autoclaved oats at temperatures of 20-25°C until good sporulation occurs. The fungus inoculum can then be harvested or stored in the culture vessels until ready for application. Wet inoculum must be applied within a few hours or else stored under refrigeration. Alternatively, the inoculum can be dried for several days at 30-40°C, stored at room temperature, and applied in the following months. Inoculum should be scattered over *O. rhinoceros* breeding sites at a rate of about 25 g dry weight/m^2 and mixed in with the top few cm of breeding substrate. Permanent breeding sites should be re-inoculated every 12 months. Temporary breeding sites such as compost heaps should be inoculated 2-3 months after they are formed. No further treatment should be required.

A comprehensive approach to the control of *O. rhinoceros* is needed. The use of *M. anisopliae* as a biological insecticide, the presence of Rhabdionvirus in the insect population, and the pursuit of a rigorous sanitation programme to remove breeding sites in the plantations should keep the insect population at an acceptably low level.

APPENDIX

In April 1975, 56 of the pits filled with compost 15-24 months previously were tested for the presence of *M. anisopliae*. Viability of the long-spored isolate after 2 years was generally higher than that found after 12 months. All test larvae in compost from pits inoculated with 139 g and 1 390 g/m^2 of oat inoculum were killed by the fungus, as were 80 % at 56 g/m^2 and 30 % at the 13.9 g/m^2 level. Pits inoculated with the short spored isolate WT10 at the rate of 56 g/m^2 contained no viable *M. anisopliae* after 24 months. Several control pits were contaminated with *M. anisopliae* so that overall 25 % of test larvae in compost from control pits were infected.

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RÉSUMÉ

Études sur l'utilisation de *Metarhizium anisopliae* pour la lutte contre *Oryctes rhinoceros*

Certains types sélectionnés de *Metarhizium anisopliae* ont été cultivés sur des grains d'avoine et examinés en tant qu'insecticides biologiques au cours d'expériences pratiques contre les *Oryctes rhinoceros*. Les résultats ont montré que *M. anisopliae* peut survivre sur les matériaux du lieu de culture pendant au moins vingt-quatre mois, leur survie n'étant pas particulièrement affectée par le type de matériel ou par les facteurs saisonniers. Les lieux de culture naturels ont été examinés trois mois après qu'ils aient été traités superficiellement par l'inoculum de grain d'avoine de *M. anisopliae*. La plupart des lieux de culture contenaient seulement des cadavres de larves d'*O. rhinoceros* tués par le champignon tandis que certains autres contenaient à la fois des larves malades et des larves en apparence saines.

L'utilisation de *M. anisopliae* est recommandée dans le cadre du programme général de lutte contre cet insecte nuisible qui s'attaque à la noix de coco.

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