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The Infectivity of *Metarhizium anisopliae* to Two Insect Pests of Coconuts

C. PRIOR

Cocoa Industry Co. Ltd., P.O. Box 1846, Rabaul, Papua New Guinea

AND

M. ARURA

Department of Primary Industry, Bubia Agricultural Research Centre, P.O. Box 73, Lae, Papua New Guinea

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Papua New Guinea isolates of *Metarhizium anisopliae* from the rhinoceros beetles, *Scapanes australis* and *Oryctes rhinoceros;* the black palm weevil, *Rhynchophorus bilineatus* (Coleoptera); and three other insect orders (Hemiptera, Lepidoptera, and Dermaptera) were all short-spored (*M. anisopliae* var. *anisopliae*). Isolates from *Scapanes* were also pathogenic to *Rhynchophorus* and *Oryctes*. When a *Scapanes* isolate was grown on brown rice and released into the frond axils of young palms, 32% of dead *Scapanes* adults collected during the following 48 weeks were infected compared to 1% in the control nonrelease palms. Some infection of *Rhynchophorus* also occurred. Infection in *Scapanes* but not *Rhynchophorus* also increased in a plot of palms adjacent to the release zone. The rice inoculum could be recovered and the fungus reisolated up to 84 days after release, but infection of *Scapanes* continued to occur after all trace of the inoculum had disappeared.

KEY WORDS: *Metarhizium anisopliae; Scapanes australis; Rhynchophorus bilineatus;* coconut pests; mycoinsecticide; biological control.

In Papua New Guinea initial damage to coconut palms by rhinoceros beetles (Coleoptera: Scarabaeidae) is frequently followed by lethal infestations of the black palm weevil, *Rhynchophorus bilineatus* (Coleoptera: Curculionidae), (Bedford, 1974), which Smee (1965) characterized as the most serious coconut pest in the country. Since *Rhynchophorus* usually attacks damaged tissue, control of rhinoceros beetle damage is vital for control of the weevil. The Asiatic rhinoceros beetle, *Oryctes rhinoceros*, is effectively controlled by the introduced *Baculovirus* of *Oryctes* in many countries and this virus is now established in Papua New Guinea (Gorick, 1980). However, Gorick (unpubl.) was unable to infect adults of the indigenous rhinoceros beetle, *Scapanes australis*, with the virus. The currently recommended control method in Papua New Guinea (Anonymous, 1983), and the Solomon Islands (Stapley, 1980) is to apply 6% a.i. gamma-HCH (Lindane) granules to the axils of the fronds at 3-month intervals or less, but control is not complete. *Scapanes* is now a major pest of coconuts in Papua New Guinea and, since it attacks mainly young palms, its seriousness will probably increase as more old palms are replanted with new hybrid material.

The entomopathogenic fungus *Metarhizium anisopliae* has been much discussed as a biological agent for controlling rhinoceros beetles (Swan, 1974). In Tonga and Samoa, artificial "trap" breeding sites for *Oryctes* were sprayed with spores and very high levels of larval infection were noted (Marschall, 1980). Very little spread to natural breeding sites occurred, presumably because the insects died before emerging from the infected breeding sites. A decline

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in palm damage was noted after release of the fungus in Tonga (Marschall, 1980) and Samoa (Young, 1974), but it was not clear if this was due to fungus-induced mortality or to the recently released *Baculovirus* (Swan, 1974; Young, 1974). Swan (1974) concluded that *Metarhizium* had failed to initiate epizootics in *Oryctes* populations and had no future as a biological insecticide against *Oryctes* larvae. However, the possibility of applying the fungus to palms to control *Scapanes* or *Oryctes* adults has not been tested. In the work described in this paper, a locally collected isolate from *Scapanes* was applied to young coconut palms to investigate its effect on *Scapanes* under field conditions. Some laboratory inoculation experiments are also described. These were carried out with a view to increasing the pathogenicity of the fungus to the target insects as described by Timonin et al. (1980).

MATERIALS AND METHODS

Collection and culture of isolates. Collections of sporulating infected insects were made in East New Britain and New Ireland Provinces in the Papua New Guinea Islands Region. Isolations were made initially onto 2% malt extract agar (Oxoid), with streptomycin at 100 units/ml. In subsequent work a modification of Doberski and Tribe's (1980) medium was found to be better. This consisted of 20 g/liter dextrose, 5 g/liter peptone, 5 mg/liter crystal violet, 0.3 g/liter chloramphenicol, and 0.14 g/liter cycloheximide (autoclaved separately), and 12 g/liter agar. Isolates were stored on slopes of 2% malt agar at 26°C. Spore lengths of 14 isolates were measured in distilled water.

For spore production, a modification of Latch's (1976) medium was used: 20 g/liter dextrose, 10 g/liter peptone, 10 g/liter casein hydrolysate, and 12 g/liter agar.

For field release, an isolate from *Scapanes* was used that had already been shown to be pathogenic to *Oryctes* and *Scapanes* in laboratory trials. Brown rice was precooked in the ratio of 1 kg rice:580 ml water until all the water was absorbed. It was then autoclaved for 30 min in 25 × 40-cm "Glad" oven bags at 150°C. The bags were inoculated with an aqueous suspension of spores and incubated for 7 days before use.

Field release. Two- to five-year-old palms growing at Vudal Agricultural College near Rabaul were divided into five zones of unequal size containing a total of 563 palms. All recorded palms were numbered. *Scapanes* and *Rhynchophorus* damage at this site was severe and had already led to the loss of many palms, with some replanting. The release zone, containing 127 palms, was located at one end of the site. One hundred control palms were also numbered in a 6-year-old block at the Lowlands Agricultural Experiment Station, Keravat, 5 km distant from the release zone. Damage was very severe in this block.

Two surveys were made in the preceding year to check the natural level of *Metarhizium* infection. In addition a pretreatment survey of all the palms at the College was made on the day before the release. All dead insects were collected and microscopically checked for infection. These insects were not replaced in the palms. The control block at Keravat was not included in this survey.

The fungus was released in December 1981, by distributing 100 g rice inoculum per palm in the axils of every frond on every palm in the release zone. Fortnightly recordings were made of all live, dead, and infected adult insects in every palm in the release zone, the adjacent nonrelease zone, the other nonrelease zones, and the control zone for 48 weeks after release. Dead and infected insects were marked with different colored pins. This enabled recorders to detect very recent infections which had not been visible at the time the insect was first recorded. During the postrelease counts, all

dead and infected insects were replaced where they were found so as not to remove the infection source from the field. One large section of the "other nonrelease zones" was not recorded from week 34 onward.

The fortnightly data were blocked into 12-week periods for analysis. The proportions of dead to infected insects in the three zones at the release site were compared with the proportion in the control zone using the chi-square test (Snedecor and Cochran, 1967).

Viability of the fungus inoculum after release. Three rice grains from each of 10 randomly chosen palms were collected at intervals after release and visually checked for *Metarhizium*. One grain from each palm was plated on modified Doberski and Tribe agar for confirmation.

Laboratory inoculation experiments on Scapanes, Oryctes and Rhynchophorus. Inoculation experiments were carried out to check the pathogenicity of isolates and to try to increase pathogenicity and extend it to other target insects. Isolations were made from two infected *Scapanes* and two infected *Rhynchophorus* collected from the release zone after release of the original isolate. These isolates were used for reinoculation, and the fungus was then reisolated from infected weevils and used to inoculate beetles and vice versa. It was never possible to obtain enough insects for replicated experiments.

Adult *Scapanes* and *Rhynchophorus* (and *Oryctes* in one experiment) were inoculated by dipping them for 5–10 sec into aqueous spore suspensions prepared from cultures on modified Latch's medium by flooding the colonies with 20 ml water plus one drop of Tween 20, brushing off the spores, and diluting to 500 ml. Spore concentrations were in the range $0.5–1.5 \times 10^7$ spores/ml. In one experiment, weevils were inoculated by scattering rice grains covered with sporulating fungus on the floor of the cage.

RESULTS

Collection and Culture of Isolates

Metarhizium was readily isolated onto modified Doberski and Tribe's agar. Colonies grew slowly and sporulated after 7–9 days. The only contaminants that were commonly encountered were *Penicillium* spp.

All Papua New Guinea isolates from four insect orders (Coleoptera, Lepidoptera, Hemiptera and Dermaptera) were "short-spored" by Latch's (1976) definition. Mean spore lengths ranged from 5.5 to 7.5 μm (4.5–8 μm). All these isolates conformed to Tulloch's (1976) definition of *Metarhizium anisopliae* var. *anisopliae*.

Cultures remained viable for 1 year on 2% malt agar. The fungus sporulated abundantly on modified Latch's medium, producing a thick spore layer after 7 days. However, several isolates lost the ability to sporulate after one or two subculturings on agar.

Field Release

Only a single infected *Scapanes* and no infected *Rhynchophorus* were found at Vudal College in the two surveys made in the year preceding release, and none were found in the prerelease survey made the day before release. The 12-weekly totals of live, dead, and infected adults in the release, adjacent nonrelease, other nonrelease, and control zones are given in Tables 1 (*Scapanes*) and 2 (*Rhynchophorus*). The data are blocked from the fortnightly counts, and are actual numbers of insects rather than percentages because of the small numbers of insects recorded at each count.

The results in Table 1 show that, in the release zone, significantly more dead *Scapanes* adults were infected than in the control zone up to 36 weeks after release of the fungus. In the first 12 weeks, significantly more dead *Scapanes* were infected in the

TABLE 1
LIVE, DEAD, AND INFECTED ADULT *Scapanes* RECORDED IN FOUR ZONES AFTER RELEASE OF *Metarhizium*

	Release Zone (127)	Adjacent nonrelease zone (96)	Other nonrelease zones (340/56)[a]	Control zone (100)
Weeks 1–12				
Live	187	146	293	262
Dead	97	39	78	97
Infected	39***	8***	2[ns]	1
Weeks 13–24				
Live	128	80	89	119
Dead	36	20	14	24
Infected	20**	3[ns]	1[ns]	1
Weeks 25–36				
Live	49	76	49	56
Dead	13	8	3	11
Infected	11**	1[ns]	0[NT]	0
Weeks 37–48				
Live	40	56	18	69
Dead	6	4	2	12
Infected	1[ns]	0[NT]	0[NT]	0
Total (Weeks 1–48)				
Live	404	358	449	506
Dead	152	71	97	144
Infected	71***	12***	3[ns]	2

Note. Fortnightly counts blocked into 12-week periods. Numbers in parentheses are the number of palms/zone.

[a] One of those zones was not recorded after week 34.

*,**,***, Significantly higher proportion of dead to infected insects compared to the control zone at $P < 0.05$; $P < 0.01$; $P < 0.001$, using chi-square test.

ns, not significantly different; NT, not tested.

adjacent nonrelease zone (50 m distant) as well. No increase in infection occurred in the more distant nonrelease zones. The increase in infection in *Rhynchophorus* (Table 2) was much smaller, but in the 48-week total the proportion of dead adults infected was significantly higher in the release zone than in the control zone.

For both *Scapanes* and *Rhynchophorus*, a large number of live adults was recorded at every count. These were not marked, and it was not possible to determine how many of them contributed to the subsequent totals of dead and infected insects and how many escaped, but it is likely that some did escape. In preparing the live insect totals for Tables 1 and 2 it was necessary to assume that no live insects were counted twice. If this did happen, the real totals of live insects would have been lower and the proportion of total insects infected would have been higher. Because of this uncertainty, no analysis was carried out on the live insect data.

There was a gradual decline in the number of live and dead (uninfected) insects recorded in all zones during the year. This may have been an artifact due to interference with the habitat caused by frequent sampling.

After 26 weeks, the release zone records were analyzed to determine the proportion of palms that had been attacked by *Scapanes* (and *Oryctes*) and/or *Rhynchophorus*, and the proportion of palms in which *Metarhizium* infection had occurred (Table 3). Seventy-six palms (60% of the total) were visited by at least one *Scapanes*

TABLE 2
LIVE, DEAD, AND INFECTED *Rhynchophorus* ADULTS RECORDED IN FOUR ZONES AFTER RELEASE OF *Metarhizium*

	Release zone (127)	Adjacent nonrelease zone (96)	Other nonrelease zones (340/56)[a]	Control zone (100)
Weeks 1–12				
Live	101	61	249	105
Dead	15	6	43	9
Infected	10^{ns}	1^{ns}	4^{ns}	1
Weeks 13–24				
Live	135	122	144	100
Dead	12	9	12	15
Infected	3^{ns}	0^{NT}	0^{NT}	0
Weeks 25–36				
Live	90	188	176	21
Dead	13	16	23	0
Infected	2^{NT}	1^{NT}	2^{NT}	0
Weeks 37–48				
Live	32	101	15	24
Dead	3	6	0	2
Infected	0^{NT}	0^{NT}	0^{NT}	0
Totals (Weeks 1–48)				
Live	358	472	584	250
Dead	43	37	78	26
Infected	15^{*}	2^{ns}	6^{ns}	1

Note. Fortnightly counts blocked into 12-week periods. See Table 1 for numbers and superscripts.
[a] One of these zones was not recorded after week 34.

during the 26-week period, and infected *Scapanes* were recorded in nearly half of these.

Ninety-nine percent of all the rhinoceros beetles recorded in the course of the trial were *Scapanes*, further evidence for the importance of this pest in young palms.

Viability of the Fungus Inoculum after Release

Rice grains were easily found in the frond axils up to 49 days after release, but were difficult to find after 84 days. By this time the wetter grains were disintegrating but the exposed and drier grains still had visible fungus on them. No grains were found after 140 days. The fungus was still visible in 70% of the grains collected at 84 days, and was fully viable in 100% of the grains plated on modified Doberski and Tribe's agar.

Laboratory Inoculation Experiments on Scapanes, Oryctes *and* Rhynchophorus

Peaks in mortality due to infection occurred at 10 (6–24) days after inoculation (*Scapanes*) and 14 (7–30) days (*Rhynchophorus*). One isolate caused infection in 15 out of 22 *Scapanes* adults. Mortality to *Rhynchophorus* was low, <20% for both isolates.

In the experiment where *Scapanes* isolates were inoculated onto *Rhynchophorus* and vice versa, both isolates were pathogenic to both target insects. One *Rhynchophorus* isolate caused mortality in 8 out of 14 adult *Scapanes*, but mortality was again low in *Rhynchophorus*. Slightly higher mortality was obtained in *Rhynchophorus* when adults were inoculated by scattering rice inoculum on the floor of the cages.

TABLE 3
PERCENTAGE INFESTATION OF PALMS IN THE RELEASE ZONE AFTER 26 WEEKS

	%
Type of insect attack (within the 26 weeks)	
Palms with at least one recorded *Scapanes* but no *Rhynchophorus*	21
Palms with at least one recorded *Rhynchophorus* but no *Scapanes*	9
Palms with at least one recorded *Rhynchophorus* and one recorded *Scapanes*	39
Palms with no recorded attack by *Rhynchophorus* or *Scapanes*	31
Frequency of infection (within the 26 weeks)	
Palms with at least one *Metarhizium*-infected *Scapanes*	28
Palms with at least one *Metarhizium*-infected *Rhynchophorus*	8

DISCUSSION

Latch (1976) found that "long-spored" isolates of *M. anisopliae* (Tulloch's *M. anisopliae* var. *major*) were more pathogenic to *Oryctes* than most "short-spored" isolates (var. *anisopliae*), but some of the latter were pathogenic even though they were obtained from other insects. In Papua New Guinea all isolates collected from coleopterans and other orders were var. *anisopliae*. One isolate, obtained from *Rhynchophorus* after the field release, was as pathogenic to *Scapanes* as other *Scapanes* isolates. Thus, there seem to be possibilities both of increasing pathogenicity to *Scapanes* by "preconditioning" the isolates in the target insects (Timonin et al., 1980) and of obtaining an isolate pathogenic to more than one of them. However, if the latter proved difficult it would also be possible to release a mixture of isolates.

Mortality to *Rhynchophorus* was low for all isolates when weevils were inoculated by the dipping method, but this method may not have been very effective due to poor retention of spore suspension on the insects' cuticle. This might also explain why high mortality (>75%) was not obtained with any of the target insects in the inoculations. It appeared that mortality in *Rhynchophorus* was higher when the insects were continually exposed to the fungus in the cage as opposed to a single exposure by dipping.

For field release, a preparation of the fungus is required which will preserve it in a sporulating and infective state for as long as possible; requirements which are well met in nature by infected cadavers. Rice autoclaved in heat-resistant plastic bags has been used as a substrate for *Metarhizium* in Brazil (Aquino et al., 1977). In Tonga, Latch and Falloon (1976) found that the fungus remained infective in *Oryctes* breeding sites for up to 2 years when released in an oat grain substrate. The brown rice inoculum released in this study was hard to detect after 12 weeks, and was undetectable after 20 weeks. However, infection continued to occur in the release zone after all traces of the substrate had disappeared, and these infections could not always be traced to infected cadavers, so it appears that the spores could still be present and infective after the substrate had disintegrated. This indicates that infection may occur from few spores, which is supported by the demonstration that *Scolytus* adults can be infected by <100 *Metarhizium* spores (Doberski, 1981).

A previous study on *Scapanes* adults recorded 2% *Metarhizium* infection at a site 10 km from the release zone during the wet season (Bedford, 1974). This compares with 10–20% infection in *Oryctes* larvae in Samoa (Marschall, 1970) and 5–10% infection in *Oryctes* adults in India during wet months (Nirula, 1955). It appears that the level of infection in *Scapanes* adults is normally low, at least in palms; the fate of adults after they leave the palms is uncertain. No infections in *Rhynchophorus* have been recorded previously in the release area, although this pest has not been extensively studied.

In the release zone the proportion of

dead *Scapanes* and *Rhynchophorus* infected by *Metarhizium* rose substantially after release. Levels of infection in *Scapanes*, but not *Rhynchophorus*, also increased in the adjacent nonrelease zone. This zone was 50 m from the release zone and on the other side of a road. No increase in infection occurred in the more distant nonrelease zones or in the control zone 5 km distant. No firm explanation for the spread of the disease can be offered, though it may have been due to newly infected insects migrating from the release zone.

Bedford (1974) commented that even a single *Scapanes* attack could allow a lethal infestation of *Rhynchophorus* to develop. Thus, the apparently low number of insects recorded in the trial still represents a dangerously high level of infestation. The data in Table 3 indicate that, in the approximately 4-year period during which palms are susceptible to *Scapanes*, most palms at the release site would suffer multiple attacks. The level of damage at the release site was very severe and would have been unacceptable in a commercial planting.

The data presented here indicate that *Metarhizium* may cause considerable mortality to *Scapanes* when applied to the palm leaf axils as a mycoinsecticide. However, a commercially acceptable level of control was not achieved in this initial trial. Three possible ways of increasing its effectiveness are more frequent and larger applications of inoculum, and increasing the virulence of the fungus to the target insect by repeated inoculation and reisolation. The susceptibility of *Rhynchophorus* to the same inoculum, which was not envisaged when the trial was planned, also needs further investigation.

More work is needed to determine how many of the live insects recorded at each count contribute to the subsequent total of dead and infected insects and how many escape. It is not known precisely how long *Scapanes* adults spend in the crowns of coconut palms before emerging and flying away to breed, nor how rapidly infection develops in the field-infected insects nor whether they may emerge from the palms and fly to breeding sites before the infection becomes fatal. If this happened, infected adults might spread *Metarhizium* to breeding sites and contaminate them, which could make the fungus more effective in controlling the population.

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