SHORT COMMUNICATIONS

Persistence of *Metarhizium anisopliae*
(Deuteromycotina: Hyphomycetes)
as a Control Agent of *Anomala cuprea*
(Coleoptera: Scarabaeidae)
in a Forestry Nursery¹

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The cupreous chafer *Anomala cuprea* (Coleoptera: Scarabaeidae) is one of the major insect pests of forestry nurseries, feeding on roots of trees such as hinoki *Chamaecyparis obtusa*. It also injures the sweet potato and peanut in agricultural fields. Recently, it is also becoming an important golf green pest.

In the early 1950s in Japan, the Forest Agency utilized *Isaria kogane* (HASEGAWA and KOYAMA, 1941; *Beauveria brongniartii*) and *Oospora* sp. (HASEGAWA and KOYAMA, 1941; probably *Metarhizium anisopliae*) to control white grubs in nurseries. These fungi had been mass-produced on silkworm pupae, and hundreds of tons of the cultured materials were dried and provided to forestry nurseries throughout Japan. However, since efficacy and cost were inferior to chemical insecticides which became popular at that time, this process was abandoned (HAMA, 1959).

White grubs were adequately controlled with benzen-hexachloride until it was banned. There has been no effective insecticidal control since then.

It is desirable to refrain from use of chemicals considering effects on the environment, and utilization of insect pathogens is once again in demand.

KUSHIDA et al. (1981) clarified that rice grain is a promising alternative to silkworm pupae or compost that had been used in the previous experiments as a medium to propagate entomogenous fungi in the soil. A strong pathogenic fungus against *A. cuprea* larvae was selected (HASHIMOTO and SHIMAZU, 1988), and we conducted an application experiment of the fungus cultured on rice to evaluate effectiveness and problems of these materials in a hinoki nursery.

MATERIALS AND METHODS

The fungal strain employed in this study was *Metarhizium anisopliae* F-200. It had been isolated from an *A. cuprea* larva collected at Hamakita, Shizuoka Prefecture, Japan. This isolate was highly pathogenic to *A. cuprea* larvae in a screening experiment of pathogenic fungi. The fungus was cultured in Sabouraud's saccharose broth with 1% of yeast extract at 25°C for 5 d in a flask on a shaker. Two hundred ml of resulting material was transferred to 500 g of brown rice with 400 ml of water which had been previously autoclaved in a 3-l flask, and incubated at 25°C. About 5 days later, when the mycelia covered all grains, they were placed in styrofoam fish trays of approximately 54 cm × 33 cm, covered with wrap film, and kept about 14 d to form conidia. Approximately 2×10^9 conidia were produced on a gram of rice grains.

The experiments were conducted in a forestry nursery of hinoki, at the Sakashita Regional Forestry Office, located at Sakashita, Gifu Prefecture in central Japan. *A. cuprea* had frequently damaged hinoki seedlings in the last several years in this area. Each experimental plot was 100 m² of hinoki seedling nursery consisting of 10 of 10 m × 1 m ridges. The fungus was applied on July 17, 1989, by mixing the cultured material with nursery soil in a mixture of 240 g/m². The seedlings were temporarily dug up during the fungus application, and then planted again.

Effects of application were then investigated. On October 30, 1989 and October 31, 1990, 5

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Table 1. Larval density of *Anomala cuprea* and root injuries on *Chamaecyparis obtusa* seedlings

Year	Treatment	Area & No. plot	No. seedlings per m ²	% TIS ^a (mean±SE)	No. Larva/plot (mean±SE)
1989	<i>M. anisopliae</i>	1 m ² × 5	45.0	91.4± 6.3	8.2±3.4
	control	1 m ² × 5	44.6	79.0±13.2	6.2±2.6
1990	<i>M. anisopliae</i>	0.5 m ² × 10	22.2	100.0± 0.0	1.8±0.8
	control	0.5 m ² × 10	20.9	93.8± 4.2	1.6±0.8

^a Percentage of "tolerable injury seedlings" (TIS; see text).

Table 2. Mortality of *Anomala cuprea* larvae reared in the nursery soil samples

Year	Treatment	No. soil sample	Source of larvae reared	No. larva per sample	Percent mortality (mean±SE)	
					<i>M. anisopliae</i>	Totals
1989	<i>M. anisopliae</i>	5	field	7.2	87.5±4.6	95.0± 5.0
		5	laboratory	10	100.0±0.0	100.0± 0.0
	control	5	field	6.0	11.3±9.1	21.3±10.4
		5	laboratory	10	0.0±0.0	6.0± 4.0
1990	<i>M. anisopliae</i>	10	field	(8) ^a	(87.5) ^a	(100.0) ^a
		10	laboratory	10	84.0±7.8	88.0± 7.7
	control	10	field	(8) ^a	(0.0) ^a	(0.0) ^a
		10	laboratory	10	6.0±1.6	20.0± 4.9

^a Numbers of larvae collected in the field in 1990 were so small that they are indicated in the total.

quadrats of 1×1 m or 10 of 1×0.5 m were randomly selected in each plot to count root injury and larval density of *A. cuprea*. All living larvae of *A. cuprea* and soil samples in each quadrat were collected. The quadrats were investigated from the ground surface to a depth of about 40 cm. Each larva collected from the quadrats was placed in a plastic cup (250 ml) with 2 cm (thickness) of the soil sample, and reared at room temperature to estimate larval infection in the field. Ten laboratory-hatched 2nd instar larvae of *A. cuprea* for each soil sample were also reared in the same way to check the viability of the fungus.

RESULTS AND DISCUSSION

Larval density and seedling injury in the treatment and control plots are shown in Table 1. Injury levels on seedlings were divided in 5 grades; un-, slightly, moderately, and heavily injured, and dead. Among these grades, un- and slightly injured seedlings are thought to be usable, and the presence of these 2 grades is expressed as tolerable injury seedlings (TIS) in Table 1. There were no

significant differences in the larval density and percentage of TIS between the treatment and control plots in both years (WILCOXON's *U*-test).

Table 2 shows the percentage of infected larvae with *M. anisopliae* in bioassay for the soil samples. Both field collected larvae and laboratory-hatched larvae reared in the soil samples from the treatment plot suffered high mortality from *M. anisopliae*. There were also some larvae killed by *M. anisopliae* in the control plot. This fact indicates the natural existence of this species, however, mortalities in the treatment plot were significantly higher than those in the control plot ($p<0.01$, *t*-test). A high rate of infection in the treatment plot was also found in 1990 with a significant difference from the control plot, and indicated that the fungus was still active in 1990.

Some examples of settlement of entomopathogenic fungi which kept pathogenicity in soil after introduction as a control agent of white grubs have been known. SHIMAZU et al. (1988) reported that *Beauveria brongniartii*, which had been introduced to control *Anomala costata*, had kept the

Fig. 1. Accumulated mortality of *Anomala cuprea* infected with *Metarhizium anisopliae* reared in soil samples from experimental plots. "Lab.": laboratory-hatched larvae. "Field": field-collected larvae.

forest soil virulent to the larvae for more than 13 years. FERRON (1981) also mentioned the residual activity of *B. brongniartii* which successfully suppressed population of *Melolontha* years after the treatment. In the present study, we also confirmed that *M. anisopliae* introduced in soil had kept high pathogenicity against *A. cuprea* larvae in the year following application. MIKUNI et al. (1982) considered *M. anisopliae* to be a soilborne fungus with an underground reproductive capacity, based on their observation on its production of new conidia in soil. This and our results indicate that *M. anisopliae* is a suitable insect control agent in soil.

Accumulated mortality of *A. cuprea* infected with *M. anisopliae* reared in the soil samples is shown in Fig. 1. Daily mortalities are corrected for natural mortality with ABBOTT's formula (ABBOTT, 1925). The median lethal times for field-collected larvae were 33 to 35 d after the collection, whereas it took 50 to 60 d after start of rearing to kill 50% of the laboratory-hatched larvae. The average periods from the start of rearing to death were also different between the groups of larvae: those for field-collected larvae were 49 and 48 d in 1989 and 1990, respectively, and those for the laboratory-hatched ones were 86 and 58 d in 1989 and 1990, respectively. These differences in incubation periods may be due to the fact that the collected larvae had already been infected in the field.

Damage to hinoki seedlings was not reduced in this experiment. However, we clarified that the introduced fungus settled in the nursery, and that

the nursery soil maintained a strong insecticidal ability against the larvae. This should greatly reduce the number of emerging adults, and subsequent population sizes. This should be proved experimentally.

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