Microbial control of scarab beetle larvae by a formulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) in a sweet potato field

Tomoko Yokoyama,¹ Makoto Hasegawa, Azusa Fujiie, Masaaki Sawada² and Katsunori Noguchi³

Chiba Prefectural Agricultural Experiment Station, Chiba 266–0006, Japan

³Katakura Chikkarin Corporation, Tsukuba Research Institute, Tsuchiura, Ibaraki 300–0061, Japan

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Abstract

Control efficacy of *Metarhizium anisopliae* RNO31 formulation against larvae of the scarab beetle and a population of this fungus were investigated in a sweet potato field. The formulation was made by addition of vegetable oil to bran pellet cultures of the fungus, drying at room temperature to a moisture content of approximately 15% and granulation to about 2 mm in diameter. Insecticidal activity of the formulation was high against the 1st stadium larvae of *Anomala cuprea* at 10^5 and 10^6 colony forming units/g air dried soil. The formulation was applied in ridges or over the entire area of experimental plots at 100 g/m² prior to planting cuttings of sweet potato stems. The percentage of undamaged roots was significantly higher in the plots with application over the entire area than in the control plots on the day of harvest. The percentages of undamaged roots in the fungus application plots were the same as in the fenthion plots. Larvae of *Maladera japonica*, *Holotrichia parallela*, *Blitopertha orientalis*, *Anomala rufocuprea* and *A. cuprea* were found in the experimental field. Numbers were very low in the plots with application over the entire area. Populations of *M. anisopliae* in these plots and in the ridge application plots showed little change from the day of application to the day of harvest.

Key words: *Metarhizium anisopliae*, sweet potato, scarab beetle, microbial control, formulation

INTRODUCTION

Larvae of the scarab beetle, such as *Anomala cuprea*, feed on tuberous roots of sweet potato, *Ipomoea batatas* L., arresting the growth of plants and degrading the commercial value of the crop. It has been difficult to obtain sufficient control, although a large quantity of insecticides has been applied to sweet potato fields to control the beetles.

We have studied their control in sweet potato and peanut fields and in turf using the entomopathogenic fungus *Metarhizium anisopliae* RNO31 (Fujiie et al., 1993b, 1994). We developed a formulation technique to use this fungus as a control agent in fields (Fujiie et al., 1993a), and conducted control experiments with the formulation. In this paper, we describe the control efficacy of the *M. anisopliae* formulation and a population of this fungus in a sweet

potato field.

MATERIALS AND METHODS

The fungal strain, *M. anisopliae* RNO31, was used as the control agent in the experiment. This strain was derived from ultraviolet irradiated protoplasts and has higher insecticidal activity against larvae of *A. cuprea* and a wider insecticidal spectrum than the original strain (Yokoyama et al., 1993). For application in sweet potato fields, the fungus was formulated according to the method described by Fujiie et al. (1993a). It was cultured on bran pellets. The culture was added to vegetable oil (salad oilTM, Daiei Corporation, Kobe, Japan), a mixture of rape, soybean and corn oils, in a weight ratio of 2.5% and dried in a moisture removal machine for 2 days at room temperature to a moisture content of approximately 15%. The dried materials were then granulated to about 2 mm

¹Present address: Chiba Prefectural Industrial Research Institute, Chiba 264–0017, Japan

²Present address: Chiba Prefectural Horticultural Experiment Station, Tateyama, Chiba 294–0014, Japan

in diameter using a flour grinder.

To assess the insecticidal activity of the formulation and determine the mass of introduction in the field, larvae of *A. cuprea* were reared with the formulation in the laboratory. The fungal formulation was mixed with rearing soil consisting of a 1 : 1 mixture of sterilized soil and humus, and conidial concentrations were adjusted to 10^4 , 10^5 and 10^6 colony forming units (cfu)/g air dried soil. The mixture was placed in the wells of 6-well tissue culture plates. Five-day-old, first stadium *A. cuprea* larvae were individually released into the wells and reared at 25°C. Dead larvae were counted daily for 3 weeks after the treatment. Twenty larvae were used per treatment.

Control efficacy of the formulation was evaluated in the field. The experiment was conducted in a sweet potato field in Narita City, Chiba, Japan. On May 17, 1994, the formulation was applied to the soil surface in ridges or over the entire area of experimental plots at 100 g/m². Following application of the fungus, the soil of the experimental field was treated with fertilizer, plowed, ridged and mulched with two layers of white and black polyethylene film. Cuttings of sweet potato stems, cultivar Benia-ka, were planted on May 18, 1994. Fenthion was applied over the entire area of plots at 9 g/m² for comparison with the fungal formulation. Control plots received no treatment except fertilizer. Each plot consisted of 5 rows of 5 m × 0.9 m ridges, and three plots were used for each treatment.

On October 12, 1994, 15 sweet potato plants were taken from the center of each plot. Tuberous roots were divided into damaged and undamaged roots, and counted. The total

weight of tuberous roots yielded from each plot was recorded. After the roots had been removed from the ridges, scarab beetle larvae were removed from soil scooped from the center portion (3 m²) of each plot, counted and identified under a dissecting microscope.

On days prior to and after application, 44 days after application and the day of harvest, three soil samples were randomly collected from each plot from the soil surface to a depth of 15 cm using a soil sampler. Each soil sample was removed from the sampler, mixed and used to determine population of *M. anisopliae*. Cfus of *M. anisopliae* in each soil sample were determined by the dilution plate method using selective medium proposed by Yaginuma (1986). The cfu was converted into a number per 1 g of air dried soil.

RESULTS AND DISCUSSION

The oil formulation of *M. anisopliae* RNO31 was suitable for field applications, because it spreads easily and quickly as fertilizers or chemicals and can be applied using a fertilizer sprayer. Insecticidal activity of the formulation was high against the 1st stadium larvae of *A. cuprea* at 10^5 and 10^6 cfu/g air-dried soil (Table 1), therefore, the formulation could be applied to fields at this conidial concentration range. Vegetable oil was added to the bran pellet culture in our formulation (Fujiie et al., 1993a). Bateman et al. (1993) reported that vegetable oil enhanced the infectivity of *Metarhizium flavoviride* against desert locusts. We also discovered that the insecticidal activity of this oil formulation against 1st stadium larvae of *A. cuprea* was higher than that of the bran pellet culture at the same concentration

Table 1. Insecticidal activity of the formulation of *Metarhizium anisopliae* RNO31 against 1st stadium larvae of *Anomala cuprea*

Concentration (cfu/g air-dried soil) ^a	Mortality (%)	Mortality (%) by <i>M. anisopliae</i>
10^6	100	90
10^5	75	65
10^4	30	20
0 (Control)	5	5

^aThe formulation of *M. anisopliae* RNO31 was mixed with rearing soil at concentrations of 10^4 , 10^5 and 10^6 cfu/g air-dried soil.

Table 2. Percentage of undamaged tuberous roots and yield in the sweet potato field treated with the formulation of *Metarhizium anisopliae* RNO31

Treatment ^a	No. of examined roots	Undamaged roots (%) ^b (mean ± SD)	Yield/15 plants (kg) ^b (mean ± SD)
Entire area application	235	91.6 ± 4.6 b	18.3 ± 0.5 a
Ridge application	182	73.0 ± 16.0 ab	17.3 ± 1.6 a
Fenthion	282	70.0 ± 12.8 ab	18.5 ± 1.7 a
Control	239	45.7 ± 18.5 a	18.5 ± 2.1 a

^aThe formulation of *M. anisopliae* RNO31 was applied to the entire area and ridge of experimental plots.

^bMeans in each column followed by the same letters are not significantly different at the 5% level by Tukey's test.

(Fujiie et al., 1993a). Thus, the addition of vegetable oil to the culture appeared to result in both facilitation of handling and enhancement of insecticidal activity.

Table 2 shows the percentage of undamaged tuberous roots by each treatment in the sweet potato field. The percentage of undamaged roots was significantly higher in the plots with application over the entire area than in the control plots, although plots with only ridge application were not significantly different from the control plots. The percentages of undamaged roots in fungus application plots were the same as in the fenthion plots. Larvae killed by *M. anisopliae* were found in the fungus application plots or during the rearing of larvae discovered in the field. The yield of tuberous roots from the 15 plants did not differ significantly among the various treatment plots.

On the day of harvest, larvae of *Maladera japonica*, *Holotrichia parallela*, *Blitopertha orientalis*, *Anomala rufocuprea* and *A. cuprea* were found in the experimental field (Table 3). Their numbers were very low in the plots with application over the entire area, however, this

was not clearly related to the percentage of undamaged tuberous roots. The insecticidal activity of the fungus differs among the species of scarab larvae, i.e.: it has high insecticidal activity against *A. cuprea* and *Popillia japonica*, medium activity against *Anomala daimiana* and *B. orientalis* and low activity against *H. parallela* and *Adoretus tenuimaculatus* (Yokoyama et al., 1993). In the ridge application plots, there were large numbers of *H. parallela* and *M. japonica* larvae than in control plots. This may due to a low activity against those species.

Populations of *M. anisopliae* in plots treated over the entire area, ridge application plots and control plots on the day of application were 1.4×10^5 , 1.3×10^6 and 1.9×10^4 cfu/g air-dried soil, respectively. On the day of harvest the corresponding numbers were 2.8×10^5 , 5.8×10^5 and 9.8×10^3 cfu/g air-dried soil, respectively (Fig. 1). They changed little from the day of application to the day of harvest in both the entire area application and the ridge application plots (Fig. 1). Mikuni et al. (1982) also reported that *M. anisopliae* was detected at a density of

Table 3. Number of scarab larvae in the sweet potato field treated with the formulation of *Metarhizium anisopliae* RNO31

Treatment ^a	Number of larvae per 9 m ²					Total
	<i>Maladera japonica</i>	<i>Holotrichia parallela</i>	<i>Blitopertha orientalis</i>	<i>Anomala rufocuprea</i>	<i>Anomala cuprea</i>	
Entire area	4	0	0	1	0	5
Ridge	13	3	0	0	0	16
Fenthion	8	0	4	0	2	14
Control	4	2	0	5	0	11

^aThe formulation of *M. anisopliae* RNO31 was applied to the entire area and ridge of experimental plots.

Fig. 1. Population of *Metarhizium anisopliae* in a sweet potato field from the day of application to the day of harvest. Vertical lines indicate standard deviations. O: entire area application, ■: ridge application, ▲: control.

10^2 – 10^4 cfu/g air-dried soil for 25 months without seasonal fluctuation of the population size in infested soil of a mulberry plantation. Shimazu et al. (1993) reported that *M. anisopliae* introduced in a forest nursery of hinoki, *Chamaecyparis obtusa*, retained a high pathogenicity against *A. cuprea* for two years. There were also reports that other entomopathogenic fungi retained pathogenicity for several years after introduction into soil (Ferron, 1981; Shimazu et al., 1988). Apparently the pathogenicity of the fungus was retained from the day of application to the day of harvest in the sweet potato field, because of the resulting high control efficacy and high population recovery.

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