SHORT COMMUNICATIONS

Insecticidal Activity of an Entomopathogenic Nematode, *Steinernema kushidai* (Nematoda: Steinernematidae) against *Anomala cuprea* (Coleoptera: Scarabaeidae) Larvae under Different Soil Moisture Conditions¹

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An entomopathogenic nematode, *Steinernema kushidai* was isolated from a soil sample collected in Shizuoka Prefecture, Japan, in 1984 (KUSHIDA et al., 1987) and described as a new species by MAMIYA (1988). The insecticidal activity of this nematode is high enough to kill larvae of scarabaeid beetles like the cuprea chafer, *Anomala cuprea*, which are difficult to control with chemical pesticides (KOIZUMI et al., 1988; MAMIYA, 1989; FUJIE et al., 1993). Thus the nematode has been receiving attention as a biological control agent of scarabaeid beetles.

For the successful application of *S. kushidai* as an effective biological pesticide, the environmental factors related to use of this nematode must be evaluated. KAYA (1990) described how soil moisture had a great impact on host finding ability and infective activity of the entomopathogenic nematode, as did temperature and soil texture. As for *S. kushidai*, the effects of soil moisture on the nematode have not been determined thus far. In the present study, we evaluated the insecticidal activity of *S. kushidai* against

A. cuprea larvae under different soil moisture conditions.

MATERIALS AND METHODS

Nematode and insect. *S. kushidai* was cultured on a modified dog food medium (OGURA and MAMIYA, 1989). Infective juveniles (J_{III}) recovered from the medium by the BAERMANN funnel method were washed three times with deionized water and stored at 15°C in shallow water until use.

The rearing method of *A. cuprea* was described in detail in our previous report (FUJIE et al., 1995). Briefly, the insect was reared individually in plastic cups (6 cm dia. and 3 cm depth) containing 30 ml of andosol (volcanic ash soil, loam), the moisture of which was maintained at 30–35% by weight. About 0.5 g of dried powdered leaves of *Quercus acutissima* were placed on the soil surface as an insect diet. The above and the following experiments were all conducted at 25°C, and the soil used throughout the experiments was andosol.

Insecticidal activity of nematodes under different soil moisture conditions. The soil was passed through a 5 mm-mesh sieve, oven-dried at 120°C for two days, and 30 ml of the soil thus obtained was introduced into each of several plastic cups. Deionized water was then added with or without 1,000 J_{III} and the soil moisture, i.e. water content, in the cups was adjusted to 0.5, 10, 20, 30 or 40%. Immediately after the moisture adjustment, 0.5 g of dried *Q. acutissima* leaves were introduced into the plastic cups, thoroughly mixed with the soil, and one third instar *A. cuprea* was released into each cup. The number of dead larvae was counted daily for ten days. All dead insects were dissected under a binocular microscope to confirm nematode infection. Four replications were made using ten larvae for each of the five soil moisture levels.

Nematode recovery from moisture-adjusted soil. Thirty milliliters of oven-dried soil was adjusted to 20, 30 or 40% moisture with deionized water containing 1,000 J_{III}. These soils were immediately placed in conical beakers (100 ml in capacity) with aluminum foil caps. On the seventh and tenth day after nematode inoculation, surviving J_{III} in the soil were recovered by the BAERMANN funnel method. Two replications were made using four beakers for each of the three soil moisture levels.

Insect feeding activity in moisture-adjusted soil. Pre-weighed third instars were individually released into

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Table 1. Insecticidal activity of *Steinernema kushidai* against third instar larvae of *Anomala cuprea* under different soil moisture conditions

Soil moisture (%)	Mortality of insects in soil ^a (% mean $\pm$ SD)	
	With nematode	Without nematode
0.5	100 $\pm$ 0 c	100 $\pm$ 0 b
10	100 $\pm$ 0 c	100 $\pm$ 0 b
20	47 $\pm$ 13 a	0 $\pm$ 0 a
30	85 $\pm$ 6 b	0 $\pm$ 0 a
40	93 $\pm$ 10 bc	0 $\pm$ 0 a

^a Mortality was examined on the tenth day after introducing insect larvae individually into cups containing 30 ml of moisture-adjusted soil with and without 1,000 infective juveniles of *S. kushidai*. Means in each column followed by the same letter are not significantly different at the 5% level by TUKEY's test.

plastic cups containing 30 ml of soil, the moisture of which was adjusted to 20, 30 or 40%. Then, a piece of dried *Q. acutissima* leaf (about 150 mg) was placed directly on the soil surface and the insects were allowed to feed on the leaf. Two days later, the leaf was removed, oven-dried at 70°C for two days, and then weighed. Weight loss of the leaf over the two days was regarded as being due to consumption by the insect. Two replications were made using five larvae for each of the three soil moisture levels.

RESULTS AND DISCUSSION

Insecticidal activity of nematodes under different soil moisture conditions

All the third instar larvae of *A. cuprea* died in soil with a water content of 0.5 or 10% (Table 1), irrespective of the presence or absence of *S. kushidai*. These results indicate that the nematode insecticidal activity cannot be evaluated under extreme soil moisture conditions. Within the soil moisture range from 20 to 40%, the nematode insecticidal activity increased significantly with increases in soil moisture (Table 1).

Insect mortality was higher at 30 and 40% than at 20%, being 85, 93 and 47%, respectively. Thus, moisture levels of 30 and 40% are considered to be suitable for nematode activity as well as for plant growth in fields.

KONDO and ISHIBASHI (1985) reported that the insecticidal activity of *S. feltiae* (= *S. carpocapsae*) against

Table 2. Recovery of *Steinernema kushidai* infective juveniles from moisture-adjusted soil

Soil moisture (%)	Recovery rate ^a (% mean $\pm$ SD)	
	Seventh day	Tenth day
20	50.6 $\pm$ 23.0	32.0 $\pm$ 7.2
30	65.8 $\pm$ 11.3	22.6 $\pm$ 16.9
40	43.5 $\pm$ 33.8	15.9 $\pm$ 3.4

^a Infective juveniles were recovered by the BAERMANN funnel method on the seventh and tenth day after inoculation of 1,000 infective juveniles into the soil. Means are not significantly different at the 5% level by TUKEY's test.

Table 3. Feeding activity of third instar larvae of *Anomala cuprea* on a dried *Quercus acutissima* leaf placed on the surface of moisture-adjusted soil

Soil moisture (%)	Dry weight of leaf consumed ^a (mg/g of larva/day, mean $\pm$ SD)
20	20.52 $\pm$ 12.74 a
30	51.57 $\pm$ 12.10 b
40	64.06 $\pm$ 10.87 c

^a Means in each column followed by the same letter are not significantly different at the 5% level by TUKEY's test.

Spodoptera litura larvae was high at the soil moisture range of 20–40%, but very low at 10–15% and 50% levels. Infectivities of *Heterorhabditis* sp. and *S. glaseri* (MOLYNEUX and BEDDING, 1984) and of *Neoaplectana carpocapsae* (= *S. carpocapsae*; SILVERMAN et al., 1982) are also known to be affected by soil moisture and by soil texture.

Nematode recovery from moisture-adjusted soil

Table 2 shows that there was no significant difference among the recovery rates of *S. kushidai* J_{III} from the soil with three different moisture levels. This result may indicate that the survival rate of the nematode is only minimally affected by the soil moisture examined. KONDO and ISHIBASHI (1985) reported that moisture conditions had little effect on nematode survival in soil, though it had a severe effect on survival in the air, as described by KAMIONEK et al. (1974).

Insect feeding activity in moisture-adjusted soil

The feeding activity of third instar *A. cuprea* on dried *Q. acutissima* leaves increased significantly with

an increase of soil moisture from 20 to 40% (Table 3). The leaves were promptly consumed by the insects at 30 and 40%. At 20% the larvae assumed a coiled posture and moved around on the soil surface only slightly. These results may explain the high mortality of the insects at 30 and 40% through an enhanced oral infection of *S. kushidai* to *A. cuprea* larvae.

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Age-Related Sexual Receptivity Change in Virgin Females of a Butterfly, *Eurema hecabe*¹

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In most butterfly species, newly eclosed females are found by searching males and mate (SCOTT, 1973; RUTOWSKI, 1984). Once mated, females become sexually unreceptive and reject courting males (e.g.,

OBARA, 1964; SUGAWARA, 1979; WICKMAN, 1985). If the females remain virgin, they actively search and solicit males (SCOTT, 1973). However, mate rejection by virgin females has been reported in several butterfly species (SUZUKI et al., 1977; OBARA, 1987). If mate rejection by virgin females is prolonged, their preoviposition period is extended and their reproductive success is reduced. Investigations into the mechanisms and significance of mate rejection by virgin females could aid in understanding their behavior.

In a period butterfly, *Eurema hecabe* (LINNAEUS), the males actively search for mates (KATO, 1986, 1989). KATO (1989) reported that adult females of *E. hecabe* are sexually active just after eclosion, but their receptivity soon drops without the occurrence of mating. In this study, we investigated age-dependent sexual receptivity in the virgin females of this species, together with ovarian maturation after adult eclosion.

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