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*UPTAKE OF BENOMYL BY THE TEA PLANT AND ITS EFFECTIVENESS AGAINST STEM CANKER (*PHOMOPSIS THEAE*)

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In *in vitro* tests, benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) was highly toxic to *Phomopsis theae*, the fungus responsible for stem canker in young tea (*Thea sinensis*). Bioassays revealed that benomyl was readily taken up by excised tea shoots and rooted cuttings in the laboratory and by potted plants in the greenhouse. The benomyl fungitoxicant was detectable in the stem, leaf and sap of treated plants and its persistence within the plant appeared to be long-lasting. When tea plants grown in soil amended with benomyl were inoculated with *P. theae*, the size of the resulting lesions was significantly reduced, as compared with the control, which indicates systemic fungitoxicity. In greenhouse tests, benomyl also gave excellent control of stem canker when sprayed on the stem at 12 oz in 100 gallons of water. Benomyl thus shows good promise as a fungicide for the control of tea stem canker.

INTRODUCTION

Stem canker, caused by the fungus *Phomopsis theae* Petch, is a serious disease in young tea plantations at high elevations in Ceylon (3) and South India (7). In Ceylon, where detailed studies on the disease were carried out recently, we found that one of the major predisposing factors for disease outbreaks is low soil moisture (6). Site factors, host resistance and some cultural treatments are also important in determining the severity of outbreaks (3, 4, 5). The disease is now controlled by the planting of resistant clones and by modification of certain cultural treatments, because adequate disease protection measures have not been found. Although a number of fungicides have been tested, none has given consistently good control. Because only a few resistant clones are available and some very desirable clones are highly susceptible to the disease, the development of a satisfactory method of chemical control is very important to the industry in Ceylon. Recently, the fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) has shown high *in vitro* and *in vivo* toxicity against *P. theae* and has also given excellent control of the disease in greenhouse tests. Benomyl is readily taken up by the tea plant from the soil and its activity within the plant is long lasting. The data relating to these observations are presented in this paper.

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METHODS AND RESULTS

In vitro toxicity of benomyl to Phomopsis theae : To determine the *in vitro* toxicity of benomyl to *P. theae,* benomyl (50% WP) was incorporated at several concentrations into Difco potato-dextrose agar (PDA) cooled to about 50° C. Tests showed that germination of conidia of *P. theae* on this medium was completely inhibited at 20 ppm, after incubation at 25° C for 24 hours. Complete inhibition of mycelial growth from agar disk inocula occurred at 0.5 ppm after incubation at 25° C for 7 days. From these tests we concluded that the minimum inhibitory concentration of benomyl for spore germination of *P. theae* is 20 ppm, and for mycelial growth 0.5 ppm. These observations indicated that benomyl is highly fungitoxic to *P. theae,* as compared with a number of other compounds tested similarly.

Uptake of benomyl by excised tea shoots : Four vigorously growing leader shoots taken from 2-year-old plants were brought to the laboratory and allowed to stand in a suspension of the fungicide (0.25%). Four similar shoots were placed in distilled water and served as checks. The uptake of benomyl by the shoots was followed by a bioassay technique using *P. theae* as the test organism. PDA plates were seeded with conidia of *P. theae* (7.0×10^6 spores/ml) and disks of stem (5 mm long) and leaf (13 mm diameter) taken from selected points of both treated and control shoots were placed on the surface of the seeded agar. The plates were incubated for 72 hours at 25° C. Clear zones of inhibition appeared around stem or leaf disks taken from only treated plants.

Stem sections and leaf disks were examined for fungitoxicity 48, 72 and 96 hours after the start of the experiment and the results are shown in Table 1.

TABLE 1 — *Uptake of benomyl by excised tea shoots*

Time of sampling (hours)	Position of sampling-distance from cut-end (cm)	Diameter of inhibition zone (mm)
48	15	55.5
	25	50.0
72	15	54.5
	25	17.0
96	15	45.0
	25	2.0

No activity was detected in the leaves of both treated and control shoots and there was no evidence of phytotoxicity.

Uptake of benomyl by young tea plants : When roots of 1-year-old plants were immersed in a suspension of benomyl (0.025%), there was positive evidence of the uptake of benomyl by the plants. The results of a typical experiment are shown in Table 2.

TABLE 2 — *Uptake of benomyl by young tea plants in the laboratory*

Time of sampling (days)	Position of sampling distance from base of stem (cm)	Diameter of inhibition zone (mm)
2	0	35.0
	10	0.0
	20	0.0
4	0	44.0
	10	25.0
	20	0.0
6	0	56.0
	10	36.5
	20	30.0
	35 (apex)	0.0
8	0	55.5
	10	36.5
	20	17.0
	35 (apex)	0.0

No activity was detected in the leaves except on the last day of sampling when one of the lower leaves exhibited slight fungitoxicity. Again, there was no evidence of phytotoxicity.

Uptake of benomyl by potted plants in the greenhouse : The uptake of benomyl by the tea plant was also studied in the greenhouse with 18-month-old potted plants. The pots were 9 inches in diameter and each contained approximately 6000 g of tea soil. The fungicide was applied to the pots as a 0.05% solution at the rate of 250 ml per pot. Eight applications were made between August 13 and September 22, which was equivalent to the addition of 1.0 g fungicide per pot. There were six treated plants and six checks in the experiment.

Uptake of benomyl was followed, as described earlier, by taking stem sections and bark and leaf disks from selected regions of the shoots at frequent intervals, beginning a day after the last application of fungicide. The results of the last sampling done 35 days after the final application of benomyl are shown in Table 3.

TABLE 3 — *Uptake of benomyl by potted tea plants in the greenhouse. Bioassay done 35 days after final application of benomyl*

	Position of sampling-distance from base of plant (cm)	Diameter of inhibition zone (mm)
A. Stem	0	43.5
	15	32.5
	30	34.5
	45	37.5
B. Leaf	0	25.0
	15	25.0
	45	40.0
C. Bark	30	34.5
	45	28.0

No inhibition zones were observed around stem and leaf disks taken from untreated plants. The results clearly indicate that benomyl is readily taken up by the roots of the tea plant and translocated to the stem, leaf and bark. Several other experiments confirmed this.

Persistence of fungitoxicant within plant : The persistence of the fungitoxicant within the plant was investigated in an experiment in which 32 potted plants (2-year-old) were treated with benomyl in the greenhouse. One g of fungicide was applied to each pot and mixed with the soil and the plants were watered in the usual manner. The presence of the fungitoxicant within the plants was determined as before by frequent sampling over the next 3 to 4 months.

The first sampling carried out 9 days after treatment revealed the presence of the fungitoxicant in the stem, but activity was confined only to the base. Twenty-two days after treatment, however, it was detected throughout the stem of the plant. Activity was first detected in the leaf 35 days after initial application of benomyl and thereafter until the end of sampling. Sampling was concluded 106 days after treatment when detectable amounts of the fungitoxicant were still present throughout the plant. The results thus showed that the fungitoxicant has long-lasting activity within the plant. There was no evidence that it was accumulating in the leaves of treated plants.

The persistence of the fungitoxicant within the plant was also investigated in another experiment in which the treated plants were transferred to fresh soil 45 days after treatment.

One gram of benomyl was applied to each of 32, two-year-old plants growing in pots in the greenhouse. A bioassay carried out 44 days after treatment revealed the presence of the fungitoxicant throughout the stem of the plants, the following day all treated plants were uprooted, roots rinsed thoroughly, and then transplanted into fresh pots. Assay for the fungitoxicant was thereafter carried out at approximately monthly intervals, two plants being sampled on each occasion by taking stem sections at 0, 10, 20 and 40 cm from the base of the plant.

The fungitoxicant was detectable in stem sections for over 6 months after benomyl-treated plants were transferred to fresh soil (Table 4). This and other similar experiments confirmed the long persistence of the fungitoxicant within the tea plant.

TABLE 4 — *Fungitoxicity of tea stem sections with time after initial application of benomyl; 1 g benomyl was applied per plant on September* 21, 1970 *and treated plants were transferred to fresh soil on November* 4, 1970.

Position of stem disk	Date of sampling						
	Nov. 17	Dec. 10	Jan. 16	Feb. 25	Apr. 3	May 29	July 5
0[a]	32.5[b]	29.5	32.5	28.0	32.0	3.5	0.0
10	36.0	26.5	21.0	23.5	14.5	2.0	0.0
20	27.7	26.5	14.5	13.0	23.0	6.0	0.0
40	20.3	11.0	4.5	9.0	7.0	0.0	0.0

[a]Position of stem disk from the base of plant (cm).
[b]Diameter of inhibition zone (mm).

TABLE 5 — *Effect of benomyl on tea stem canker.*

Treatments[a]	Mean canker length (cm) Experiment I	Experiment II
6 applications of benomyl before inoculation	0.68	2.62
6 applications of benomyl before and 6 after inoculation	0.61	0.59
6 applications of benomyl after inoculation	2.70	4.93
Control	3.89	8.97
LSD .001	1.68	6.16
.01	1.28	4.68
.05	0.95	3.49

[a]At each application 250 ml of a 0.05% solution of benomyl was given per plant.

The fungitoxicant has also been detected in the sap of treated plants. Sap was collected by cutting the stem of the plant about 6 inches above ground level and applying suction to the cut end. The sap thus collected was concentrated and applied to sterile filter paper disks placed on seeded agar, where clear zones of inhibition developed around the paper disks. No activity was seen in the sap of untreated plants.

In vivo toxicity of benomyl to P. theae : To determine the *in vivo* toxicity of benomyl to *P. theae,* two experiments were carried out in the greenhouse. In one, 1-year-old plants of clone TRI 2024, growing in pots, were given the following treatment : 1) Six applications of fungicide prior to inoculation : 2) Six applications of the fungicide before inoculation and six after inoculation : 3) Six applications of the fungicide after inoculation : 4) Control—untreated.

Benomyl was applied to the soil as 0.05% solution at the rate of 250 ml per plant per application. The interval between applications varied between 2 and 7 days. There were 10 plants per treatment and all plants were inoculated once on the collar by using a culture of *P. theae,* after the preinoculation applications of the fungicide were completed in treatments 2 and 3. After inoculation, plants were watered lightly for about a month to induce the formation of large cankers (6). The length of the resulting cankers was measured about 3 months after inoculation.

In the second experiment, the highly susceptible clone KEN 16/3 was used instead of TRI 2024, and this resulted in larger cankers. The treatments were the same as in the previous experiment.

The results showed that all three fungicidal treatments caused significant reductions in the size of the cankers produced (Table 5). In this regard, treatments 1 and 2, which received six applications of benomyl before inoculation, were superior to treatment 3, which received all six applications after inoculation. Further, when treatments 1 and 3 are compared, it is evident that applying benomyl before inoculation is more effective than applying it after. This may be because the pathogen had commenced activity before toxic levels of the fungitoxicant had built up within the plant. In the second experiment, treatment 2 was highly effective in suppressing canker development because only one of the 10 inoculations produced a measurable canker, and the remaining nine inoculations had healed over (Fig. 1).

FIG. 1—*Effect of Benomyl on tea stem canker*
Right—Plant treated with Benomyl before and after inoculation
Left—Untreated control.

Control of stem canker by benomyl sprays : A preliminary experiment on control, in which benomyl was applied as a spray on the stem, was conducted in the greenhouse. Treatments consisted of wounding 2-year-old plants (clone KEN 16/3) by removing disks of bark, 7 mm in diameter, and spraying these wounds to run-off with the fungicide or water (control). Four hours later, disks of agar supporting fungal growth were placed on the wounds and bound with polythene tape. There were 10 plants per treatment and each plant was inoculated twice on the stem at 5 and 30 cm from the soil. Inoculations were made on April 8 and the resulting cankers were measured and recorded on June 26. The treatments and the results are shown in Table 6.

TABLE 6 — *Control of tea stem canker by benomyl sprays.*

Treatment	% Successful inoculations	Mean length of cankers (cm)
benomyl at 4 oz in 100 gal.[a]	25.0	2.35
benomyl at 8 oz in 100 gal.	10.0	0.42
benomyl at 12 oz in 100 gal.	0.0	0.00
Control	90.0	6.71

[a]Imperial gallons.

The results show clearly the effectiveness of benomyl against stem canker especially at the highest concentration tested.

DISCUSSION

In vitro tests indicated that benomyl is highly active against the mycelium of *P. theae* and this probably accounts for its effectiveness against stem canker at relatively low concentrations in the greenhouse tests. Although complete germination of conidia was inhibited at 20 ppm, growth of the germ tube was restricted to approximately one spore length or less at 1 ppm.

The experiments on the uptake of benomyl provide ample evidence that benomyl is readily taken up by the tea plant, when applied to the soil, and translocated to the stem and leaf. Bioassays also indicated that the fungitoxicant was present in the sap of treated plants, but there was no evidence that it was accumulating in the leaf tips or margins as reported by Biehn and Dimond (1) and Peterson and Edgington (2). Furthermore, no phytotoxic symptoms were seen on any of the treated plants in this study.

No attempt was made to study the nature of the fungitoxicant, but its long persistence within the plant suggests it is highly stable. It thus appears that one application of benomyl to the soil could provide treated plants with systemic fungitoxicity lasting several months.

The greenhouse investigations on control show that benomyl is highly effective against stem canker as both a systemic and a protectant fungicide. Its effectiveness at low concentrations, its systemic activity, and its apparent lack of phytotoxicity indicate great possibilities for benomyl as a routine fungicide for field control of stem canker in young tea plantations.

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