FACTORS LIMITING THE CONTROL OF VERTICILLIUM WILT IN TOBACCO OBTAINED WITH BENZIMIDAZOLE FUNGICIDES

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Summary

Applications of benomyl or thiophanate-methyl to the soil, at transplanting time, reduced the loss of yield caused by verticillium wilt in tobacco plants, but would not have been economic except in limited circumstances. The efficacy of treatments was markedly influenced by soil type and by varietal resistance to the disease. Treatments using wettable powder preparations were generally more effective than those using granules. Plants raised in soil treated with benomyl sometimes had a distorted growth habit.

INTRODUCTION

Benomyl has been used to control verticillium infections in a number of plant species (Biehn, 1970; Booth *et al* 1971; Busch and Hall, 1971; Ebben and Last, 1969; Lockhart *et al* 1969). Several workers have obtained good control with benomyl in greenhouse tests but have had difficulty in obtaining the same level of control in the field (Erwin and Buchenauer, 1971). Good control of verticillium wilt in tobacco was obtained in our initial trials (Hartill, 1971) but in a trial designed to evaluate practical methods of fungicide application poor disease control was obtained. This later trial was conducted on a heavier soil type than those used previously and we decided to investigate how soil type and other factors might affect disease control. Thiophanate-methyl was included in the last of these trials as we had found previously that it gave a similar level of disease control to that obtained with benomyl (unpublished).

EXPERIMENTAL METHODS

Pot trials

Fire-clay pots, 20 cm diameter, were filled with a loam/peat potting mixture and drenched with 250 ml of the appropriate fungicide suspension. After the drench had drained, the soil in the pot was mixed with a large spatula to improve fungicide incorporation. The amount of fungicide applied was calculated on the basis of uniform incorporation into the soil.

Seedlings were raised in boxes. When the seedlings were 10 cm tall they were pulled roughly, so as to break off the smaller roots, and inoculated by standing in bright sunlight for 1 hour with the roots immersed in a 10^6/ml suspension of *Verticillium dahliae* Kleb. conidia. They were then planted into the treated soil.

Field trials

Field trials were conducted at sites known to be heavily infected with *V. dahliae*. Trials were designed to compare methods of applying fungicides to the soil; to measure the response to identical fungicide treatments at sites differing in soil type; and to compare varietal response to fungicide treatments.

(i) *Application methods*

The application methods used were chosen so that they could be mechanised by making simple modifications to existing equipment. Four methods were compared.

1 'Planting water' treatments were made by pouring 200 ml of the appropriate fungicide suspension into each planting hole at the time of transplanting; in all other treatments a similar amount of plain water was used to set the transplants, as is recommended in commercial practice.

2 Fungicide suspensions were incorporated in the soil by spraying them between the ridging discs at the time of ridging.

3 Benomyl granules (5% a.i.) were applied before planting by pouring them evenly in a line along the ground and then forming a ridge over them. Subsequent observations showed that the granules were dispersed unevenly within the ridge.

4 The roots of the plants were dipped in a 100 ppm suspension of benomyl thickened with 0.5% agar before transplanting. When these plants received their final mechanical cultivation, 4 to 5 weeks after transplanting benomyl granules were injected into the ridge at a point where assimulating roots were likely to be present.

Previous greenhouse trials (unpublished) had shown that the addition of 1% v/v Tween 20 (polyoxyethlene sorbitan monolaurate) enhanced the activity of benomyl in clay soils. This ajuvant was further tested here using application methods 1 and 2.

(ii) *Soil types*

The sites chosen for the trials could be divided into two soil classes; one a brown granular loamy clay soil, the other an alluvial sand/silt soil.

(iii) *Varieties*

Six tobacco varieties were used. They were:—

Hicks	very susceptible, yielding less than 20% of potential at infected sites.
Yellow Gold) Virginia Gold)	susceptible, yielding less than 30% of potential at infected sites.
NC 75	intermediate, yielding less than 50% of potential at infected sites.
Waimea	moderately resistant, yielding approximately 60% of potential at infected sites.
Kuaka 21	resistant, yielding approximately 90% of potential at infected sites.
McNairs 121	resistant, not field tested but approximately equivalent to Kuaka 21.

(iv) *Assessments*

Before reaping the approximate total number of leaves per plot was determined. At each reaping leaves with no obvious external symptoms (hereafter termed symptomless leaves) were counted and weighed.

Symptomless leaves were typical of those taken from infected crops in commercial practice but occasionally there had been some invasion of the vascular tissue.

The progress of the disease within the crop was followed by estimating the proportion of unharvested leaves affected by the disease at fortnightly intervals.

(v) *Other*

In all trials normal cultural practices were followed as far as possible; flowering heads were removed before the corollas open and leaves were reaped at intervals as they matured.

RESULTS

(i) *Application techniques and soil type*

Identical field trials were carried out at two sites, one with a loamy clay soil, the other with an alluvial sand/silt soil. Plants in untreated plots had similar infection levels, and gave similar yields of symptomless leaves, at both sites. Some decrease in the incidence of the disease was obtained by applying benomyl in the planting water or as a spray incorporated in the ridge but differences were only significant in the sand/silt soil trial ($p < 0.05$) (Table 1). The fungicide was not effective when applied as granules either before planting or at the last cultivation, five weeks later. Adding Tween 20 to the fungicide suspensions did not improve disease control. Most benomyl treatments gave better disease control and greater increases in yield with Waimea plants than with Yellow Gold plants, although in untreated plots, plants of the Waimea variety had less verticillium infection than Yellow Gold plants. There was an indication that the treatments followed the same order of efficacy in the loamy clay soil trial but differences were smaller and non-significant.

TABLE 1: COMPARISON OF METHODS OF APPLYING BENOMYL TO CONTROL VERTICILLIUM WILT OF TOBACCO ON A SAND/SILT SOIL

benomyl kg/ha	*Method of application*	*Symptomless leaf/plant (% of total)*		*Weight of symptomless green leaf/plant (g)*	
		Yellow Gold	*Waimea*	*Yellow Gold*	*Waimea*
	S.E.	(± 7.2)		(± 60.4)	
nil	(untreated control)	15 b	54 d	149 c	526 c
2.5	in the planting water	28 ab	82 ab	314 ab	802 a
2.5	in the planting water + 1% v/v Tween 20	29 a	82 ab	360 ab	794 a
5.0	sprayed on to soil as ridge was formed	38 a	76 bc	386 a	698 ab
5.0	sprayed on to soil as ridge was formed + 1% v/v Tween 20	24 ab	78 bc	264 bc	812 a
2.5	granules applied before the ridge was formed	20 ab	70 bc	186 c	731 a
	100 ppm root dip at transplanting + 2.5 kg/ha as granules placed in the ridge at final cultivation	20 ab	63 cd	204 c	632 bc

In untreated plots symptoms appeared at the same time in both varieties in both field trials (Figs. 1A and 1B). The subsequent rate of increase in disease development was lower in Waimea plants than in Yellow Gold plants. In plots treated with benomyl, either in the planting water or as a spray incorporated into the soil, there was a delay of approximately one week in the appearance of symptoms in Waimea plants in both trials. In treated plots of Yellow Gold plants there was a similar delay in the onset of symptoms in the sand/silt soil trial but not for the trial on the loamy clay soil. Once symptoms had appeared in Yellow Gold plants disease development was not further affected by treatment but in Waimea plants fungicide treatments did depress the subsequent rate of symptom development.

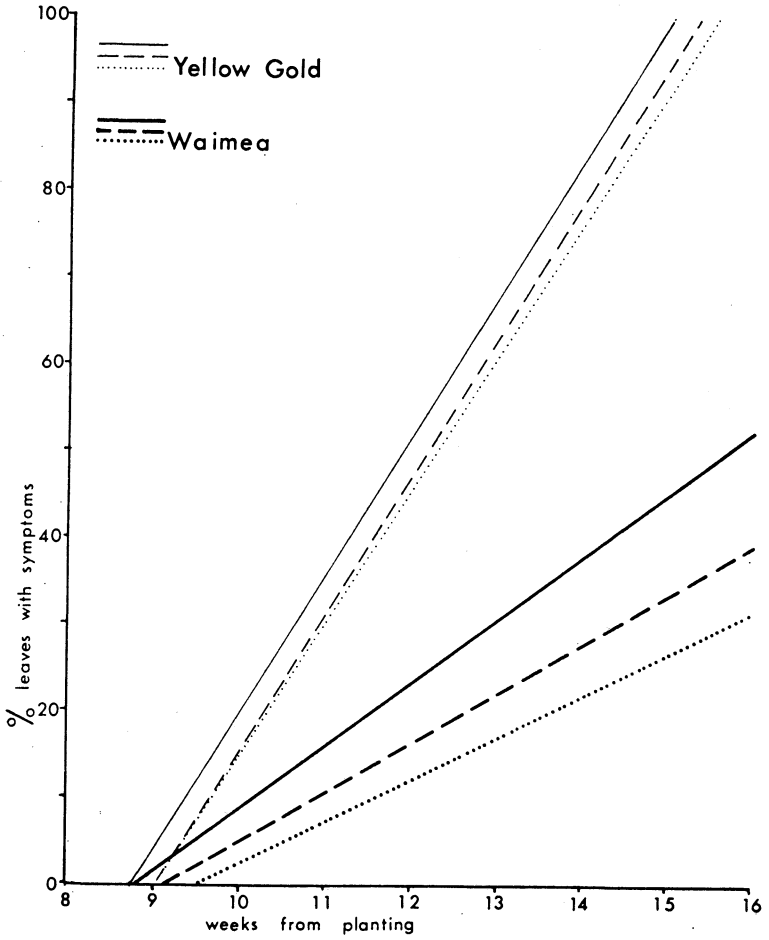

Fig. 1A Effect of fungicide treatments on the rate of symptom expression, loamy clay soil trial. The lines are the regressions of leaves with symptoms against time. Solid lines indicate untreated plant, broken lines indicate plants treated with benomyl in the planting water and dotted lines indicate plants treated by incorporating a benomyl spray into the ridge.

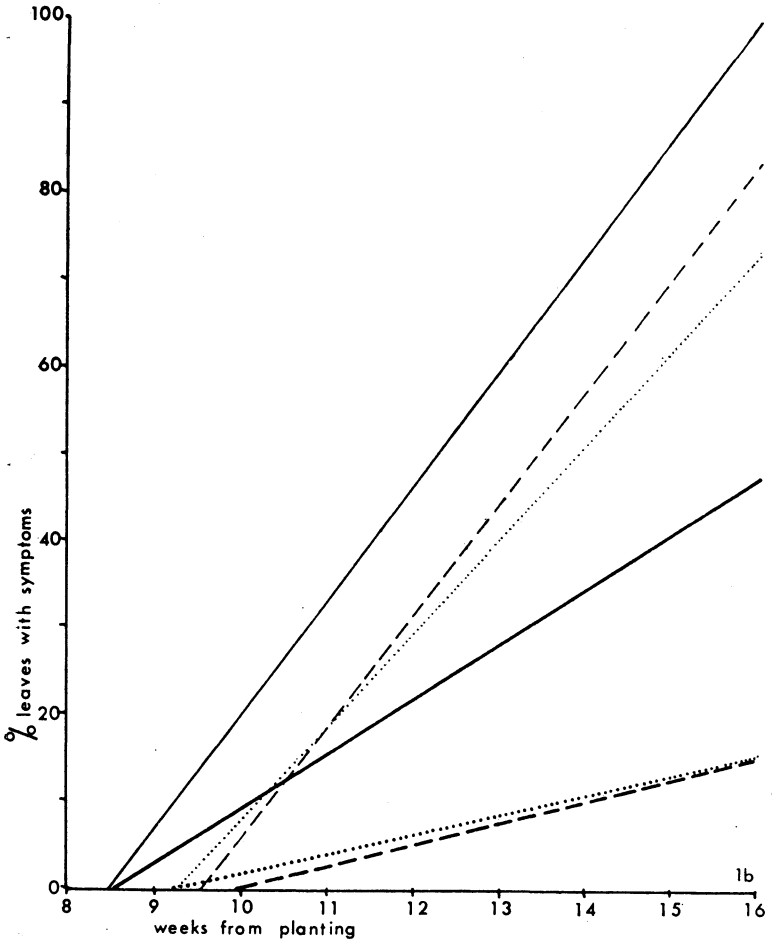

Fig. 1B Effect of fungicide treatments on the rate of symptom expression, sand/silt soil trial. For details see Fig. 1A.

(ii) *Fungicide effectiveness and varietal resistance*

Two green-house trials were carried out to compare the effectiveness of different rates of benomyl, incorporated in the potting mixture, in controlling verticillium wilt in a series of varieties exhibiting different levels of tolerance to the disease. Increasing the amount of benomyl incorporated in the soil reduced the disease incidence in all varieties. All plants grown in soil containing 25 ppm benomyl remained disease free. Plants of the most susceptible variety, Hicks, were not protected by 10 ppm benomyl in either trial. Yellow Gold plants were protected by 10 ppm benomyl but not by 5 ppm. Waimea plants were protected by all rates of benomyl tested. A number of growth measurements were made during these trials and it was found that infection did not affect the development of plants of any of the varieties tested before the lower leaves had started to senesce.

In a field trial on a site with a loamy clay soil we compared two fungicides, benomyl and thiophanate-methyl, applied in the planting water, on three varieties known to have some tolerance to verticillium wilt (NC 75, Waimea and Kuaka 21). There were small increases in the numbers and weights of symptomless leaves reaped from plants treated with fungicides (Table 2). These increases were largest in the least tolerant variety NC 75, and least in the most tolerant variety, Kuaka 21.

TABLE 2: CONTROL OF VERTICILLIUM WILT IN THREE TOLERANT VARIETIES BY SYSTEMIC FUNGICIDES

Treatment Fungicide (2.5 kg/ha)	*Variety*	*Symptomless leaves/plant*		*Symptom development up to weeks from planting †*	
		% of total	*green wt (gms)*	*Onset (weeks from planting)*	*Subsequent rate increase (% of new leaf infection/week)*
	S.E.	(±3.9)	(±9.6)	not analysed	
None	Waimea	62	612	7.2	5.1
None	NC 75	29	312	7.5	9.5
None	Kuaka 21	94	849	7.5	1.2
benomyl	Waimea	65	639	10.2	6.1
benomyl	NC 75	43**	425***	8.7	9.1
benomyl	Kuaka 21	96	853	10.2	1.4
thiophanate-methyl	Waimea	78***	715***	10.5	6.3
thiophanate-methyl	NC 75	42**	483***	8.4	9.1
thiophanate-methyl	Kuaka 21	96	899**	10.9	1.5

, * = differs significantly from corresponding untreated value at p= .01 or p = .001 level respectively.
† = as calculated by line fitting

Symptoms were first observed in all three varieties at approximately the same time. The subsequent rate of increase in the number of leaves affected was much slower in Kuaka 21 plants than in plants of the other two varieties. In plants treated with fungicides the initial appearance of symptoms was delayed by up to three weeks but the disease then developed as fast in treated as untreated plants.

(iii) *Phytotoxic effects*

In these trials plants treated with benomyl were sometimes distorted, particularly where the Waimea variety was used. Plants at all trial sites were affected to varying degrees and one trial on a sand/silt soil was abandoned due to the severity of the malformations. The symptoms included leaves with forked midribs, leaves with no lamina attached to parts of the midrib, leaves with enations and plants with highly irregular internodes so that the leaves frequently appeared to be in whorls.

DISCUSSION

In these trials and those reported earlier (Hartill, 1971) benomyl has given variable control of verticillium wilt in tobacco and only in some trials would an economic return have been obtained from the use of the fungicide. Benomyl appeared to be most effective when used:—

(a) as a wettable powder suspensions either mixed in the planting water or incorporated into the soil, (b) when used on a relatively light textured soil, and (c) when the variety used had some tolerance to the

disease. Tween 20 was not an effective adjuvant when used in field trials although it was effective when used in previous pot tests. When benomyl was used with the highly resistant Kuaka 21 variety it reduced the small incidence of wilt only slightly. A further limitation to the possible use of benomyl in tobacco is its tendency to cause growth distortions on some soils.

In these and other trials thiophanate-methyl has given control of verticillium wilt comparable to that obtained with benomyl.

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