BLUE MOULD (*Peronospora tabacina* Adam) OF TOBACCO IN NORTH QUEENSLAND: SOME ASPECTS OF CHEMICAL CONTROL.

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SUMMARY.

The results of screening trials to assess the efficiency of various fungicides and fungicidal mixtures for blue mould control are detailed and discussed. Field spray trials are described and a theory of acquired resistance following blue mould attack is developed to explain the anomalous results in some trials. Evidence drawn from field experiments is presented to substantiate this theory.

I. INTRODUCTION.

Flue-cured tobacco is an important crop in North Queensland. The producing areas in 1958 were the Mareeba-Dimbulah district (4,400 acres), the Burdekin (1,225 acres), and Ingham (150 acres). The average yield is between 700 and 800 lb. of leaf per acre. This compares very unfavourably with the yield of flue-cured tobacco obtained in the United States of America. While it is probable that many factors contribute to this reduced yield, it is plain that the incidence of tobacco blue mould in the field in North Queensland makes substantial inroads on the crop. Estimates of percentage loss on individual farms range as high as 44 per cent. in a crop planted from healthy seedbeds (Angell 1957). The destruction of bottom leaf by spotting is very considerable, but in some seasons, particularly when wind storms are experienced, this loss is aggravated considerably by the collapse of crops in which the incidence of systemic infection is high.

Since the Second World War and consequent on the completion of various water conservation schemes in the tobacco-growing areas, there has been a rapid expansion in the acreage of tobacco grown under irrigation in North Queensland. It was with the increase in the number of irrigated farms that blue mould emerged as an ever-present and serious problem in the field. This appears to be related to the early or spring planting which is now the rule for such crops. The seedbeds are planted in June and July and transplanting takes place during August and September. The plants are subsequently exposed to weather conditions, in the form of low minimum temperatures and frequent heavy dews during September and October, which enable the disease to become established. If rain eventuates the inoculum potential increases rapidly and an epiphytotic develops.

No resistance to the disease has been noted in any of the flue-cured varieties grown on a commercial scale in North Queensland up to the present.

II. EFFECTS OF THE FIELD DISEASE ON THE PLANT.

In the initial stages of infection in the field the plant develops large chlorotic lesions on the bottom leaves. On the lower surfaces of these lesions a purplish down, comprising the conidiophores and conidia, is produced. As the lesions age they become necrotic. Such necrotic spots drop out either in the field or during curing. On badly affected leaves the spots are so numerous as to coalesce; the leaves become entirely chlorotic, develop a practically continuous layer of down on the lower surface, wilt and die very quickly. As the infection progresses the fungus may invade the conducting tissues of midrib and stalk and establish a systemic infection (Angell and Hill 1932).

Systemically infected plants commonly exhibit wilting symptoms and stunted leaves may be produced following vascular penetration. Sucker growth is often abnormal and sucker leaves may be narrow and mottled. The most serious aspect of this phase of the disease is, however, the loss of plants by lodging due to weakening of the infected stems at their bases where vascular invasion is usually most intense.

Subsequent foliage infections on systemically infected plants show as much smaller spots or flecks with greatly inhibited sporulation or as a breakdown of veins often accompanied by puckering of the lamina around the blighted structure.

III. SCREENING OF FUNGICIDES FOR BLUE MOULD CONTROL.

The benzol fumigation treatment of tobacco seedlings (Angell, Allen and Hill 1936) when used as recommended by the Queensland Department of Agriculture and Stock (Pont 1956) has given satisfactory control of the disease in seedbeds in North Queensland. However, with the increase in importance of blue mould as a field problem, it became necessary to investigate the possibility of control by fungicides in the field. As a preliminary stage in this project, the efficiency of a large number of fungicides was assessed in a series of screening trials conducted in the years 1951, 1952, 1956, 1957 and 1958*.

The fungicidal control of blue mould has received considerable attention in the United States. The use of the dithiocarbamates in seedbeds has been well investigated since fermate was first used for that purpose (Anderson 1942), and both ferbam and zineb (sprays and dusts) have been shown to control the disease in that country (Todd 1955).

A cuprous oxide-cottonseed oil treatment was developed for mould control in the United States (Clayton *et al.* 1938). It was later shown that even though the oil was not toxic to spores of the blue mould fungus it made tobacco more resistant to the disease. Cuprous oxide alone was almost ineffective (Clayton *et al.* 1943). These workers also demonstrated that the salicylates as a group were good blue mould fungicides.

* The results of the first 1959 screening trial became available as this article was going to press. The more interesting of these have been included in Tables 1 and 2.

The fungicidal value of the salicylates was further investigated at a later date (Graham *et al.* 1947) and benzyl salicylate again found to give satisfactory results. In addition, the synergism of the cuprous oxide-cottonseed oil mixture used by Clayton *et al.* (1943) was discussed and the synergism of mixtures of salicylates and various other organic fungicides was demonstrated.

Recently mixtures of cottonseed oil with wettable powder suspensions of organic fungicides have been tested for the control of field blue mould in the United States (Rich and Taylor 1957).

Malachite green has been used for the control of downy mildew of onion and suggested as a control for other downy mildews (McWhorter and Pryor 1937). It has also been shown to inhibit sporulation of the onion downy mildew fungus (Yarwood 1941).

(1) Methods and Procedure.

The screening trials were conducted with seedbed plants. Standard sized seedbeds 10 ft. by 4 ft. were used. Each was divided into five plots to give a plot size of 2 ft. by 4 ft. Treatments were replicated three, four or more times, depending on their number. A randomised block layout was used (Fig. 1).

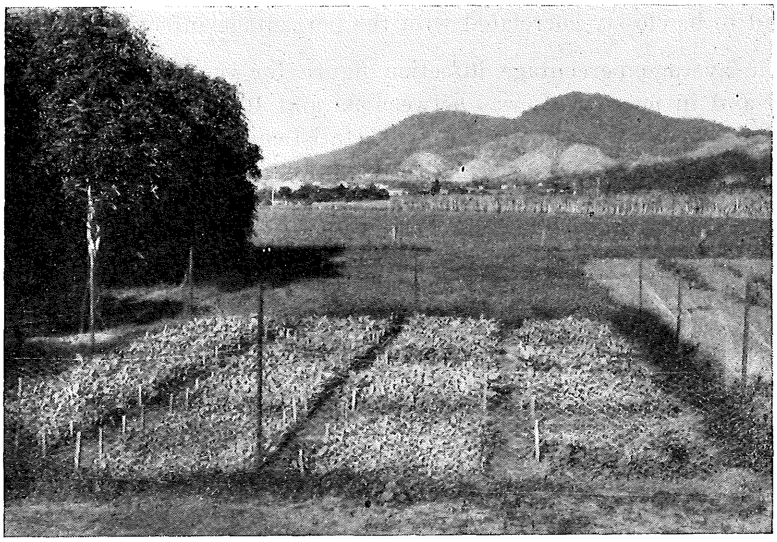

Fig. 1.

A Typical Seedbed Screening Trial.

Treatments were applied twice in seven days—generally on the Monday and Friday of each week while the trial lasted.

Spray treatments were applied with a bucket pump equipped with a single Rega Cyclone nozzle. Spray drift between plots was prevented by enclosing each plot in plywood screens while it was being sprayed. Sprays were applied from each side of the bed with the nozzle held as nearly parallel to the ground as possible in order to ensure maximum coverage of the lower surfaces of the leaves.

Dust treatments were applied with a Rega Bonza duster. Drift was similarly prevented by enclosure in screens. To ensure maximum coverage the plot being treated was totally enclosed except for the opening on the side from which the dust was being applied. Plots were dusted successively from each side of the seedbed.

When the seedlings had reached a suitable size, generally about five weeks after planting, all beds were inoculated with a strong aqueous suspension of conidia of *Peronospora tabacina.* The beds were watered uniformly with the suspension from a 3 gal. watering can with a fine rose.

An unsprayed check treatment was included in each trial.

Spraying terminated when blue mould was well developed in the check plots, and the results were assessed. This was achieved by counting the number of diseased seedlings in a sample of approximately 150 plucked from the centre of each plot. This figure was then expressed as percentage infection. A rating for intensity of infection was given at the same time. In all cases this rating was found to be closely correlated with the percentage infection figure.

The average percentage infection figure for the check plots was never below 96 and in most trials was between 99 and 100. The difference between treatments was always so obvious that statistical analysis of the data was never necessary.

The results are presented in Tables 1 and 2. In these tables the percentage blue mould infection figures originally obtained have been shown as ratings. The conversion from percentage infection was made as follows: 0=nil; 1=1–10 per cent. infected plants; 2=11–20 per cent. infected plants, and so on to 10=91–100 per cent. infected plants.

The scale used for phytotoxicity rating was:

0	Nil.
+	Scattered necrotic spotting but no setback in growth.
++	More general necrotic spotting. Slight stunting.
+++	Necrosis and stunting without death.
++++	Severe necrosis and stunting. Death of many seedlings.

(2) Discussion.

The results of the screening trials described in this article confirm that copper fungicides used alone are relatively inefficient for blue mould control. In Table 1 it will be seen that none of the coppers had a rating lower than 6. The home-made cuprous oxide mixture used in these trials was the molasses mix

Table 1.

COMBINED RESULTS OF 1951–1959 SCREENING TRIALS USING SINGLE INGREDIENTS.

Fungicide.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Cuprox (50% copper as copper oxychloride)	3·75 lb.	(2) 1951	7	0
		1956	10	0
,,	5 lb.	(1) 1952	9	0
Home-made cuprous oxide (25% copper as cuprous oxide)	3·75 gal.	(3) 1952	6	0
		1956	9	+
		1958	8	0
Copper oxydul ultra (80% copper as cuprous oxide)	4 lb.	(1) 1956	9	++
Duphar Colloidal Copper (15% copper)	6 pints	(1) 1956	9	++
Crag Potato Fungicide (copper zinc chromate)	2·5 lb.	(1) 1956	10	+
Copper Nirit (5% copper —45% thiocyanodinitrobenzene)	3 lb.	(1) 1957	2	+++
Blitox Ipsilon (12% copper)	5 lb.	(1) 1957	7	0
Phygon (50% dichloronaphthoquinone)	1 lb.	(1) 1951	5	0
S.R. 406 (50% captan)	2 lb.	(2) 1951	7	0
		1952	10	0
Fermate (76% ferbam)	4 lb.	(2) 1951	3	0
		1952	7	0
Zerlate (80% ziram)	4 lb.	(2) 1951	4	0
		1952	6	0
Methasan (100% ziram)	4 lb.	(1) 1951	3	0
Fulasin ultra (90% ziram)	1·5 lb.	(1) 1956	2	0
Zebtox (65% zineb)	3·75 lb.	(5) 1956	1, 3	0, 0
		1958	2, 10*	0, 0
		1959	7*	
Curit (72% zineb)	3·75 lb.	(1) 1958	10*	0
Dithane M22 (70% maneb)	2 lb.	(1) 1956	5	0
Dispersible Tetroc (chloranil)	4 lb.	(3) 1957	1	0
		1958	1, 10*	0, 0
TZU. 4311 (40% thiram ; 20% ziram ; 20% tetra methyl dithiocarbamine-acid-methyl arsine)	4 lb.	(1) 1956	1	++++
,,	2 lb.	(1) 1956	6	++++
Shell Fungicide WL 1491 (polymeric thiocarbamate)	3·75 lb.	(1) 1958	10*	0
Phaltan (N trichloromethyl thiophthalimide)	3·75 lb.	(1) 1958	10*	0
Goodrite Zac (zinc dimethyl dithiocarbamate cyclohexidamine)	3·75 lb.	(1) 1956	4	++
P.M.F. (4% w/w mercury as phenyl mercury dinaphthyl methane disulphonate)	0·5 lb.	(1) 1956	5	++
Quat (1% w/v cetyl trimethyl ammonium bromide ; 5% w/v β naphthoxy-acetic acid)	2·5 gal.	(1) 1956	9	Hormone injury
Agrimycin (15% streptomycin ; 1·5% oxytetracycline)	2·34 lb.	(1) 1956	7	+
Hexamine	3 lb.	(1) 1957	9	0
Benzyl salicylate	1 lb.	(1) 1959	6*	0

COMBINED RESULTS OF 1951–1959 SCREENING TRIALS USING SINGLE INGREDIENTS—*continued*.

Fungicide.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Methyl salicylate	2 lb.	(1) 1959	10*	0
Bismuth salicylate	1·5 lb.	(1) 1959	10*	
Salicylanilide	3·75	(1) 1959	9*	
Bulbosan (7·5% trichlorotrinitro-benzene)	Dust	(3) 1957 1959	1, 2 7*	0, 0
Zineb 10%	Dust	(1) 1959	2*	0

* The second 1958 trial and the 1959 trial were severe ones in that conditions for infection and blue mould ev elopm ent were particularly good.

which was at one time the standard recommendation for the control of blue mould in seedbeds in Queensland (Mandelson 1933). Zineb had low ratings in the 1956 trials and in the first 1958 trial. The rating of 10 in the second 1958 trial can be explained by the fact that this was deliberately made a severe test, in which the disease was allowed to become established in the plots before the first spray applications were made. Ziram gave good results in a 1956 trial and ferbam had a low rating in the 1951 test. Another promising organic fungicide was chloranil, which was tried in 1957 and 1958. The thiocyanodinitrobenzene-copper oxychloride mixture used in 1957 gave good control of the disease but was extremely phytotoxic.

The only dust used, viz., trichlorotrinitrobenzene, proved to be extremely efficient in the 1957 trials. The performance of this fungicide was undoubtedly enhanced by the excellent coverage obtained with the method of application described above.

The most interesting features of this series of trials appear in Table 2, where the data for the various fungicide and fungicide-oil mixtures are presented. The chief points are listed below:

(*a*) The increase in fungicidal efficiency gained by using such mixtures is obvious and may be gauged by examining the case of cuprous oxide and its various mixtures. From a consideration of Tables 1 and 2 it is possible to make the following deductions:

(i) Cuprous oxide is less efficient than cuprous oxide plus white oil, which in turn is less efficient than cuprous oxide plus white oil plus malachite green.

(ii) Cuprous oxide plus white oil is less efficient than cuprous oxide plus cottonseed oil, and cuprous oxide plus white oil plus malachite green is less efficient than cuprous oxide plus cottonseed oil plus malachite green.

(iii) Cuprous oxide plus cottonseed oil plus malachite green is less efficient than cuprous oxide plus cottonseed oil plus benzyl salicylate.

Table 2.

COMBINED RESULTS OF 1956–1959 SCREENING TRIALS USING MIXED INGREDIENTS.

Fungicidal Mixture.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Home-made cuprous oxide	3·75 gal.	(1) 1956	4	++
Zebtox	3·75 lb.			
Home-made cuprous oxide	3·75 gal.	(1) 1957	7	++
White oil	2½ pt.			
Home-made cuprous oxide	3·75 gal.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	4	++
Home-made cuprous oxide	3·75 gal.	(3) 1956	3	+++
White oil	2½ pt.	1957	2,2	+++, +++
Malachite green	1·6 oz.			
Home-made cuprous oxide	3·75 gal.	(3) 1957	1, 1	++, ++
Cottonseed oil	6 pt.	1958	2	+++
Malachite green	1·6 oz.			
Home-made cuprous oxide	3·75 gal.	(1) 1958	1	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Cupravit Blue	5 lb.	(2) 1956	6	++
White oil	2½ pt.	1957	3	+++
Malachite green	1·6 oz.			
Copper oxydul ultra	3 lb.	(1) 1957	0	++++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Copper oxydul ultra	2 lb.	(1) 1957	2	+
Cottonseed oil	6 pt.			
Zebtox	3·75 lb.	(1) 1957	1	0
White oil	2½ pt.			
Zebtox	3·75 lb.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	2	+
Zebtox	3·75 lb.	(2) 1957	1, 1	++, ++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Zebtox	3·75 lb.	(2) 1957	1	+
Cottonseed oil	6 pt.	1958	2	++
Malachite green	1·6 oz.			
Zebtox	3·75 lb.	(1) 1959	1*	+
Benzyl salicylate	1 lb.			
Zebtox	3·75 lb.	(1) 1959	3*	–
Methyl salicylate	2 lb.			
Zebtox	3·75 lb.	(1) 1959	4*	–
Bismuth salicylate	1½ lb.			
Zebtox	3·75 lb.	(2) 1957	0	0
Cottonseed oil	6 pt.	1958	1	++
Benzyl salicylate	8 oz.			
Zebtox	3·75 lb.	(1) 1958	2	++
Cottonseed oil	6 pt.			
Benzyl salicylate	5·3 oz.			

COMBINED RESULTS OF 1956–1959 SCREENING TRIALS USING MIXED INGREDIENTS—*continued.*

Fungicidal Mixture.	Concentration. (Quantity used per 100 gal.).	No. of Times and Years Tested.	Blue Mould Rating. (1–10).	Phytotoxicity Rating.
Zebtox	2 lb.	(1) 1958	4	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Zebtox	3·75 lb.	(1) 1959	2*	++
Cottonseed oil	6 pt.			
Benzyl salicylate	1 lb.			
Zebtox	3·75 lb.	(1) 1958	2	+++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Malachite green	1·6 oz.			
Curit	3·75 lb.	(1) 1958	4*	+++
Cottonseed oil	6 pt.			
Benzyl salicylate	12·8 oz.			
Curit	3·75 lb.	(1) 1958	5*	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Curit	3·75 lb.	(1) 1958	3*	++
Cottonseed oil	6 pt.			
Methyl salicylate	3·75 lb.			
Curit	3·75 lb.	(1) 1958	7*	+
Cottonseed oil	6 pt.			
Methyl salicylate	1·8 lb.			
Fulasin ultra	2 lb.	(1) 1957	1	+++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Fulasin ultra	2 lb.	(1) 1957	1	++
Cottonseed oil	6 pt.			
Maneb	2 lb.	(1) 1957	1	+++
White oil	2½ pt.			
Malachite green	1·6 oz.			
Dispersible Tetroc	4 lb.	(2) 1957	1	++
Cottonseed oil	6 pt.	1958	3	++
Dispersible Tetroc	4 lb.	(1) 1958	3	+++
Cottonseed oil	6 pt.			
Malachite green	1·6 oz.			
Dispersible Tetroc	4 lb.	(1) 1958	3	++
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.			
Benzyl salicylate	8 oz.	(1) 1957	1	0
Cottonseed oil	6 pt.			
Benzyl salicylate	8 oz.	(1) 1957	1	++
Cottonseed oil	6 pt.			
Malachite green	1·6 oz.			
Zebtox	10%	(1) 1959	2*	++
Zinc salicylate	2% dust			

* This second 1958 trial and the 1959 trial were severe ones in that conditions for infection and blue mould development were particularly good.

(*b*) In the case of the zineb combinations there are indications of similar though less obvious differences in the efficiency of zineb alone and with the various additives

(*c*) As would be expected, the concentrations of the ingredients in the mixture have an effect on its fungicidal efficiency. Zineb (3·75 lb.—100) plus cottonseed oil plus benzyl salicylate (8 oz.—100) is superior to either zineb (2 lb.—100) plus cottonseed oil plus benzyl salicylate (8 oz.—100) or zineb (3·75 lb.—100) plus cottonseed oil plus benzyl salicylate ($5\frac{1}{2}$ oz.—100).

(*d*) In most cases the mixtures are more phytotoxic than the constituent fungicides used alone. The phytotoxicity is aggravated particularly by the inclusion of malachite green in the mixture.

(3) Conclusions.

The superiority of the organic fungicides zineb, ziram, ferbam and chloranil over the various copper compounds used in these trials was shown. Improved efficiency resulted from the addition of cottonseed oil, white oil, cottonseed oil plus malachite green, white oil plus malachite green, cottonseed oil plus benzyl salicylate or cottonseed oil plus methyl salicylate to either copper or organic fungicides. Phytotoxicity to tobacco seedlings generally increased with the use of fungicidal mixtures.

This increase in efficiency shown by mixtures was undoubtedly due to the synergism reported by other workers with tobacco blue mould fungicides.

IV. FIELD SPRAY TRIALS FOR BLUE MOULD CONTROL.

During the years 1952 to 1958 a series of field trials of blue mould fungicides was conducted at various sites in the Mareeba-Dimbulah area. The fungicides used in these trials were generally selected after consideration of the results of the screening trials which preceded them. However, when zineb became available in 1954, it was used in a field trial without prior testing, due principally to the reports of its efficacy for blue mould control which were received from the United States.

These field experiments will be considered in two groups; firstly, the experiments of 1952, 1953, and 1954, in the course of which the puzzling behaviour of blue mould fungicides under field conditions first became apparent; and, secondly, the experiments of 1956, 1957, and 1958, in which the problem of control was investigated in more detail.

(1) Methods and Procedure.

The experimental layout was in all cases a randomised block. The plot size varied in the early experiments but in 1956 was standardised at three rows. The centre row was regarded as the datum row for the assessment of results. The length of the row was never less than half-a-chain.

The plants were sprayed when small with a Rega knapsack (Moles Patent type) spray equipped with either a Cyclone nozzle or a Rega No. 6 adjustable nozzle. In the later stages of growth a portable power spray was used. After early trial with various types of nozzles the Rega No. 6 adjustable was chosen as the standard. A pressure close to 100 lb. per sq. in. was used, as pressures greater than this injured tender foliage. The aim of the operator was to get the fungicide on to the lower surfaces of all leaves on the plant.

The main features of the early trials are summarised below, after which the later trials are discussed in more detail.

(2) Field Trial, 1952.

(*a*) *Treatments.*—The 1952 trial consisted of 12 replications of 3 treatments:—home-made cuprous oxide (1½ gal. stock–40 gal.); ferbam(4 lb.–100 gal.); and untreated.

Five applications were made at 7-day intervals. Spraying commenced when the plants were two weeks in the field and terminated four weeks later, which was about four weeks before harvest.

(*b*) *Method of Rating.*—Each plant was rated for blue mould damage at the completion of spraying on a scale ranging from 0 (=complete absence) to 10 (=complete defoliation). The results appear in Table 3.

Table 3.

MEAN RATING FOR BLUE MOULD DAMAGE IN 1952 FIELD TRIAL.

Treatment.	Mean.	Sig. less than (1% level).
A. Cuprous oxide	1·15	C
B. Ferbam	0·72	A, C
C. Nil	2·01	

(3) Field Trial, 1953.

(*a*) *Treatments.*—Eight replications were made of 6 treatments:—home-made cuprous oxide (1½ gal. stock–40 gal.), primed and unprimed; 80 per cent. ziram (2 lb.–100 gal.), primed and unprimed; and unsprayed, primed and unprimed.

Four applications were made at 10-day intervals. Spraying commenced when the plants were three weeks in the field and terminated about two weeks prior to harvest.

(*b*) *Method of Rating.*—(i) Overall rating. Immediately prior to the first harvest plots were rated on a scale ranging from 0 (=complete absence) to 5 (=average of 20 per cent. of leaf surface affected).

(ii) Detailed rating. About one week after the first harvest 20 plants in each datum row were given ratings for blue mould infection at three different levels, viz., ankle, knee, and hip high. The above scale was used. Results appear in Table 4.

Table 4.

EQUIVALENT MEAN RATING FOR BLUE MOULD DAMAGE IN 1953 FIELD TRIAL.

Treatment.	Overall.			Ankle.			Knee.			Hip.		
	Un-primed.	Primed.	Mean	Un-primed.	Primed.	Mean.	Un-primed.	Primed.	Mean.	Un-primed.	Primed.	Mean
Cuprous oxide	2·1	2·0	2·1	0·7	0·6	0·6	1·5	1·6	1·5	0·5	0·7	0·6
80% Ziram	2·4	2·5	2·4	1·3	1·0	1·1	1·6	1·6	1·6	0·7	0·5	0.6
Nil	1·7	1·7	1·7	2·0	1·7	1·9	1·1	1·2	1·1	0·5	0·5	0.5
Mean ..	2·1	2·0	2·0	1·3	1·1	1·2	1·4	1·5	1·4	0·6	0·6	0,6
	Nil << Ziram Nil < Cuprous oxide			Cuprous oxide << Nil Ziram < Nil			Nil << Cuprous oxide Nil << Ziram			No significant differences		
	No significant differences between primed and unprimed.											

Square root transformation used in analysis.

(4) Field Trial, 1954.

(*a*) *Treatments.*—Seven replications were made of three treatments:—home-made cuprous oxide (3 gal. stock–40 gal.); 65 per cent. Zineb ($1\frac{1}{2}$ lb.–40 gal.); and untreated.

Six applications were made at 7-day intervals. Spraying commenced when the plants were four weeks in the field and terminated after nine weeks in the field, about two weeks before harvest.

(*b*) *Method of Rating.*—(i) Overall rating. One week after spraying was terminated those plants which were flowering were rated on the scale used in the previous experiment.

(ii.) Detailed rating. One week later a maximum of 30 plants in each plot was examined. Top and bottom halves of these plants were rated separately. The same scale was used. Results appear in Table 5.

Table 5.

MEAN RATING FOR BLUE MOULD DAMAGE IN 1954 FIELD TRIAL.

Treatment.	Overall.	Bottom Half.	Top Half.
Cuprous oxide	1.4	2.06	1.8
65% Zineb	1.3	1.9	2.04
Nil	1.85	2.1	1.9

(5) Discussion of the 1952-1954 Field Trials.

These experiments demonstrated that the fungicides used were of little or no use for blue mould control in the field.

In 1951 a limited control was obtained. However, this trial was completed four weeks or more before harvesting commenced and subsequent experience has indicated that the position can change drastically in the period preceding harvest.

In 1952 early observations had indicated that the fungicides were giving some degree of control of the disease. However, a surprising situation was encountered when the plants were rated immediately prior to harvest and it was found that the intensity of infection on the unsprayed plants was significantly less than that on plants treated with either of the fungicides. The detailed readings subsequent to harvest revealed that, while the sprayed plants had less mould on the older foliage, the position was reversed on leaves higher up the plants.

In 1954 somewhat similar though less definite results were obtained. In this trial, although the overall ratings showed less mould on the sprayed plants, the detailed examinations later showed that while the amount of infection was less on the lower halves of sprayed plants than it was in this zone on unsprayed plants, it was greater on the upper halves of those plants sprayed with zineb than it was in a similar position on unsprayed plants.

While lack of control of the disease might be attributed to the inefficiency of the fungicides or of the schedules employed, one would not expect more intense infection on sprayed plants than on unsprayed plants.

Consideration of these data led to the adoption of the theory of acquired resistance, resulting from blue mould infection in the field. This matter will be discussed in more detail later.

(6) Field Trial, 1956.

Preventive spraying for blue mould control demands regular and efficient coverage of the surfaces of newly expanded foliage. Observations had indicated that one reason for the breakdown of control on sprayed plants in previous experiments was that such coverage was difficult to obtain because of the rapid growth of the tobacco plant. It was, therefore, decided to investigate the effects of more frequent applications and the 1956 trial was designed primarily for this purpose. The choice of fungicides was influenced by the results of the 1956 screening trials.

(*a*) *Treatments.*—Four replications were made of 8 treatments:—home-made cuprous oxide (3 gal. stock–40 gal.) plus white oil (1:320) plus malachite green (1:10,000); 65 per cent. zineb ($1\frac{1}{2}$ lb.–40 gal.); 90 per cent. ziram (4/5 lb.–40 gal.); and untreated.

Two schedules were used:—(i) One application per week; and (ii) two applications per week. Schedule 1 treatments received nine applications and

Schedule 2 treatments 18. Spraying commenced on Sept. 21, when the plants had been one week in the field, and was terminated on Nov. 20 just prior to harvest.

(*b*) *Method of Rating*.—On Nov. 22, subsequent to the final spray application, 10 plants in the datum row of each plot at or near the flowering stage were inspected and detailed recordings of blue mould incidence were made. The number of leaves on each plant was counted and each leaf was given a percentage rating for blue mould infection based on a visual estimate of the percentage of the lamina infected with the disease. The intensity of infection on each plant was gauged by calculating an average figure for percentage blue mould per leaf. The results are summarised in Tables 6 and 7. In Table 8 will be found particulars of the number of live leaves per plant.

Table 6.

INTENSITY OF BLUE MOULD INFECTION IN 1956 FIELD TRIAL.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	9·3	..	..	..	9·3
Schedule 1	..	7·2	27·0	16·5	16·9
Schedule 2	..	2·0	10·3	8·2	6·8
Mean ..	9·3	4·6	18·7	12·4	11·9

Marginal differences:

Zineb $\gg$ untreated, cuprous oxide.

Zineb $>$ ziram.

Ziram $>$ cuprous oxide.

Schedule 1 $>$ untreated, Schedule 2.

The variability was very high and not homogeneous. This was due to the infection pattern, which will be discussed below. When this factor is considered, the percentages taken over Blocks 3 and 4 only are better indications of the effects of the treatments. The relevant means for these blocks are given in Table 7.

Table 7.

INTENSITY OF BLUE MOULD INFECTION IN BLOCKS 3 AND 4 OF 1956 FIELD TRIAL.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	14·2	..	..	..	14·2
Schedule 1	..	10·6	34·1	19·2	21·3
Schedule 2	..	2·4	10·1	11·3	7·9
Mean ..	14·2	6·5	22·1	15·2	14·5

Individual differences:

Cuprous oxide Schedule 2 $\ll$ all others.

Zineb Schedule 1 $\gg$ all others.

Ziram Schedule 1 $\gg$ all except zineb.

Table 8.

NUMBER OF LIVE LEAVES PER PLANT IN 1956 FIELD TRIAL.

——	Untreated.	Cuprous oxide.	Zineb.	Ziram.	Mean.
Untreated ..	22·0	..	..	..	22·0
Schedule 1	..	24·9	21·4	23·4	23·2
Schedule 2	..	26·0	25·0	25·5	25·5
Mean ..	22·0	25·4	23·2	24·5	24·4

Marginal differences:
Cuprous oxide >> untreated.
Cuprous oxide > zineb.
Ziram > untreated.
Schedule 2 > Schedule 1, untreated.

Since the internal stem infection is such a serious phase of the disease, it was necessary to check the effect of treatment on the development of systemic mould. This was done by examining 25 plants in the datum row of each plot. With the aid of a knife the bark at the base of the stalk was raised and the presence or absence of the characteristic internal discoloration was noted. The percentage stem mould for the different treatments was as follows:—Schedule 1: cuprous oxide 4, zineb 1, ziram 1. Schedule 2: cuprous oxide 2, zineb 2, ziram 1. Untreated 44.

(*c*) *Discussion.*—By Nov. 5, three weeks after it had first appeared, the disease was prevalent but not serious in most of the unsprayed plots and was present to a minor degree in those plots treated with ziram and cuprous oxide. The incidence in the zineb-sprayed plots was negligible. On Nov. 13, one week after storm rain on Nov. 6, the disease was present in epiphytotic proportions in Blocks 3 and 4 and portions of Blocks 1 and 2. It was quite evident that those plots sprayed with zineb and ziram, particularly Schedule 1 treatments, were far worse affected than either those treated with cuprous oxide or the unsprayed plots. It was also quite evident that the infection was most intense on the middle leaves on these zineb-treated and ziram-treated plants. Later it extended to basal leaves (Figs. 2 and 3).

From the result presented above it can be seen that cuprous oxide plus white oil plus malachite green Schedule 2 was the only spray treatment which reduced blue mould infection to a significant degree. The plots treated once a week with zineb and ziram had a significantly greater intensity of infection than had the untreated plots.

These results were not entirely unexpected. The phenomenon of acquired resistance encountered previously in this work was undoubtedly responsible for the failure of the disease to intensify on the unsprayed plants. A somewhat similar experience has been described for another downy mildew type disease—downy mildew of cabbage (Cox 1957, p. 270), who found that "by harvest time the check plots were the 'cleanest'."

Fig. 2.

1956 Field Trial. Left-hand side: zineb once each week. Right-hand side: cuprous oxide once each week.

The inexplicable feature of the results reported here, however, was the extent of the breakdown in the protection afforded by dithiocarbamate sprays under epiphytotic conditions. Similar difficulties have been encountered in Victoria when nabam and zineb are used for tobacco blue mould control under conditions favourable to intense blue mould infection (R. G. Paddick, personal communication 1959).

When the figures for live leaves per plant listed in Table 8 are examined, it will be seen that particularly in the case of the Schedule 2 treatments there have been increases in the numbers of leaves on sprayed plants. The one treatment showing a decrease is zineb Schedule 1. It should also be noted that the incidence of stem mould has been reduced from an average of 44 per cent. in the unsprayed plots to an average of 2 per cent. in the sprayed plots.

It would, therefore, appear from the results of this experiment that early mould infections which confer a degree of immunity on the host also destroy valuable lower leaves and are associated with a high incidence of systemic mould.

(7) Field Trial, 1957.

The concentration of cuprous oxide employed in the 1956 trial gave rise to undesirable deposits on sprayed leaf, particularly when the mixture was applied twice each week. Spray burn was also a problem under certain conditions. In 1957 a weaker cuprous oxide mixture was used, viz., $1\frac{1}{2}$ gal. stock to 40 gal. water. Other materials were introduced as a result of the latest screening trials. The following concentrations were used:—zineb $1\frac{1}{2}$ lb.–40 gal.; chloranil 4 lb.—100 gal.; cottonseed oil (C.O.) ·75 per cent.; benzyl salicylate 1:1,600; malachite green (M.G.) 1:10,000.

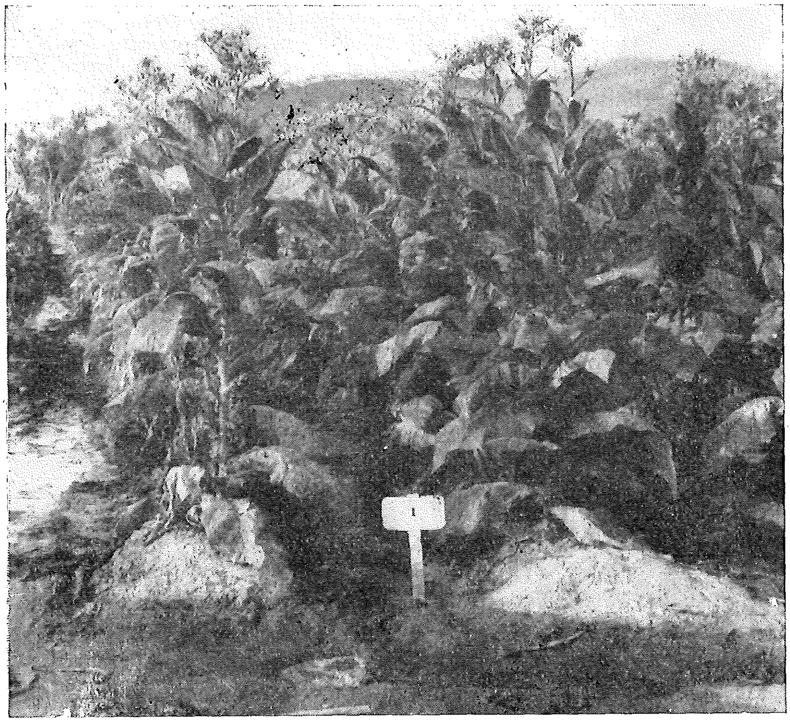

Fig. 3.

1956 Field Trial. Left-hand side: zineb once each week. Right-hand side: unsprayed.

Since previous experience had shown that conditions suitable for the development of an epiphytotic did not usually occur before some six weeks after planting out, treatments commencing at planting time were compared with treatments commencing six weeks later. In three treatments (K, L, O) the plants were primed twice during the first six weeks.

The possibility that low-volume application of oil-based fungicides would be of use, particularly in the later stages of growth, prompted the inclusion of early spraying plus late misting treatments. The machine used to apply the oily mists was a "Swingfog" with a misting attachment fitted. Cuprous oxide (a commercial preparation containing 80 per cent. copper) and zineb were each used at the rate of 1 lb. to 1 gal. oil.

(*a*) *Treatments.*—There were 3 replications of 16 treatments. Particulars of the latter are given in Table 9.

Table 9.

AVERAGE BLUE MOULD RATING FOR TOP AND CENTRE ZONE ON NOVEMBER 11, FIELD TRIAL, 1957.

Treatment.	Time of Application.	Mean Rating.	Significantly less than
K. Primed. Cuprous oxide + C.O. + M.G.	late	0·8	(1%) P (5%) M, E, F
J. Benzyl salicylate + C.O. + M.G. ..	late	1·2	(5%) F, P
B. Zineb + C.O.	early + late	1·3	(5%) P
D. Benzyl salicylate + C.O. + M.G. ..	early + late	1·3	(5%) P
A. Cuprous oxide + C.O. + M.G. ..	early + late	1·3	
G. Cuprous oxide + C.O. + M.G. ..	late	1·3	
C. Chloranil + C.O.	early + late	1·5	
I. Chloranil + C.O.	late	1·5	
N. Zineb + C.O. .. .. Zineb mist	early late	1·6	
L. Primed. Zineb + C.O.	late	1·7	
O. Primed. Nil spray		1·8	
H. Zineb + C.O.	late	1·9	
M. Cuprous oxide + C.O. + M.G. .. Cuprous oxide mist	early late	2·3	
E. Cuprous oxide + C.O. + M.G. ..	early	2·4	
F. Zineb + C.O.	early	2·6	
P. Nil spray		2·7	

The experimental block was planted on Sept. 10. The initial sprays of the early treatments were applied on Sept. 17. The last spray applications in the early treatments (E, F, M and N) were made on Oct. 21 and 22. The first spray applications in the late spray treatments (G, H, I and J) were made on Oct. 28 and 29. Misting with oil-based fungicides (M and N) commenced on Oct. 29. Final applications of mists and sprays were made on Nov. 11 and 12. Thus the early spray treatments (E, F, M and N) were repeated six times at weekly intervals and the late spray treatments (G, H, I, J, K and L) four times. The plots which were sprayed each week from time of commencement (A, B, C and D) received 10 sprays.

(*b*) *Method of Rating.*—On Nov. 11, approximately nine weeks after planting out, a rating for blue mould incidence was made on 15 plants in each datum row. Each plant was rated in three positions. Counting downwards from the first leaf exceeding 9 in. in length, the foliage was divided into three zones comprising the top five leaves, the centre five leaves, and the remaining bottom leaves. The following rating scale was used:—

0=Nil.

1=Scattered spots on one leaf; odd spots or nil on remainder.

2=Scattered spots on two leaves; odd spots or nil on remainder

3=Concentrated spots on one leaf; scattered spots on two leaves; odd spots or nil on remainder.

4=Concentrated spots on two leaves; scattered spots on remainder.

5=Concentrated spots on three leaves; scattered spots on remainder.

6=Concentrated spots on four leaves; scattered on one.

7=Concentrated spots on all leaves.

On Nov. 18, during the week following the final spray application, 10 plants at or near the flowering stage in the datum row of each plot were inspected and detailed readings on blue mould incidence were carried out. A leaf count was made on each plant commencing at and including the first leaf to exceed 15 in. in length. Estimates of percentage blue mould infection were given to each leaf as in the 1956 trial. The plants were again divided into three zones (top, centre and bottom), each zone in this case consisting of six leaves. Where leaves were killed they were given a rating of 100 per cent. blue mould infection. An average percentage mould infection figure was calculated per leaf for each zone.

Table 10.

Average Blue Mould Rating for Bottom Zone on November 11, Field Trial, 1957.

Treatment.	Time of Application.	Mean Rating.	Significantly less than
B. Zineb + C.O.	early + late	0	(1%) J, E, O, G, P (5%) H, I
N. Zineb + C.O. Zineb mist	early late	0·1	(1%) J, E, O, G, P (5%) I
D. Benzyl salicylate + C.O. + M.G.	early + late	0·2	(1%) J, E, O, G, P
A. Cuprous oxide + C.O. + M.G.	early + late	0·4	
C. Chloranil + C.O.	early + late	0·5	
F. Zineb + C.O.	early	0·5	(1%) G, P
K. Primed. Cuprous oxide + C.O. + M.G.	late	0·5	(5%) J, E, O
L. Primed. Zineb + C.O.	late	0·6	
M. Cuprous oxide + C.O. + M.G. Cuprous oxide mist	early late	1·0	(1 P %)
H. Zineb + C.O.	late	1·2	
I. Chloranil + CO.	late	1·2	
J. Benzyl salicylate + C.O. + M.G.	late	1·8	
E. Cuprous oxide + C.O. + M.G.	early	1·8	(5%) P
O. Primed. Nil spray		1·8	
G. Cuprous oxide + C.O. + M.G.	late	2·2	
P. Nil spray		3·2	

Results are given in Tables 9, 10 and 11. In Table 9 the figures are simplified by combining the ratings for top and centre as there was little difference between these regions at this time.

Table 11.

PERCENTAGE BLUE MOULD PER LEAF IN EACH OF THREE ZONES ON NOVEMBER 18, FIELD TRIAL, 1957.

Top.		Centre.		Bottom.	
Treatment.	Equiv. Mean %.	Treatment.	Equiv. Mean %.	Treatment.	Equiv. Mean %.
D	1·1	K	8·1	B	9·9
J	1·1	J	10·2	D	10·0
K	1·1	G	12·8	N	18·4
I	1·3	D	13·8	A	21·2
G	1·7	L	14·5	K	24·1
N	2·0	A	15·5	H	31·3
O	2·0	I	16·0	L	34·9
P	2·2	H	18·4	I	38·6
L	2·3	B	18·5	J	43·4
C	2·4	O	19·5	F	43·4
B	2·5	N	29·0	G	45·1
E	2·6	E	33·6	M	50·7
A	2·7	P	36·7	E	61·1
H	2·8	M	44·2	C	62·8
F	4·5	C	46·0	O	70·8
M	4·9	F	50·7	P	85·7
D, J, K < F, M I < M		K << C, F ; < E, P, M J << F ; < P, M, C G, D, L < M, C, F A, I < C, F H, B, O < F		B, D << O, P ; < E, O N, A, K << P ; < O H, L, I < P	

Prior to analysis percentages were transformed by means of the inverse sine transformation.

(c) *Discussion.*—Until Oct. 22 blue mould infection was scattered in the experimental area with the exception of Block 3, where there had been a rapid spread. In this block at this date the pattern was much as would be expected with those plots receiving early spray treatments showing less disease than the unsprayed plots. An interesting feature at this time was the reduced amount of blue mould in treatments K, L, and O, which had been primed once at this juncture.

By Nov. 11, subsequent to a rain group which commenced on Oct. 30 and lasted until Nov. 6, all plants in the three blocks were infected. The intensity of infection was increasing rapidly in Blocks 1 and 2. In Block 3, however, due to the effect of acquired resistance, the severity of infection did not show a substantial increase. At this date it was plain that the treatments which involved weekly sprays from the time of commencement (A, B, C and D) had considerably reduced blue mould damage on the lower leaves. Treatments M and N also had less infection in this zone. The late-sprayed treatments commenced on Oct. 28 prior to the beginning of the rain group and consequently treatments H, I, J, K and L conferred a degree of protection on lower leaves. Spraying for the first six weeks only produced no reduction of mould in the upper zone of foliage.

Between Nov. 11 and Nov. 18, when the final assessments of foliage damage in the various treatments were made, the intensity of infection reached a maximum. In the bottom zone the benefits of spraying were plainly shown by the reduced incidence of mould on the lug leaves in the best treatments. There was little to choose between the zineb, benzyl salicylate and cuprous oxide sprays in this respect. Zineb applied as an oily mist also appeared to be effective but its use is accompanied by the risk of serious leaf burn.

For the top and central zones the main features shown were—

(i) The improvement in the position of the unsprayed and the late-sprayed treatments (treatments L and H excepted). This was no doubt due to the acquired resistance factor, which could have operated to the advantage of all these treatments, particularly those in Block 3, which had been exposed to infection for six weeks before treatment commenced.

(ii) The deterioration in the position of treatments B (zineb) and A (cuprous oxide), which was reminiscent on a much smaller scale of the breakdown of zineb in the 1956 trial when inoculum potential was high. The anomalous position of treatments L and H mentioned above could also have been due to this breakdown. Treatment C (chloranil) gave reasonable control of the disease until an epiphytotic developed, when it also lost its efficiency. The superiority of treatment I over treatment C might well be linked with the acquired resistance factor.

(8) Field Trial, 1958.

Screening trials had demonstrated the usefulness of zineb-salicylate mixtures (Table 2), and accordingly a zineb-methyl salicylate spray was chosen for use in the 1958 field trial. The composition of this spray was:—zineb $1\frac{1}{2}$ lb–40 gal. + cottonseed oil 0·75 per cent. + methyl salicylate (M.S.) 1 lb.–27 gal. Zineb plus a wetting agent (Triton B1956) was included so that the behaviour of the two mixtures could be compared if an epiphytotic of the disease occurred. In treatment G the seedlings were dipped in the zineb-methyl salicylate mixture before planting out.

(*a*) *Treatments.*—There were three replications of eight treatments, particulars of which appear in Table 12.

The experimental area was planted on Sept. 11. Spray applications commenced on Sept. 29. The late-spray treatments B and D commenced on Nov. 3. The final applications were made on Nov. 17. The plots sprayed twice each week from time of commencement received 15 applications; those that were sprayed once each week from that time received eight applications; and the late-sprayed treatments received three applications.

Table 12.

EQUIVALENT MEAN PERCENTAGE BLUE MOULD PER LEAF AND EQUIVALENT MEAN STEM MOULD PER TREATMENT IN 1958 FIELD TRIAL.

Treatment.	Application.	Nov. 7.	Nov. 18.	Nov. 18.			Stem Mould.
				Top.	Centre.	Bottom.	
A. Primed. Nil spray ..		14·2	4·7	0	0·4	11·9	26·7
B. Primed. Zineb + C.O. + M.S.	late only	12·9	3·9	0	0·3	9·1	19·6
C. Nil spray		14·1	5·7	0	0·6	15·4	24·0
D. Zineb + C.O. + M.S. ..	late only	14·8	2·4	0	0·8	7·5	20·3
E. Zineb + C.O. + M.S. ..	weekly	4·4	2·9	0	0·3	8·6	2·5
F. Zineb + C.O. + M.S. ..	twice each week	2·0	1·4	0	0·8	3·2	1·3
G. Zineb + C.O. + M.S. (seedling dip)	weekly	5·0	2·7	0	0·3	9·1	2·9
H. Zineb + Triton B1956 ..	weekly	8·3	4·1	0·3	0·8	11·9	16·5
		E, F, G << A–D F << E, G, H E < H < D	No sig. diff.	Observed means. Not analysed		No sig. diff.	E, F, G << A–D, H

Analysis in terms of log X.

(*b*) *Method of Rating.*—On Nov. 7, soon after commencing the late-spray schedule, all the plants in the datum row of each plot (usually 35) were inspected and leaf counts were made which included all leaves exceeding 9 in. in length. Each leaf was given a percentage rating for blue mould damage as in previous trials and a mean figure was calculated as before.

On Nov. 17 and 18, similar readings were made on 10 plants at the flowering stage in each datum row. The same method of disease assessment was used and a mean percentage infection figure was calculated. In addition, the plants were divided into three zones of foliage, each consisting of six leaves, and mean percentage infections per leaf were calculated for each zone. Dead leaves were regarded as 100 per cent. infected.

At intervals from Nov. 18 to Dec. 15, all the plants in this trial were examined for stem infection. A mean figure for percentage stem mould was calculated for each treatment.

(*c*) *Discussion.*—During the course of this experiment a serious outbreak of blue mould failed to eventuate, so the fungicides and schedules used were not subjected to the harsh test of an epiphytotic as had occurred in previous years.

Practically 100 per cent. of the plant population was infected with the disease on Oct. 31, but to a minor degree only. The intensity of infection had probably reached a peak on Nov. 7, when the average percentage infection per leaf for the worst affected treatment was 15·6. After this date, fresh infection was at a minimum, due perhaps to the combined effects of acquired resistance and unsuitable weather conditions.

At Nov. 7 it was too soon to assess the value of the late-spray treatments B and D, for these had received their first spray application only on Nov. 3. All the other zineb treatments had effectively reduced blue mould. Priming at four and seven weeks after planting out (A and B) gave no benefit in this trial.

On Nov. 18, as a result of the factors mentioned above, the overall percentage infection was low and the differences between treatments not significant. There is a suggestion that late spraying had some effect.

There was a certain amount of phytotoxicity associated with the use of the zineb plus cottonseed oil plus methyl salicylate mixture. This was particularly noticeable on those plants sprayed twice each week. The symptoms appeared on the lowest leaves and varied from an abnormal chlorosis (tending towards a bronzing) to necrotic patches of variable size. Bottom leaf from plants which had been sprayed repeatedly with this mixture also did not cure satisfactorily.

This trial confirmed the results obtained in screening trials which demonstrated the superiority of the zineb plus cottonseed oil plus methyl salicylate mixture over zineb alone for blue mould control. In the absence of suitable conditions, it was not possible to state whether this spray possesses the major disadvantage of zineb alone—i.e., the breakdown in efficiency which occurs when conditions are very favourable for disease development. In addition, the adverse effect of the mixture on curing excludes any possibility of its recommendation to growers until further work has been done on formulation and method of application.

(9) GENERAL CONCLUSIONS FROM THE FIELD TRIALS.

Early trials gave little indication that control of blue mould by any of the fungicides used was possible under field conditions. The 1956 trial, however, showed that the addition of white oil and malachite green to cuprous oxide improved the performance of this fungicide and the disease was controlled by applications twice each week. The benefits of spraying were rendered less obvious by low blue mould readings on unsprayed plants due to acquired resistance.

In the 1957 trial the plants were once again subjected to an epiphytotic. The most successful treatment, taking into consideration the whole growing period, was benzyl salicylate plus cottonseed oil plus malachite green (D, J). Some phytotoxicity problems associated with this mixture are yet to be overcome.

Treatments involving zineb and cottonseed oil (B, N) were very effective in reducing mould on the lower leaves under conditions of moderate infection but lost efficiency when infection intensified. The reason for the breakdown at a critical time shown by the dithiocarbamate sprays is obscure. An attempt to overcome this in the 1958 trial by the addition of methyl salicylate and by spraying at more frequent intervals failed to yield an answer, as the usual late epiphytotic did not eventuate.

Regarding schedules, the most successful in the 1957 trial involved applications once each week from one week after planting out until immediately prior to harvest. Late-spray schedules initiated prior to the onset of wet weather proved of more benefit than early schedules which terminated at this time, but the acquired resistance effect probably operated to the advantage of the late-schedule sprays.

The control of mould on the lower leaves during the early part of the season is associated with a marked reduction in the amount of basal stem mould. Some of the zineb combinations are quite effective in this respect.

In summing up the results of these field trials it is apparent that a blue mould fungicide which can be recommended without reservation to tobacco growers is not yet available. However, mixtures have been tested which have improved on the performances of standard fungicides. It is also plain that successful spray treatments are less likely to result in spectacular reductions in leaf mould infection than in less obvious but equally important reductions in the incidence of systemic infection.

V. ACQUIRED OR INDUCED RESISTANCE TO TOBACCO BLUE MOULD.

Acquired immunity to certain plant virus diseases is a well-known reaction of the host (Price 1940) and has been used as a control measure in some cases. Acquired immunity or resistance of a plant to a fungus disease is not a common occurrence, however. The subject has been reviewed in some detail by Chester (1933).

The resistance of tobacco seedlings, which have been infected with tobacco blue mould and recovered, to further attack by the fungus has been described (Wolf 1939, Clayton *et al.* 1938) and it has been noted that, when infected tobacco seedlings are transferred to the field and stem infection develops, leaf-spotting on such plants is seldom as pronounced as that on plants from healthy seedbeds (Angell 1957).

Earlier in this paper a theory of acquired resistance to blue mould infection was adopted in order that the results of certain early experiments could be interpreted. This theory received support after an independent authority had studied the data from these early experiments (G. S. Taylor, personal communication 1957).

It is possible that the results obtained in Florida (Cox 1957) with a related disease, downy mildew of cabbage, previously commented on, were due to the operation of a similar effect.

(1) Discussion of Evidence Provided by Field Trials.

The results of the 1956 field spray trial were expressed graphically by plotting the average percentage blue mould infection for a leaf against the serial number of that leaf (Fig. 4). The number of leaves used for comparison

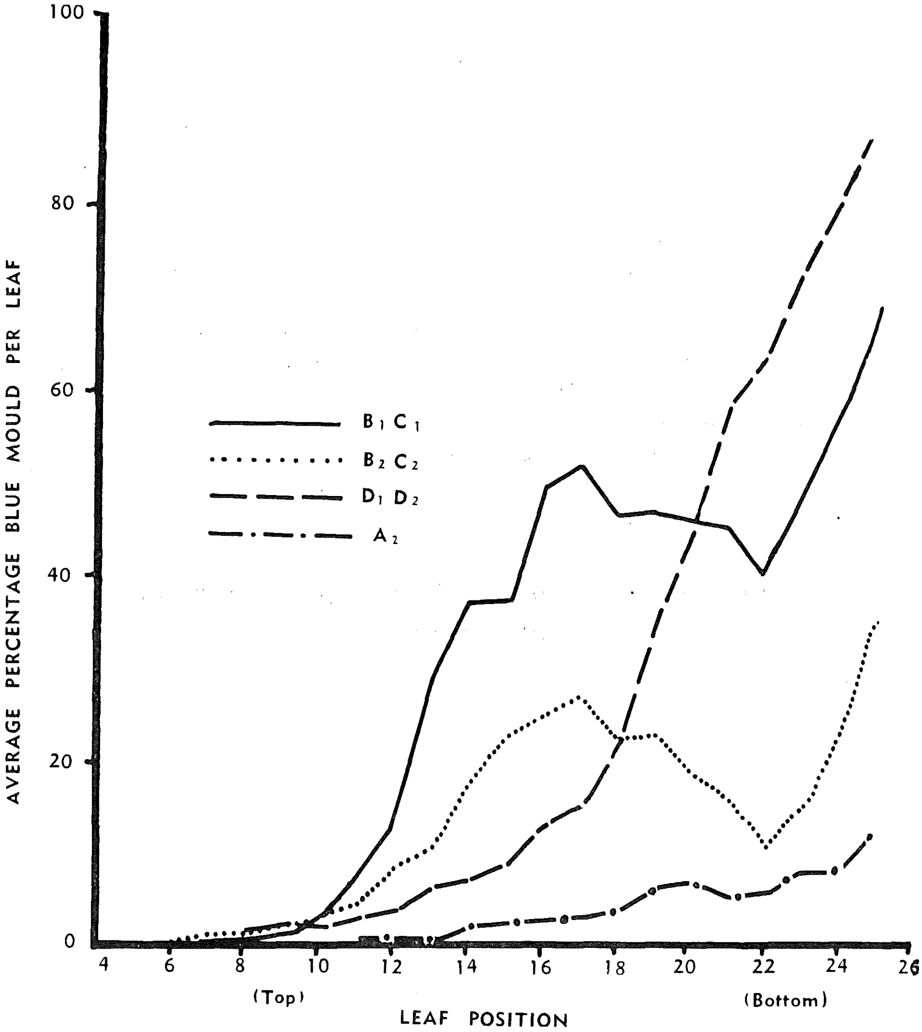

Fig. 4.

Resistance to Blue Mould in Relation to Fungicidal Treatment. 1956 Field Trial. A_2 — Cuprous oxide + white oil + malachite green, twice a week. B_1, C_1 — Mean zineb and ziram plots, twice a week. B_2, C_2 — Mean zineb and ziram plots, once a week. D_1, D_2 — Mean unsprayed plots.

purposes was 25, the average number of leaves for the best spray treatment. These were numbered from 1 (top) to 25 (bottom). Dead leaves were given values of 100 per cent. blue mould infection.

A mean of the percentage blue mould figures for the four replications was taken and in order to obtain representative examples of different spray effects the following selections or combinations of treatments were used—cuprous oxide + white oil + malachite green, twice each week (A_2), representing an efficient fungicide; a combination of the figures for zineb and ziram weekly (B_1, C_1) and twice each week (B_2, C_2), representing a fungicide liable to break down under stress; and a combination of the two nil treatments (D_1, D_2).

If the acquired resistance theory were to be substantiated, the following types of curves would be expected:—

(*a*) The curve for unsprayed treatments. This should be quite steep, indicating infection and death of lower leaves with subsequent reduced infection on upper leaves due to resistance. This is well illustrated in Fig. 4.

(*b*) The curves for those treatments which were ineffective when the inoculum potential was at a maximum. These would be expected to commence their final steep drop much further towards the left, indicating the disposition of susceptible foliage higher on the plant due to the protection afforded by these sprays earlier and the consequent lack of acquired resistance. The location of the maxima would be fixed by the position on the plants of the most intense infection. In Fig. 4 the zineb-ziram curves for both weekly and twice-each-week applications have been included. These both conform to the anticipated general shape with the twice-each-week exhibiting the less extensive infection.

(*c*) The curve for an efficient treatment. This should be quite flat, showing little increase with increase in position number, indicating low mould incidence on all leaves. This is well illustrated by the curve for the twice-each-week cuprous oxide spray.

In considering the effect of acquired resistance in the 1957 spray trial, the unsprayed plots only have been selected to illustrate the point. In this experiment blue mould was widespread in Block 3 prior to Oct. 22 but was virtually absent from Blocks 1 and 2. Apparently due to the acquired resistance the unsprayed plots in Block 3 showed much less injury than those in the other two blocks on Nov. 18, i.e., subsequent to the November epiphytotic. The actual state of affairs is shown in Tables 13 and 14.

Table 13.

Blue Mould Rating in Unsprayed Plots on October 22, Field Trial, 1957.

Treatment.	Block 1.		Block 2.		Block 3.	
	Bottom.	Top.	Bottom.	Top.	Bottom.	Top.
O ..	·6	·1	·2	·1	1·9	0·8
P ..	·1	0	·2	0	3·1	1·2

Table 14.

AVERAGE PERCENTAGE OF BLUE MOULD PER LEAF IN EACH OF THREE ZONES ON NOVEMBER 18, FIELD TRIAL, 1958.

Treatment.	Block 1.			Block 2.			Block 3.		
	Bottom.	Centre.	Top.	Bottom.	Centre.	Top.	Bottom.	Centre.	Top.
O ..	60·4	13·5	2·3	80·3	26·0	1·8	25·7	1·7	0·4
P ..	92·9	47·1	3·4	76·4	27·0	1·2	9·7	2·5	0·2

At the Oct. 22 examination each plant was divided for the purpose of rating into two approximately equal zones from the uppermost leaf exceeding 9 in. in length downwards. The rating was on a scale from 0 (nil) to 5 (chlorosis or concentrated spotting on all leaves).

On Nov. 18 the examination and rating was made as described for the Field Trial, 1957.

The results of the disease assessments made on Nov. 18 on the unsprayed plots are shown in graph form in Fig. 5. As in Fig 4 this was done by plotting the average percentage blue mould per leaf against the position number of the leaves.

Once again the steep curves typical of non-sprayed plants with acquired resistance were the result. However, the protection provided by the early infection in Block 3 is made quite apparent by the position of the curve for this Block.

In 1958 a trial was designed to ascertain whether a resistance to blue mould could be induced by a deliberate early infection with the disease and, if so, whether any practical importance could be attached to it.

In one treatment seedlings were infected at an early age in the seedbed. Eradication was then attempted with benzol fumigation subsequent to the appearance of sporing blue mould lesions but was not successful. Large numbers of inoculated seedlings developed vascular infection with consequent high mortality both in the beds and on transfer to the field.

In another treatment the seedlings were inoculated a few days prior to transplanting and had not developed obvious mould lesions when they were shifted. In this treatment, too, a large percentage of the plants were infected with stem mould and many died.

Conditions in 1958 were not conducive to field infection and it was impossible to detect differences between any of the treatments. However, the data did demonstrate a relationship between systemic infection and reduced intensity of leaf infection (Table 15).

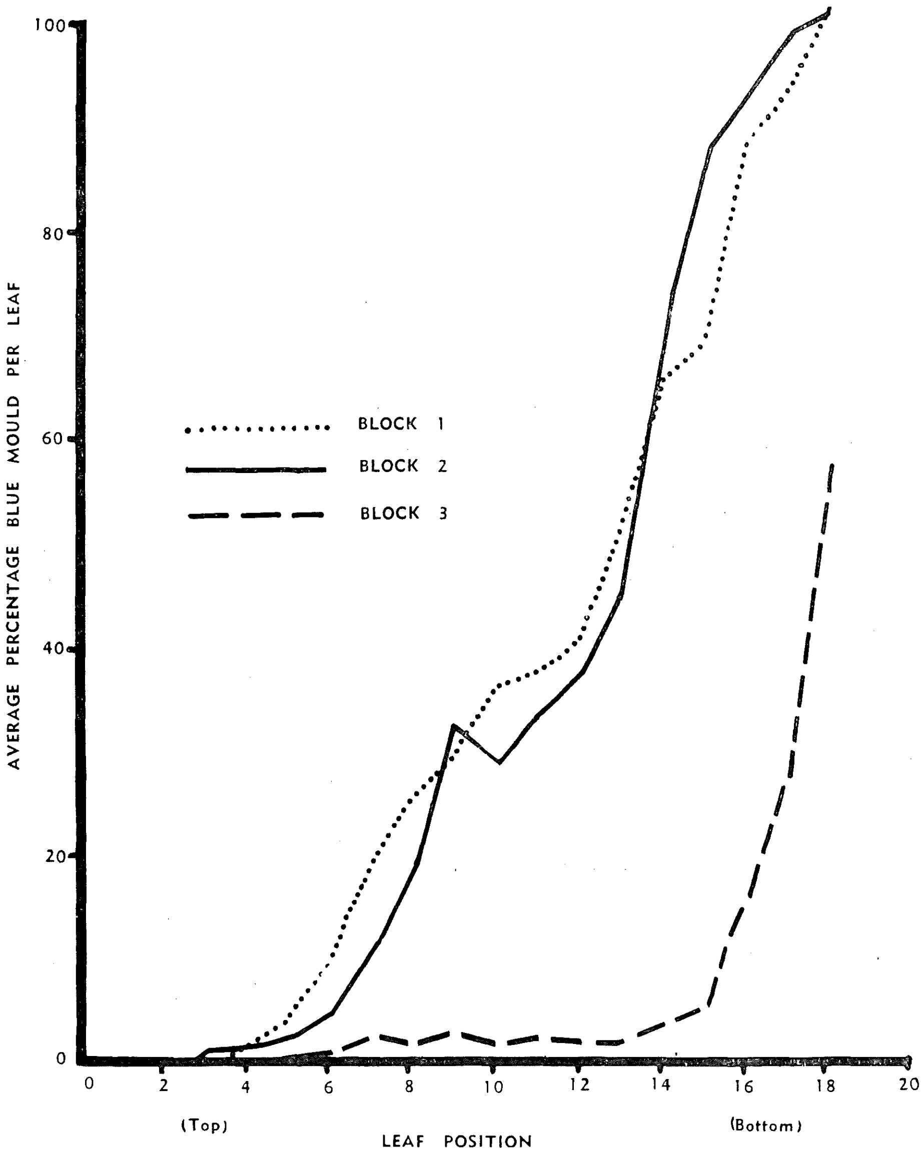

Fig. 5.

Effect of Early Blue Mould Infection on Subsequent Mould Development. 1957 Field Trial. Blocks 1 and 2, late infection. Block 3, early infection.

Under the conditions of this experiment and in the absence of an epiphytotic there was no evidence to presume that any infection other than a systemic one induces a resistance to subsequent leaf infection. This is not surprising, for it has been said (Butler and Jones 1949) that "it is remarkable fact that the only cases in which it has been fully demonstrated that infection can lead in plants, as in animals, to immunity from subsequent re-infection occur in the most completely systemic plant diseases that are known, those due to viruses.''

Table 15.

ASSOCIATION BETWEEN STEM MOULD AND LEAF MOULD IN 1958 ACQUIRED RESISTANCE TRIAL.

Plants without Stem Mould.		Plants with Stem Mould.	
Average No. of Leaves.	Average Percentage Blue Mould.	Average No. of Leaves.	Average Percentage Blue Mould.
13·3	4·8	12·1	0·5

(2) Conclusions.

The evidence put forward here suggests that tobacco plants, when exposed to blue mould infection, acquire a resistance to subsequent re-infection. However, this resistance is coupled with systemic infection which in seedlings may lead to stunting and death. On field plants the results of systemic infection are often not so obvious but lug leaf is destroyed and weakened stalks usually result with consequent danger of lodging before the crop is harvested. It is unlikely therefore that advantage can be taken of this phenomenon for reducing loss from the disease.

It is, however, necessary to take into account the possibility of acquired resistance when interpreting routine fungicide trials in order to explain what otherwise appear as anomalous results.

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