TOBACCO BLUE MOULD (Peronospora tabacina Adam)IN NORTH QUEENSLAND.2. EPIDEMIOLOGICALSTUDIES BEARING ON THE DEVELOPMENT AND
CONTROL OF THE DISEASE

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SUMMARY

In North Queensland, blue mould (*Peronospora tabacina* Adam) persists on out-of-season hosts comprising plant crops, standover crops, volunteer plants and the native host *Nicotiana glauca* Graham. There is some evidence of seedborne infection. The oospore stage of the fungus appears to be absent.

A uniform distribution of inoculum does not exist early in the season. Early mould outbreaks are predictable following a study of the location of sources of infection. The build-up of mould in early-planted crops ensures a high intensity of inoculum by the time the major field plantings are made.

Initial aerial infections are slight and may be expected three weeks after field plantings where a source of inoculum is close. Economic loss and coincidental build-up of mould to an intensity where widespread aerial dispersion of spores takes place may be expected some three weeks after initial infections. The relationship of rate of spread to intensity of inoculum is discussed.

Stem mould is correlated with leaf mould severity at an early age and is found to increase as time from leaf infection increases.

A north-south row direction was found to alleviate mould losses, probably because of better penetration of sunlight.

The relationship between rainfall and the development of the disease is discussed. Dew alone is sufficient to ensure the spread of blue mould throughout a crop but the increase in intensity of infection to epidemic proportions depends on adequate rainfall.

I. INTRODUCTION

In accordance with a resolution passed at the fourth meeting of the Queensland Tobacco Advisory Committee, a comprehensive survey of the incidence of blue mould (*Peronospora tabacina* Adam) in tobacco crops in North Queensland was carried out by the junior author in the 1958 and 1959 seasons. Much of the information presented in this article has been assembled from observations made in the course of this survey. In addition, use has been made of experimental data and observational evidence accumulated by the senior author during work on various aspects of the blue mould problem at the Parada Tobacco Experiment Station from the 1956 season onwards.

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Tobacco in North Queensland is now grown mainly as an irrigated crop. The acreage grown under irrigation is increasing yearly and it is expected that upwards of 12,000 acres of irrigated tobacco will eventually be planted each season in the Mareeba-Dimbulah district alone.

Irrigation has enabled planting to be carried out much earlier in the year than was the case when the crop was rain-grown and transplanting to the field necessarily coincided with early summer storm rains. In the main tobacco districts of the north the majority of first field plantings are made in August. This provides a continuity of plantings extending from August through till early October or even later in the year if the few remaining rain-grown crops are included. In some years in some districts, particularly those along the Burdekin River, off-season crops which are planted in February-March and harvested during the winter are common. Of recent years, the time of planting of the earliest plantings of the main crop has been progressively moved forward until in 1960 the first crops were transplanted in April-May. If this planting policy is persevered with, it will mean that there will be tobacco in the field for most of the year in these districts.

Seedbed blue mould has waned in importance in North Queensland since the benzol vapour fumigation treatment was adopted by growers and remains a problem only when benzol treatment is neglected or carelessly handled.

Substantial losses are, however, caused by blue mould in field plantings. The effect of field blue mould on the tobacco plant has been described previously (Pont 1956, 1959). In estimates made by Angell (1957) the percentage loss in crops due to the combined effects of leaf mould and stem mould ranged up to 44 per cent. The surveys carried out by Hughes in 1958 and 1959 showed that losses due to leaf mould were as high as 40 per cent. He assessed the average loss per farm surveyed from leaf and stem mould at 11.4 per cent. in 1958 and 13.6 per cent. in 1959. In neither year did late rains with attendant epiphytotics develop and in neither year could mould be regarded as severe.

II. METHODS

(a) Data from Field Surveys, 1958 and 1959

The field surveys conducted in the 1958 and 1959 seasons involved all the major tobacco-growing districts and were carried out from mid-May to December in each year. In 1958, 173 farms and in 1959, 183 farms were surveyed. Each of these farms was visited at intervals of three weeks. On each farm a datum area of approximately 2 ac of tobacco was selected and this was taken from the first field plantings.

(i) 1958 Survey

The information sought and disease assessments made at the respective inspections were as follows for the 1958 survey.

EPIDEMIOLOGICAL STUDIES OF BLUE MOULD

(1) Seedbed Phase.—Seedbed notes comprised:—plant variety; benzol usage (in both volume and evaporating surface used to a unit area of seedbed); frequency of fumigation; time of commencement and cessation of fumigation; type and condition of seedbed covers (an airtight cover is the aim of North Queensland tobacco growers, the covering material being galvanized iron or plastic sheeting); preplanting seedbed sterilization; watering practices; proximity of inoculum where obvious; condition of seedlings; date of field planting; and presence or absence of sporing blue mould. Seedbeds were re-inspected and examined for sporing blue mould at the date of field planting.

(2) Field Phase.—Four field inspections were made of each datum area.

First Inspection.—Three weeks after field planting the percentage of mouldaffected plants for each datum area was established. Eight random lots each of 50 consecutive plants were examined for the presence of sporing mould. The percentage of plants affected was calculated from these 400 plants.

Notes were taken on fertilizer application; irrigation and cultivation; use of fungicides; plant height, colour and texture; and, where of interest, farm topography and soil type.

Second Inspection.—Six weeks after field planting mould severity was assessed as follows:

Severity = $\frac{\text{Number of plants infected}}{\text{Number of plants inspected}} \times \frac{\text{Average severity rating}}{\text{for 100 plants inspected.}}$

The number of plants inspected was the same as at the first inspection eight random lots each of 50 consecutive plants and in addition four lots each of 25 consecutive plants on which individual severity ratings were made; in all, 500 plants.

All severity ratings were made on the leaf showing most infection. The rating used was:

0—Nil infection.

1—Scattered spotting.

2-Concentrated spotting.

3—Spotting involving more than 50 per cent. of the leaf area or one leaf lost per plant.

Again at this inspection the numbers of irrigations and cultivations were noted, as well as primings, use of fungicides, plant height, colour and texture and approximate date of harvest.

Third Inspection.—Nine weeks after field planting, percentage mould loss figures were compiled for each datum area. Mould loss (calculated as percentage number of leaves lost) was assessed for each plant in eight random lots of 25 consecutive plants. The average percentage loss for each datum area was calculated as the average of these individual ratings. In addition, the number of plants obviously affected with stem mould or systemic mould was recorded by noting the number of plants lodged, stunted or deformed in eight random samples of 50 consecutive plants. Irrigation, cultivation, fungicide usage, crop height, colour and texture were noted as before.

Fourth Inspection.—This inspection was conducted after harvest had commenced (that is, 12-15 weeks after field planting). To estimate systemic mould incidence, stems were cut on one side near ground level and the presence or absence of brown discolouration recorded. Eight lots of 25 consecutive plants were cut in each datum area. When complicating factors such as leaf-miner tunnelling contributed to a stem discolouration no results were recorded.

At this inspection notes were made on row direction and row and plant spacing.

(ii) 1959 Survey

For the 1959 season figures on average price per pound of leaf on sale and returns per acre were kindly supplied by the Tobacco Leaf Marketing Board. These are figures for the whole of the growers' leaf, which was made up in part only from the datum area.

A random selection of farms was made in the 1958 survey, but in 1959 blocks of farms were selected and considerable time was taken to locate and record preplanting sources of inoculum in order to study the spread of blue mould. As districts were studied as a whole in this year, individual plantings of different age were being inspected. The second field inspection was modified by omitting the severity rating. No stem cutting was carried out in 1959.

(b) Data from Experimental Plots, Parada

In the experimental work at Parada Tobacco Experiment Station, records of epidemiological observations were made by charting the block in question and recording at weekly intervals the position of all fresh blue mould infections. Infection dates were regarded for the purposes of this work as the dates on which infection was visible.

The figures representing intensities of infection are values for average percentage blue mould infection per leaf calculated for the datum plants in the randomized unsprayed plots in blue mould control experiments. In arriving at these average values each leaf was assigned a figure for percentage blue mould infection based on a visual estimate of the percentage of the lamina showing blue mould lesions. The expression total amount of blue mould/number of leaves then gave the average value in percentage per leaf. In order to obtain a truer estimate of the amount of damage, the figure employed for "number of leaves" was the average number of live leaves in the best spray treatment plots, and where the number of live leaves in the plot under consideration was less than this the leaves lost were recorded as being 100 per cent. mould-affected.

Stem mould infections were recorded by raising the bark at the base of a plant at ground level on both sides of the stalk in a line parallel to the row and recording the presence or absence of the characteristic discolouration. By sampling various portions of the block for stem mould incidence at successive dates and by referring to time of infection as shown on the block maps, it was possible to correlate stem mould incidence with date of infection.

III. SOURCES OF INOCULUM

(a) Standover and Volunteer Plants

In most of the North Queensland tobacco-growing districts a succession of tobacco plants exists from season to season. These plants comprise undestroyed crop residues, stem and root suckers from these, and seedlings which emerge following seed dissemination by wind or mechanical means. The perpetuation of the fungus on such material provides inoculum for seedbeds and field plantings, a fact which has been described fully by Hill and Angell (1933). The absence of standover and volunteer plants in some districts is reflected in the absence of mould in seedbeds and in the delay in its appearance in field crops.

Farm inspections in the Burdekin district prior to seedbed plantings showed sporing blue mould on volunteer plants on almost all farms. This district grows alternate crops which are irrigated and seedling growth within these crops is abundant. In the Dimbulah district volunteer plants showed on very few farms, resulting in an average distance from infection of over one mile at the time of preparation of seedbeds. In the Mary River district no infection could be found on volunteer and standover plants at the time of seedbed preparation; this district is situated over 20 miles from the nearest tobacco-growing area.

The spread of the pathogen from the stem of a tobacco plant into new axillary shoots has been described in southern Australia (Hill and Angell 1933; Mandryk 1960). Although in North Queensland axillary and root suckers commonly show blue mould infection, it is doubted that this infection arises from internal stem infections. In an isolated district of North Queensland, standover plants were inspected for blue mould infection. Although these plants had experienced severe mould in the previous season, and at the time of inspection axillary suckers were plentiful, no blue mould was recorded. These plants would have endured summer temperatures above 104° F maximum, which may have been responsible for the non-survival of the fungus (Lucas 1958).

(b) Nicotiana glauca

Nicotiana glauca has been known to be a host of *P. tabacina* for many years. In North Queensland it appears to be confined to the Burdekin River catchment area and is ecologically confined to the immediate area of the river bank. Preferring broken soil, it grows frequently on eroded embankments. Plant inspections in 1959 showed very few plants infected and all of these were on the eastern river bank and were subjected to morning shade. Stem infection was observed in plants severely affected. The short-term eradication of this host would be practical.

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(c) Oospores

In the United States of America Wolf (1939) collected oospores in necrotic leaves during each of seven seasons and stated that they may be presumed to occur wherever the pathogen exists. According to Wolf, McLean, and Dixon (1936) and McGrath and Miller (1958), primary blue mould infection of seedlings in North America is usually caused by the thick-walled overwintering oospores. These spores are not common in southern Australia (Adam 1925; Angell and Hill 1932). In North Queensland, microscopic examination on numerous occasions of necrotic leaf tissue and infected stems has failed to locate oospores.

A study of cropping histories on different lands and the time of appearance of blue mould indicates that the oospore stage of the fungus is of no importance in North Queensland.

(d) Seedborne Infection

Evidence of seed transmission of blue mould was supplied by Angell (1929) and Angell and Hill (1932).

A study of blue mould outbreaks in well isolated districts where the possibility of aerial infection is unlikely indicates that seedborne infection may be one source of blue mould in North Queensland.

Some such isolated outbreaks may be related to the use of particular seed stocks. One seedbed planting with over 20 miles isolation and no local source of inoculum comprised 10 seedbeds. Two-and-a-half of these were sown to one unsterilized seed stock while $7\frac{1}{2}$ were sown on the same day to another stock. Infection occurred in the first $2\frac{1}{2}$ seedbeds, while none occurred in the remaining beds. The farm had not grown tobacco or seedlings before and all seedbeds were similarly treated and planted to the same variety.

The importance of seedborne inoculum is difficult to assess in the field. Many growers begin benzol fumigation soon after seedling emergence and any infection which might show is corrected and passes unobserved. During the 1959 season 50 per cent. of North Queensland's tobacco was grown from seed saved by individual growers and their friends. Much of this seed was not treated with silver nitrate.

(e) Seedbeds

Since the discovery of the efficacy of benzol as a seedbed fumigant, seedbed mould has become less of a problem. Correct seedbed management using the recommended schedule of 1 sq. in. of evaporating surface to 1 sq. ft. of airtight seedbed cover applied every third night has in all cases achieved effective control of mould. There is therefore now no essential reason for seedbed mould to be a source of field infection.

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In the 1958 season a survey of benzol fumigation on 138 farms showed that 28 per cent. of the farmers were using ineffective schedules. However, only 9 per cent. of the growers experienced seedbed blue mould. In the 1959 season, on 131 farms inspected 30 per cent. of the seedbeds were being ineffectively fumigated with benzol; only 13 per cent. of them experienced seedbed blue mould. A study of the sources of inoculum and the isolation distances involved shows that the discrepancy between the number of farms prone to seedbed infection and the number actually infected is due to escape from infection.

(f) Masked Infection of Seedlings

Angell (1957) stressed the importance of what he termed "masked infection" in seedlings when, due to the use of inadequate benzol and dry conditions, blue mould can infect the seedlings but the usual symptoms, observable on plants in the open, are masked. Following transplanting blue mould immediately appears on the developing leaves of infected seedlings.

Mandryk (1957) investigated the effect of different concentrations of benzol vapour applied under glass-covered iron trays at various intervals after inoculation with *Peronospora tabacina*. Among other things he found that higher concentrations of benzol (20 ml and 30 ml per 115,943 c.c.) applied in later stages of incubation (3-4 days after inoculation) usually did not eliminate the pathogen in all plants and resulted in masking of the disease.

The standard Departmental recommendation for North Queensland of $\frac{1}{2}$ pt (284 ml) of benzol apportioned between two containers each 5 in. x 4 in. for each 10 ft x 4 ft x 1 ft iron seedbed cover gives a concentration approximately equivalent to that obtained by Mandryk with his 30 ml dosage.

It has been consistently demonstrated in Departmental experiments and by successful growers that this dosage, when properly applied every third night, prevents the development of sporing blue mould in seedbeds. Due to this fact and to the phytotoxic effects of benzol when applied under vapour-tight covers even when treatment every third night is practised, more frequent treatment has not been considered desirable. During the 1958-1959 surveys, field inspections three weeks after planting, when presumably any masked infection would have shown up, disclosed no evidence of this occurring.

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In order to investigate the possibility of masked infection following the standard seedbed recommendations, a series of three seedbed experiments, in which the frequency of benzol application was varied from the normal, was carried out at Parada Tobacco Experiment Station during the 1957-1959 seasons.

In the three trials the treatments were applied to small beds 4 ft x 2 ft under iron covers 4 ft x 2 ft x 1 ft; 80 ml benzol was exposed under each cover in an evaporating tray 4 in. x 2 in. x 1 in. supported above the seedlings in a central position. Two treatments were employed in each experiment. These were replicated five times in a randomized block layout. Covers were applied before 4.30 p.m. and removed at 7.30 a.m. and benzol residues were measured each morning. About two weeks before transplanting and on a day following a night on which both treatments coincided, the seedlings were watered with a heavy suspension of conidia of *P. tabacina*.

At transplanting time seedlings were pulled at random from each plot. In the field the treatments were further randomized and three field plots, one in each of three blocks, were planted from each seedbed plot to give a 10×3 layout.

Results were assessed by weekly counts of mould-infected plants and by examining each plant for stem mould infection 9-11 weeks after planting out and prior to commencement of harvest.

The following treatments were applied:—1957: benzol 1 sq. in./1 sq. ft. every night and every third night; 1958 and 1959: benzol 1 sq. in./1 sq. ft. every second and every third night.

Experiment 1 (1957) was abandoned because of the pronounced phytotoxicity of the nightly benzol treatments. Many of the seedlings receiving this treatment were killed. Growth of the remainder was retarded so much that none was suitable for planting out. Seedlings treated every second night were in both 1958 and 1959 less thrifty (more stunted and lighter in colour) than those receiving treatment every third night. The size difference persisted for the first 3 or 4 weeks in the field but by harvest time no appreciable differences could be noted.

Results of the 1958 and 1959 experiments are presented in Table 1. There is no indication that masked infection had occurred in the beds receiving less frequent treatment.

Table 1

Leaf Mould and Stem Mould Development in Plants Which had Received Benzol Treatment Every Second and Every Third Night

		After	5 Weeks	Percentage	Percentage	
Year	Treatment	Total No. of Plants	No. of Infected Plants	Infected Plants After 8 Weeks	Stem Mould Prior to Harvest	
1958	2nd night	354	110	88	8	
	3rd night	359	76	90	5	
1959	2nd night	395	1	100	50	
	3rd night	404	1	100	44	

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In 1958 minimum temperatures ranged from $43^{\circ}F$ to $62^{\circ}F$ during the course of the experiment. Volume concentrations (calculated on the basis of a 10 ft x 4 ft bed) ranged from 165 to 330 ml, with a mean for the whole experiment of 235 ml. In 1959 the minimum temperature range was $31^{\circ}F$ to $64^{\circ}F$, and volume concentration 175 to 320 ml with a mean of 220 ml.

IV. DISTRIBUTION OF INOCULUM

(a) Means of Distribution

The shedding of conidia from the conidiophores can apparently be brought about either by a reduction in relative humidity or by mechanical shock. Pinckard (1942) found that conidia were produced but not released in a saturated atmosphere. Mature spores were shed when the atmosphere subsequently became Cruickshank (1958) concluded that either change in humidity or drier. mechanical shock may be involved in conidial discharge, that each may act independently of the other and that their effect increases with the magnitude of the change in relative humidity or the size of the shock. Waggoner and Taylor (1958) investigated the dispersal of spores from blue mould lesions and found that evidently the maximum number of spores are dispersed early in the morning and few or no spores are airborne during the afternoon or night. They estimated that a blue mould lesion produced about 2 x 10⁵ spores per day; however, only 1 spore in $4 \ge 10^3$ produced a lesion even at close range. Despite this dilution factor and the apparent very limited viability of the conidia in the absence of water and the presence of sunlight (Lucas 1958; McGrath and Miller 1958), the disease is wind-borne for considerable distances in North America. For example, Stover and Koch (1951) attributed widespread outbreaks of the disease in Ontario, Canada, to wind-borne conidia from Kentucky or Ohio, over 125 miles to the south-west.

In addition to airborne spread, other means of transport have to be considered. For instance, the moths of the tobacco leaf miner, *Gnorimoschema operculella* Zell., have been demonstrated to be vectors of the disease (Angell, Hill, and Currie 1930). It seems possible, therefore, that other insects may carry the conidia on their bodies. In the course of the surveys in North Queensland in 1958 and 1959, instances of what appeared to be infection due to the carriage of spores on the person were noted. In such cases the first blue mould spots in a tobacco field appeared near points of entry to the planting. Undoubtedly the movement of persons from one crop to another and one district to another aids the distribution of blue mould inoculum.

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The distribution of infected seedlings is a very important means of mechanical spread of blue mould. In this way inoculum may be transported many miles in a very short time. Mould-affected seedlings are generally easily available for distribution among growers who are short of planting material, whereas a reliable source of mould-free seedlings is usually more difficult to obtain.

The inevitable result of planting infected seedlings is severe economic loss from stem mould and such plantings are frequently abandoned at an early age. A far more widespread and more important aspect is the establishment early in the season of a concentrated source of inoculum.

Angell (1957) drew attention to the relation of blue mould infected seedbeds to losses from field mould on farms in the Mareeba-Dimbulah district. He quoted a mean percentage crop loss of 6.86 on farms where healthy seedlings were used, compared with $25 \cdot 3$ per cent. in crops which had been planted with diseased seedlings.

(b) Characteristics of Airborne Spread

Although mechanical spread may be important in establishing new infections early in the season, airborne dispersal of inoculum is by far the most important means of inoculum distribution.

Survey work has shown the presence of inoculum prior to planting at an average distance of one mile from most seedbed or field sites, but by no means must a uniform distribution of inoculum be presumed to exist. Early in the season, outbreaks of blue mould most often occur close to sources of inoculum and early outbreaks are predictable following a study of the location of these sources.

When plantings are made from infected seedbeds or when later in the season groups of farms become infected, the inoculum concentration is increased many thousands of times and a general and uniform airborne distribution of spores may occur. In the 1959 season late in September such a fall-out of spores occurred and enveloped all farms to the west of Mareeba, some of these 14 miles from the source of inoculum, which was in this case 100 or more infected farms in the Mareeba district itself (Figures 2–4).

Spore survival of the fungus is considered a very important factor in airborne infection. Survey work showed that when dispersion of spores takes place at a time when conditions favour spore viability, all farms exposed to infection become infected. The amount of infection is roughly proportional to the area of leaf exposed, indicating that cultural practices and varietal characteristics lend little or nothing towards plant resistance.

There is the suggestion that a continual fall-out of viable spores does not exist; rather a fall-out of viable spores produces infection and then no further infection takes place until a spread of blue mould occurs from the first points of infection.

As initial infections by air are usually slight, it is uncommon for mould to appear before the three weeks' stage when healthy planting material is used.

(c) Spread Within a Field

Dispersal of airborne spores under conditions favouring spore viability results in a scattered field infection. The initial spots of blue mould are few and frequently overlooked by a grower.

The appearance of 100 mould spots per acre as a result of a fall-out of spores during weather conditions conducive to spore survival is high and indicates that a source of inoculum is close. A more common distribution would be 15 spots per acre.

After initial infection has taken place, the rate of spread of the disease throughout a crop depends largely on weather conditions. A normal increase of mould would result in the loss of only one or two of the lower leaves three weeks after the recording of the first mould spots in the field. After this time the build-up is likely to be much more rapid. Where infection occurs late in the life of a crop and shaded conditions prevail within that crop, severe economic loss has been noted in two weeks.

Mechanical transference of inoculum, and airborne spread providing isolated foci of infection, have provided opportunities to study the airborne spread of blue mould from point sources of infection. The rate of spread from such sources has been recorded in three instances. Successive spore generations could be observed as the fungus spread in roughly circular stages away from the source. The damage at the source of infection was in each case much more than at the perimeter and the result a "hole in the crop." The rate of spread of the fungus is sufficiently slow for this effect to be conspicuous.

In all three cases, within the first three weeks mould spots appeared only as far as 300 yd from the focus of infection. In the following week a spread of one mile was recorded in the one district where contiguous farms enabled observations to be made. This indicates that the concentration of inoculum at a source must be considered to fully understand the field rate of spread of blue mould within a district.

In Figure 1 is shown the rate of increase of blue mould in one field sampled in 1959. On each of 17 days from September 3 to 25 at least 400 plants were examined in random counts of 50 plants and the presence of blue mould noted. No leaves were removed and first infections were noted soon after the plants were three weeks in the ground.





From this graph may be seen the low initial infection leading to a sudden field build-up in three weeks.

This rate of increase is considered representative of most North Queensland farms. The weather conditions corresponding to the above period were cool and cloudy in early September with rain recorded on September 1, 2, 5, 10 and 12. Total precipitation was 0.17 in.

(d) Rate of Spread within a District

As mentioned earlier, local outbreaks of blue mould early in the season occur close to sources of infection. When discussing the rate of build-up on an individual farm it was noted that district rate of spread is dependent to some extent on the concentration of inoculum at a source. It is not unusual to find a build-up of mould taking place on a single farm or on a number of farms without spread from these areas. Spore survival is considered the most important factor in rate of spread of blue mould throughout a district. As the distance of spore travel will affect spore viability, distances between farms within a district and between districts will determine the district rate of spread.

The series of maps in Figures 2-5 shows the progress of the disease throughout the Mareeba-Dimbulah districts in 1959. Although a few minor sources of infection such as groups of volunteer plants occurred, the uniformity of distribution of infection within fields throughout the Dimbulah district leaves no doubt that the source was the heavily infected Mareeba district.

Figure 2 shows the geographical distribution of blue mould in field plantings on August 10. This early mould was due to transplanting mould-affected seedlings.

Figure 3* shows the distribution on September 10. A local spread of blue mould has occurred embracing farms up to four miles distant from the original source of infection. When compared with Figure 4 it can be seen that outlying districts escaped early infection due to their isolation. However, once a large source of inoculum was built up at Mareeba, aerial infection served to distribute inoculum to all outlying districts.

Figure 4 shows the geographical distribution of blue mould in field plantings on October 10. By the end of October mould had increased in farms in the outlying districts to cause economic losses.

Figure 5 shows the geographical distribution of lodging from stem mould. Data for this map were collected in late November. From comparison with Figure 3 it is noted that stem mould lodging has occurred only where plantings experienced early and severe mould due to close proximity to initial sources of infection.

Many tobacco seedbeds and some field plantings escape blue mould infection. It has been determined that an isolation distance of one mile from a small source of inoculum (e.g. a group of volunteer plants) gives a splendid chance of seedbed escape from infection.

Numerous growers far removed from a source of infection do not use benzol fumigation and seldom experience blue mould attack.

^{*}A mould outbreak occurred at Dimbulah in a field planting made from infected seedbeds. A local spread was not recorded from this planting as all farms in the area west of Mareeba experienced a fall-out of spores from the Mareeba district.



Fig. 2.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on August 10.



Fig 3.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on September 10.



Fig. 4.—Airborne distribution of blue mould, Mareeba-Dimbulah district, 1959. Distribution on October 10.



Fig. 5.—Airborne distribution of blue mould, Mareeba–Dimbulah district, 1959. Distribution of lodging from stem mould in late November.

Escape from infection due to isolation becomes increasingly obvious after field plantings have been made. While inoculum concentrations are low, those farms closest to sources of inoculum are first infected, and those some miles removed remain healthy.

Although the evidence suggests that a large number of farms could dispense with benzol fumigation of seedbeds and rely on escape from infection, one would be reluctant to advocate such a practice, as small sources of inoculum in the form of volunteer plants are often present on a farm although undetected by the grower.

V. RELATIONSHIP BETWEEN STEM MOULD AND LEAF MOULD

During the 1958 season, 30,000 stems were cut on 150 farms and records made of the presence or absence of the characteristic brown discolouration. No stem discolouration was recorded where crops had experienced no leaf mould. The number of plants showing stem mould in a planting was found to be correlated with the severity of mould in the crop at an earlier age. In Figure 6 is shown the correlation between mould severity at six weeks of age and the percentage of plants exhibiting stem infection later. All ratings were made on the leaf worst affected and according to the method described on page 3.

In Table 2 the amount of stem mould is related to time of first infection The number of farms on which observations are based is given in brackets. As mould severity at six weeks is dependent on time of infection, the data in the table are in conformity with Figure 6.

	Infection before Three Weeks	Infection after Three Weeks
Percentage internal stem discolouration, 1958	23.5 (49)	9.4 (81)
Percentage stem lodging, 1959	15.1 (21)	0.9 (72)

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Table	4

Relation of Stem Mould Damage to Early and Late Infection

The number of farms on which the observations are based is shown in brackets.

It is apparent that almost a direct relationship exists between the severity of leaf mould at an early age and the amount of stem mould at maturity. The severity of mould at an early age depends largely on the distance of the crop from a source of infection. The avoidance of leaf mould attack at an early stage of plant development by eliminating nearby sources of infection is recommended as a practical field control for stem mould.

The relation of stem mould to date of infection is brought out in data taken from field mapping at the Parada Tobacco Experiment Station during the 1958 and 1959 seasons.



Fig. 6.—Correlation of stem infection with leaf mould severity at six weeks.

In 1958 an analysis was made of stem mould incidence in a block of over 3,500 plants, planted on September 11. The incidence of leaf mould was mapped in this block at weekly intervals from October 6, when the first diseased plants were counted, until October 27, when all plants were infected.

Stem mould incidence was determined in a series of six examinations commencing on November 14. At each inspection date the plants in portion of the block were rated as positive or negative. By December 22 all plants had been examined. The results are detailed in Table 3.

Similar data are presented in Table 4 for a block of about 1,850 plants surveyed in 1959. In this case the date of planting was September 17. Two inspections for stem mould were made, one half of the block being rated on each of the dates of November 21 and December 2.

Allowing for some uneven distribution of stem mould throughout the datum blocks, there was a tendency in both years for percentage stem mould to increase as the interval between date of infection and date of inspection lengthened. From Table 3 it will be noted that 43 days after initial field infection stem cutting revealed only 12.6 per cent. stem mould infection. It is considered from general observations that stem mould will be of little significance until a minimum of five weeks has elapsed since leaf mould first appeared.

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Date of Inspection for Stem Mould	14-x	14-xi-58		18xi58		28-xi-58		5-xii-58		12-xii-58		22-xii-58	
Date of Infection	No. of Plants Leaf Mould	Stem Mould (%)											
6-x-58 (26 days from planting)	36	8.3	95	12.6	96	22-9	82	25.6	35	37.1	109	28.4	
13-x-58 (33 days from planting)	170	11.7	288	11.1	278	19-7	322	20.4	149	42-9	273	42-4	
20-x-58 (40 days from planting)	104	10.5	172	14·2	198	28.7	161	27.9	96	54·1	174	42.5	
27–x–58 (47 days from planting)	51	15.6	145	8.9	135	28.8	153	25.4	72	38.7	127	32.2	
Average		11.6		11.6		24.5		23.8	•••	44·6	•••	38.4	

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ODSERVATIONS	UN THE	RELATIONSHIP	DET WEEN	I EKCENTAGE	DIEM	MOULD	AND	TIME OF	DAMPLING,	I AKADA,	1950

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EPIDEMIOLOGICAL STUDIES OF BLUE MOULD

Table 4

Date of Inspection for Stem Mould	21x	i–59	2-xii-59			
Date of Infection	No. of Plants Leaf Mould	Stem Mould (%)	No. of Plants Leaf Mould	Stem Mould (%)		
26–x–59 (39 days after planting)	93	31	103	29		
2-xi-59 (46 days after planting)	555	28	423	46		
9-xi-59 (53 days after planting)	360	41	314	55		
Average		32.9		47·4		

Observations on the Relationship between Percentage Stem Mould and Time of Sampling, Parada, 1959

There was little obvious correlation between date of infection and final percentage stem mould in these observations. This is to be expected in the light of the low intensity of infection characteristic of early infections.

Since the incidence of stem mould is proportional to intensity of leaf infection, it is to be expected that the factors which influence intensity of leaf infection affect stem mould development also. In the surveys during the 1958 and 1959 seasons this relationship was verified for row direction.

VI. CLIMATIC FACTORS AFFECTING THE INCIDENCE OF BLUE MOULD IN THE FIELD

In the United States of America the optimum temperature for the production of conidia of *P. tabacina* is evidently around 60° F. Clayton and Gaines (1933) determined the optimum at $60-62^{\circ}$ F. They later found (Clayton and Gaines 1945) that alternating high and low temperatures (77° diurnal and 60° nocturnal) were more favourable for sporulation than a constant temperature of 60° F. However, the effect of temperature on sporulation was modified by changes in the physiology of the host because exposure to prolonged periods of low light intensity enabled sporulation to take place at temperatures above 80° F. Dixon, McLean, and Wolf (1936) considered the optimum for sporulation was somewhat lower, namely 56° F. Wolf (1957) stated that germination does not take place except in a film of water or in a saturated atmosphere. Dew provides sufficient moisture. In America, periods of cloudy, rainy weather are regarded as blue mould weather and temperatures above 85° F are definitely inhibitory to the fungus. Outbreaks of the disease are arrested by the advent of several bright days with temperatures in excess of 85° F (Wolf 1957). In Australia, Cruickshank (1958) has investigated the critical relationship between sporulation intensity and diffusion pressure deficit of the host tissue and relative humidity of the air in the immediate vicinity of the abaxial leaf surface. Threshold values of D.P.D. and R.H. for maximum sporulation were 3–4 atmospheres and 97 per cent., respectively. The minimum period of exposure to optimal conditions necessary to give rise to maximum sporulation was found to be three hours.

2

In North Queensland, blue mould build-up takes place in the field in the absence of rainfall. During September and October long dry periods characterized by hot sunny days and cool nights are common. Maximum temperatures are likely to be well in excess of 85° F, but minimum temperatures seldom are higher than 65° F. Heavy dews are common. The rapid development of field mould under such conditions is illustrated in Figure 7, which shows the progressive increase in percentage infected plants in a block of irrigated tobacco at Parada Tobacco Experiment Station during October 1958. The seedlings were transplanted on September 10 and the first infections were mapped on October 6; the block was 100 per cent. infected on October 27. Also on the graph are maximum daily temperatures and mean nocturnal temperatures (2-hourly readings from 10 p.m. to 6 a.m.). The duration of any dews which occurred during this period was noted with a 7-day dew recorder (Theis and Calpouzos 1957). Only 0.02 in. of rain fell during the 7 weeks' period to October 27.



Fig. 7.-Blue mould development during the absence of rain, Parada, 1958.

Epiphytotics of the disease are, however, dependent on rainfall for their development. It is unusual if rains do not occur at some time during the growing period of the North Queensland crop and any precipitation of sufficient magnitude to be accompanied by high humidities, depressed daytime temperatures and reduced light intensity can give rise to a sudden increase in intensity of infection. Isolated storms may lead to a local intensification of the disease but a succession of storms within the one district or several days of showery weather within one week are more important contributing factors.

Experience at Parada Tobacco Experiment Station has shown that the amount of damage in fields exposed to "blue mould" weather is, nevertheless, variable and depends to a large extent on the amount of early infection. This is due to the operation of the acquired resistance effect (Pont 1959).

The main weather influences prevailing during a period which proved ideal for blue mould development in an out-of-season crop are illustrated in Figure 8. The crop in question was a mixed block of blue mould susceptible and resistant







Fig. 9.—Weather conditions associated with an epiphytotic of blue mould during the normal growing season, Parada, 1957. Intensity of infection on November 18 was 40 per cent. (Compare Fig. 10.)

lines planted at Parada Tobacco Experiment Station on March 16, 1960. Blue mould was absent until May 9, when flowering had commenced, but on this date a scattered infection resulting from limited airborne inoculum was noted along the windward side of the block. Following 1.10 in. of rain during the period May 13–18, the infection spread rapidly, and by May 25 all susceptible plants were infected, with an intensity of infection ranging as high as 50 per cent.

The weather conditions associated with an epiphytotic during the normal growing season are given in Figure 9. Here the incidence of the disease in the unsprayed plots in a blue mould spray trial at Parada Tobacco Experiment Station in the 1957 season is depicted. As a comparison, the data associated with a non-epiphytotic year at the same station are presented in Figure 10. The weather data plotted are daily maximum and minimum temperatures and rainfall.

In 1957 the planting date was September 10. The first blue mould was noted on October 7. All plants were infected by November 11. The intensity of infection on November 18 was 40 per cent. It should be pointed out here



Fig. 10.—Weather conditions associated with blue mould development in a non-epiphytotic season, Parada, 1958. Intensity of infection on November 18 was 11 per cent. (Compare Fig. 9.)

that, due to their acquired resistance, plants which were infected early escaped serious damage in the November epiphytotic. The mean figure for intensity of infection has, therefore, been calculated from late infections only.

In 1958 the block was planted on September 11. Blue mould appeared by October 3 and all the unsprayed plants were diseased on October 31. The intensity of infection on November 17 was 11 per cent.

It can be seen that although the rate of spread from plant to plant is much the same in each year the intensity of infection is much greater in the year of high rainfall.

In Figure 11 weather conditions during the growing seasons of 1956 and 1958 in the Burdekin district of North Queensland are compared. The 1956 season was a wet one in this area with well distributed rainfall throughout the entire growing season of the main crop. Losses from leaf mould were heavy and the lodging of stalks weakened by systemic infection reached serious proportions.



Fig. 11.—Comparison of weather conditions in a year of heavy (1956) and light (1958) losses from blue mould in the Burdekin district.

In 1958 dry weather prevailed during the growing season of the main crop. Blue mould was widespread but never severe. Systemic mould was responsible for little obvious loss.

VII. FACTORS OTHER THAN CLIMATE INFLUENCING INTENSITY OF INFECTION

It has been noted following a general airborne spore distribution such as illustrated in Figures 2-5 that the amount of initial infection per field is dependent on the leaf area exposed. That some of these plantings were young, some old, some watered and some dry, each on land with different cropping histories and frequently different fertilizers, demonstrates that plant predisposition for infection is little influenced by cultural practices.

However, once initial infection is established the intensity of subsequent infection can be influenced by several factors which will now be considered.

(a) Row Direction

The effect of row direction is very pronounced. An east-west planting favours the disease, probably due to the shading of one plant by its neighbour. The effect of morning shade was noted also when *Nicotiana glauca* plants were being inspected for blue mould infection. The plants on the eastern river bank showed infection, while those on the western bank showed no infection.

Table 5 shows an analysis of percentage leaf loss for each row direction. Recordings from different districts are listed separately, as are the years when observations were made.

			East-West		North-South					
	-	No. of Farms	Average Loss (%)	Range of Loss (%)	No. of Farms	Average Loss (%)	Range of Loss (%)			
1958			-							
Mareeba-Dimbulah		37	10.5	0-30.6	30	7.6	0-19.4			
Burdekin		26	13.0	1.7-39.6	3	7.2	6.7- 7.4			
Total	·	63	11.7	0-39.6	33	7.4	0–19·4			
1959	-		-							
Mareeba-Dimbulah		20	13.25	0-40	27	8.9	1-21			
Burdekin	•••	28	12.5	0–30	6	11.6	5–28			
Total		48	12.8	0-40	33	9.4	0-28			

Table 5

EFFECT OF ROW DIRECTION ON DAMAGE BY LEAF MOULD

An analysis of field notes reveals that there is also a difference in stem mould due to row direction. This is related to the difference in leaf mould severity at an earlier stage of growth. During the 1958 season, recording of discolouration in cut stems was taken as an indication of the presence of stem mould. The results in relation to row direction are shown in Table 6.

		Table (5	
CORRELATION O	F S	TEM INFECTIO 1958 Seas	ON WITH ROW	v Direction,
Row Direction		No. of Farms	Average Percentage of Plants with Stem Infection	Range (%)
East-West		56	19.9	0-83.5
North-South	••	36	12.8	0–68

(b) Row Width and Plant Spacing

A survey of 150 farms in 1958 indicated that leaf mould was less where plant spacings and row widths were greatest. These results were not supported in the 1959 survey. Row spacing varied from 3 ft 6 in. to 4 ft 6 in. and plant spacing from 18 in. to 27 in.

(c) Water Usage

The application of irrigation water early in the life of the crop was found in the 1958 and 1959 seasons to have little effect on leaf mould severity. Table 7 shows an analysis of percentage leaf loss at harvest time for plantings subjected to various water applications during the first six weeks after transplanting. The number of farms inspected for each group is included in brackets.

Table 7

Average Per of Wat	CENTAGE LEAF L ER APPLICATION W	OSS FOR DIFFERE N DURING THE VEEKS	ENT FREQUENCIES First Six
Year	Planting Water Only	Planting Water plus Water after 4 weeks	Water from Planting Onwards
1958 1959	10·7 (37) 10·6 (72)	10·7 (63) 9·0 (70)	7·6 (13) 7·6 (15)
Average	10.6 (109)	9.8 (133)	7.6 (28)

The number of farms inspected for each group is given in brackets.

The effect of early irrigation practice on the subsequent expression of stem mould was also investigated. Table 8 shows the percentage of plants affected with stem mould for the three irrigation groupings. The data for 1958 were obtained by stem cutting, while those for 1959 represent lodging. There is a tendency for a reduction in stem mould damage with increase in water.

Table 8

AVERAGE PERCENTAGE STEM MOULD AND LODGING IN RELATION TO FREQUENCY OF IRRIGATION

Year	Planting Water Only	Planting Water plus Water after 4 weeks	Water from Planting Onwards
1958	16.3 (66)	15.6 (59)	10.6 (15)
1959	8·0 (38)	3.8 (63)	0.1 (14)

The number of farms inspected for each group is given in brackets.

In 1958 a replicated irrigation experiment was carried out at the Parada Tobacco Experiment Station comparing normal watering, light watering and withholding water for the first six weeks. The results were statistically inconclusive.

To sum up, there is as yet no definite evidence that withholding water alleviates the leaf mould position. The average time taken from transplanting until harvesting begins is 10 weeks. This time varies with individual farmers and may be reduced to eight weeks or extended to 14 weeks, depending on watering procedure and cultural methods. It is considered that the extension of the growing period by withholding water contributes to stem mould losses.

(d) Fungicides

During the 1959 season close attention was paid to farms which used fungicides on a field scale. Zineb was the fungicide most widely used. Where growers sprayed before the appearance of blue mould, i.e. before the initial infections took place, a practical field control was obtained. In Table 9 is a comparison of zineb sprayed with unsprayed showing percentage leaf loss at harvest. All of these farms were located on the Burdekin irrigation area.

Table 9

PERCENTAGE LEAF LOSS AT HARVEST OF SPRAYED AND UNSPRAYED CROPS, BURDEKIN, 1959

Percentage Leaf Loss	0	1	2	3	4	5	6	7	10	11	12	15	16	17	19	20	26	27	29	30
Unsprayed	1		1	1		6		4	3	1	1	3	3	2	1	1	1	1	1	1
Sprayed	24	1	1	2					1			• ·				••			••	

Figures represent number of farms in each category.

The average leaf loss of 32 farms unsprayed was 12 per cent. and that of 29 farms sprayed was 0.7 per cent.

An analysis of spray concentrations and frequency of application shows that for those farms using zineb an average of $1 \cdot 2$ lb. of zineb in 36 gal of water was applied per acre every 4–5 days.

In the Mareeba-Dimbulah district only a few growers used zineb on a field scale. Seven growers who applied zineb had an average leaf loss of 4 per cent., while 52 who did not spray suffered an average loss of $9 \cdot 8$ per cent.

The success of zineb is attributed to the preventive effect of the spray. Earlier in this paper it was shown that initial blue mould infections occur through the establishment of a few isolated spots within a field and subsequent field build-up takes place from these points. Zineb spraying prior to infection seems quite capable of controlling the disease with this low concentration of inoculum.

Analyses of mould loss, zineb usage and leaf values have been compiled for the 1959 season for the Mareeba and Burdekin districts. From Table 10 it may be seen that zineb spraying does not reduce the value of the leaf. Zineb treated leaf is apparently acceptable to the manufacturers and presumably poses no taint problem. It is of interest to note also from this table that leaf value is altered very little by mould damage.

No. of Farms	Average Leaf Loss (%)	Zineb Usage	Average Price of cured leaf (pence per lb)		
Mareeba-					
7	4.0	Yes	150.5		
16	4.0	No	146.9		
17	8.5	No	141.7		
16	17· 0	No	141.0		
Burdekin-					
24	0.7	Yes	125.3		
26	14.0	No	124.7		
			1		

Table	10
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Analysis of Mould Loss and Leaf Values With and Without Zineb Spray, 1959

Because of its success in the 1959 season, zineb will be more widely used by tobacco growers in future, particularly by those in the Burdekin districts. However, it has been found (Pont 1959) that, under conditions of high inoculum potential, such as were experienced when wet weather led to a sudden intensification of the disease in a block of plants in which it had previously been confined mainly to the unsprayed plots, the protection afforded by regular zineb treatments was lost. Under these conditions a massive infection developed in plots which had been sprayed with this fungicide, and due to the resistance which unsprayed plants had acquired by virtue of their early infection, the leaf loss on the zineb-sprayed plants was greater than that on the untreated ones.

For this reason the known synergism of mixtures of salicylate and other organic fungicides (Graham *et al.* 1947) has been utilized in experimental work to augment the efficiency of zineb in an attempt to find a mixture which can be recommended without reservation to tobacco growers.

Mixtures containing zineb and methyl salicylate and zineb and benzyl salicylate which were found to be particularly effective in screening trials (Pont 1959) were used in field trials in 1958 and 1959 respectively at the Parada Tobacco Experiment Station. However, the late epiphytotics characteristic of the 1956 and 1957 seasons at this Station were not duplicated in these later years. The question of the relative efficiency of zineb alone and of these mixtures under field conditions extremely favourable to disease development has, therefore, not yet been answered.

VIII. SOME ASPECTS OF CONTROL

The foregoing discussion brings up certain aspects of the control programme which are considered of primary importance. These are briefly referred to here.

(a) Elimination of Sources of Inoculum

In North Queensland the important source of inoculum is the carryover of blue mould on the vast number of volunteer plants originating from seed shed from seed-heads on untopped plants or suckers. These seedlings are more of a problem when fields which have grown tobacco are fallowed or are sown to irrigated truck crops or other field crops such as cotton and peanuts. They seem to be less troublesome when the fields are not cultivated and grasses and weeds take over. Standover plants appear to play a minor role in the survival of the fungus, but nevertheless have to be considered.

Legislation was introduced in August 1959 in the form of an Amendment to the Regulations under the *Tobacco Industry Protection Act of* 1933, which gives Inspectors under the Act authority to order the destruction in a prescribed fashion of the following classes of plants:—

(1) Tobacco plants on an area of land on which harvesting has been completed, whether they be from the planted crop or regrowth from the planted crop or volunteers.

(2) Tobacco plants remaining in areas laid down as seedbeds and no longer required for transplanting into the field or not in a fit condition because of age or disease to be used for such a purpose.

(3) Tobacco plants growing outside an area or areas specifically planted for the production of tobacco seedlings or tobacco leaf.

The destruction of these sources of infection is incumbent on every tobacco grower. Individual efforts at eradication by conscientious growers delay the appearance of the disease but are of little eventual benefit unless all growers co-operate at the same time.

In the Burdekin districts the possibility of eradicating *Nicotiana glauca*, which appears to be ecologically confined to the levee soils, should be considered.

(b) Standardized Planting Dates

The legislation mentioned can be rendered ineffectual by the presence of out-of-season standing crops affected by mould. Off-season planting, that is, field planting in the autumn, is undesirable, particularly along the Burdekin River where tobacco farms are contiguous, since it provides large populations of overwintering plants on which, due to low temperatures and periods of drizzly rain, blue mould incidence is likely to be exceedingly high.

1

This trouble could be avoided by enforcing a standardized date for first planting. The eradication legislation could be strictly enforced for two months prior to this date to ensure a break in the life cycle of the fungus.

Strict enforcement of the existing legislation in conjunction with the standardization of planting dates would go a long way towards providing an escape from the disease.

(c) Use of Healthy Seedlings

The transplanting of mould-infected seedlings almost invariably leads to crop failure but more importantly establishes a concentrated source of inoculum early in the season. The use of the benzol vapour treatment as recommended will enable the planting of healthy seedlings. However, due to various causes, there is a shortage of seedlings on some farms in most years and there is usually an interchange of planting material when growers are forced to seek extra plants to make up deficiencies. The interchanged seedlings usually come from beds on which benzol treatment has been terminated and are very often infected. A certified source from which a grower could draw healthy seedlings as required would obviate indiscriminate traffic and planting of infected material.

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