

FIELD AND LABORATORY STUDIES ON
TWO TOBACCO LEAF-SPOT DISEASES.

by

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ACKNOWLEDGMENTS.

INTRODUCTION.

The tobacco crop in Southern Rhodesia is subject to attack by two fungus diseases which cause more loss of leaf every year than any other factor, namely brown or Alternaria spot (Alternaria longipes Ell. & Ev. Mason) and frog-eye (Cercospora nicotianae Ell. & Ev.). The former disease, although appearing in a milder form on the sand veld, is very destructive on the heavier types of soil suggesting that the trouble is at least in part nutritional. The latter disease is widespread throughout the colony and is, on the basis of acreage affected, the cause of a greater loss of leaf than the former.

Work at the Tobacco Research Station, Trelawney during the last two seasons has been largely directed towards a more complete understanding of these diseases. In the case of brown spot, particular attention has been paid to the nutritional aspect and the way in which the fungus persists from year to year, while the frog-eye problem has been tackled more directly by the study of positive control measures.

Practical considerations have been the primary object of the work but the problems have also been approached from an academic viewpoint and the fundamental aspects of the diseases have also been studied.

The work on Alternaria longipes was started originally while the writer was at the Imperial College of Science & Technology, London. Certain portions of this work have

been repeated here in modified form under the more natural conditions of the Rhodesian climate, but the scope of the study has been generally extended along new lines.

Each disease has been given separate treatment for although both are caused by air-borne fungi the methods of control under investigation differ widely.

PART I.

BROWN SPOT CAUSED BY ALTERNARIA LONGIPES (Ell. & Ev.) Mason.

PART I.

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I. HISTORICAL SURVEY.

Brown spot of tobacco was first recorded from N. Carolina, U.S.A. in 1892 by Ellis & Everhart (6) and was assigned to Macrosporium longipes n. sp. Since 1892, various reports have been made from several other tobacco-growing states in the U.S.A.

In 1924, a brown spot disease became conspicuous on tobacco in Florida. Tisdale (19) isolated the fungus in 1925 and found it to be an Alternaria. Tisdale & Wadkins (19) considered that the difference between their isolations and those of Ellis & Everhart did not warrant the creation of a new species, but since the spores were produced in chains in their cultures, they proposed a new combination - Alternaria longipes (Ell. & Ev.). Proof of the pathogenicity of the fungus was established by Tisdale & Wadkins in 1927.

Hopkins (9) reported the disease in Rhodesia in 1931 under the name of Alternaria leaf-spot (A. tabacina (Ell. & Ev.) Hori). In 1939, however, having examined papers by Tisdale & Wadkins and Gulyás (8), Hopkins (10) came to the conclusion that the fungus concerned in Rhodesia was identical with A. longipes and A. tabacinum of Tisdale & Wadkins and Gulyás respectively. Since Mason (14) in 1928 had already set up the binomial A. longipes (Ell. & Ev.) Mason, Hopkins decided to adopt this name for the Rhodesian fungus.

Tisdale & Wadkins (19) carried out the first detailed observations on the disease, studying the physiology of the fungus and the susceptibility of several tobacco varieties. They found no varietal differences in susceptibility but stressed the necessity of maintaining vigorous growth to avoid infection.

Hopkins (11) has observed the disease in the field over a number of years and has suggested that susceptibility is governed by the nitrogen balance in the plant. Susceptible leaf on analysis was found to be low in minerals, especially phosphorus and potash. Hopkins has also observed potash deficiency symptoms in severely infected plants in the field.

Tobacco grown on the same soil for the second or third season in succession has also been found to be more susceptible to brown spot, presumably due to a fall in the level of available mineral nutrients.

Hopkins states that the fungus has rarely been encountered on seedlings. In 1948, he reported atypical infection on young seedlings, while transplants were found to have more typical lesions. (12).

The writer carried out experiments with A. longipes isolates from Rhodesia, in England during 1948. Work was confined, however, to greenhouse and laboratory investigations.

The main conclusions arising from this work are summarised below.

- 1) Susceptibility of tobacco was influenced by the nutrient

supply in sand culture. Medium N, and deficient P and K favoured the disease. Plants grown at high N, P and K levels were least susceptible. High P and K fertilizers in soil did not significantly reduce the disease. Nitrogen applied as $(\text{NH}_4)_2\text{SO}_4$ produced more susceptible plants than NaNO_3 .

2) Tobacco seedlings were found to be only slightly susceptible: infection was atypical. Susceptibility increased with age. 3) The fungus was found to be slightly pathogenic to potato, tomato, Capsicum sp., Nicandra physaloides and Datura stramonium.

II. NUTRITIONAL AND FERTILIZER STUDIES.

1. Greenhouse experiment: The effect of mineral nutrition on susceptibility to infection by *Alternaria longipes*.

The writer observed in a previous experiment that the susceptibility of tobacco to A. longipes was influenced by the ratio of N:P:K supplied to plants in sand culture (17). In this experiment, only three levels of each element were used, making a total of 27 treatments.

The present experiment was modified to include a larger range of each of the three elements N, P, K and each was varied independently of the other two. Two sources of nitrogen were compared as to their effect on susceptibility of tobacco, namely, sodium nitrate and ammonium sulphate.

Tobacco seedlings were grown in sandy loam in seed pans. At the three to four leaf stage, seedlings were pulled, the roots washed to free them from soil and transplanted into

washed sand contained in 5" pots. Pots were painted on the inner surface with bitumen paint to eliminate mineral effects from the clay. The experiment was arranged as a randomized block with three replications of each treatment.

Nutrient application.

Nutrient treatments were commenced 7 days after potting, the plants being potted on the 3rd. June 1949. Nutrient solutions were made up to provide between 0 and 100 mgms. of each element per week, and minor elements and iron and calcium in addition. When one of the elements was varied, the other two were held at constant levels.

By the 12th. August 1949, it was obvious that the plants were not receiving enough nutrients so that the next application was trebled and all subsequent applications were doubled. The original rates of application are shown in Tables I to V, but the ratios remained the same at the increased rates.

Inoculation.

Inoculum was prepared by washing spores from a 5 week-old culture of A. longipes on cornmeal agar (T.R.S. 25, highly virulent in the field) and filtering through folded muslin.

Inoculum was applied by spraying the leaves with a De Vilbiss atomiser. Plants were inoculated on the 7th. September 1949. No results were observed by the 16th. and the plants were then reinoculated. The absence of any reaction may have been the result of low night temperature and low humidity. Greenhouse temperatures were thereafter

maintained between 18° and 30° C., artificial heat being supplied by Valor Perfection oil stoves.

Disease estimation.

Disease reaction was poor due largely to the difficulty of maintaining a satisfactory humidity at this time of year.

The number of infected leaves per plant and the appearance of plants was recorded 10 days after the second inoculation. An arbitrary index of severity of infection was given to each infected leaf on plants as follows: 0, 10, 20, 30. The index was multiplied by the number of leaves corresponding to it and a total was made up for each plant. e.g. a plant with three leaves infected, scored 10, 10, 20, would have a total of 40. Figures in the Tables show the mean indices for three replications.

The three series for N, P and K were carried out together so that results may be compared one element with another.

Table I. Composition of nutrient solutions.

| Salt | | Nitrogen | Phosphorus | Potassium |
|---|----------------|-----------|------------|-----------|
| | | cc./week. | | |
| NaNO ₃ | 10 mgms. N/cc. | 0 - 10 | 2 | 2 |
| (NH ₄) ₂ SO ₄ | " " | 0 - 10 | 2 | 2 |
| Na ₂ HPO ₄ | " P/cc. | 1 | 0- 10 | 1 |
| K ₂ SO ₄ | " K/cc. | 1 | 1 | 0 - 10 |
| Minor elements | | 1 | 1 | 1 |
| Iron | | 0.5 | 0.5 | 0.5 |
| Calcium | | 0.5 | 0.5 | 0.5 |

The minor element, calcium and iron solutions were as follows:

Minor Elements: H_3BO_3 3 gms., $MnSO_4 \cdot 5H_2O$ 2 gms., $ZnSO_4 \cdot 7H_2O$ 0.3 gms., $CuSO_4 \cdot 5H_2O$ 0.1 gm., $MgSO_4$ 2 gms.
Water 1000 cc.

Calcium solution: $CaCl_2$ 12 gms., Water 1000 cc.

Iron solution: $FeSO_4$ 15 gms., Tartaric acid 15 gms. Water 1000 cc.

Results.

Nitrogen Series.

The basic nutrients are shown in Table I. There were two sets of pots in this series, one using $NaNO_3$, the other $(NH_4)_2SO_4$.

Nitrogen was added in doses of 0 - 100 mgms. per week or multiples of these figures. Inoculation results are given in Tables II and III and are illustrated in Fig. 1.

Table II. Influence of N on the growth and susceptibility of tobacco to *A. longipes*.

a) $NaNO_3$.

| N added mgms. | Appearance plants | Mean No. leaves infected/plant | Severity index. |
|---------------|-------------------|--------------------------------|-----------------|
| 0 | +++ N deficiency | nil | 0 |
| 10 | ++ " " | 2.0 | 26.6 |
| 20 | slightly yellow | 1.0 | 3.3 |
| 40 | " " | 1.0 | 10.0 |
| 60 | normal | 1.0 | 16.6 |
| 80 | very green | 1.0 | 10.0 |
| 100 | " " | 1.6 | 16.6 |

The main effect of nitrogen as NaNO_3 was on the growth of the plants. Optimum supply of nitrogen at the particular P and K levels used was 60 mgms. Higher or lower concentrations were noticeable by the colour of the plants.

Relationship of nitrogen to disease reaction was complex. Plants receiving no nitrogen were not affected. Maximum infection occurred at the 10 mgm. nitrogen level. There was certainly a higher infection index at the higher nitrogen levels, but the lowest index recorded was at the 20 mgm. level, an intermediate concentration.

Table III. Influence of N on the growth and susceptibility of tobacco to *A. longipes*.

b) $(\text{NH}_4)_2\text{SO}_4$.

| N added mgms. | Appearance plants | Mean No. leaves infected/plant | Severity index. |
|---------------|-----------------------|--------------------------------|-----------------|
| 0 | +++ N deficiency | nil | 0 |
| 10 | ++ " " | 1.0 | 10.0 |
| 20 | slightly yellow | 1.6 | 16.6 |
| 40 | very green, crinkled. | 2.3 | 23.3 |
| 60 | " " | 1.6 | 30.0 |
| 80 | " " | 1.6 | 30.0 |
| 100 | " " | 1.3 | 33.3 |

Figures for disease severity in $(\text{NH}_4)_2\text{SO}_4$ series differed somewhat from those in the NaNO_3 series. There was a progressive increase in disease from the low to the high

levels. The total of the severity indices for the $(\text{NH}_4)_2\text{SO}_4$ series exceeded the total of the NaNO_3 series by a considerable amount. Thus $(\text{NH}_4)_2\text{SO}_4$ produced more susceptible plants than NaNO_3 when differences of level were eliminated.

The plants in this series were of a peculiar texture, the leaves being very crinkled and mottled. This was particularly noticeable at the higher levels of $(\text{NH}_4)_2\text{SO}_4$. It would appear that the NH_4 ion is in some way detrimental to the good growth of tobacco and therefore predisposes plants receiving this source of nitrogen to infection. The effect of NH_4 on increasing the susceptibility of tobacco to attack by A. longipes has been noted previously in soil (17). Besumont (2) discusses the effect of NH_4 and its toxic effect for tobacco in America and states that this source of N predisposes plants to parasitic attack.

Phosphorus Series.

Basic nutrients are given in Table I. Phosphorus was added as Na_2HPO_4 in doses of 0 - 100 mgms. per week or multiples of these figures.

Table IV. Influence of P on the growth and susceptibility of tobacco to *A. longipes*.

| P added mgms. | Appearance plants | Mean No. leaves infected/plant. | Severity index. |
|---------------|---|---------------------------------|-----------------|
| 0 | ++ P deficiency: necrotic spotting; elongated petioles. | 4.0 | 123.3 |
| 10 | slightly yellow. | 1.6 | 36.3 |
| 20 | " " | 0.3 | 6.6 |
| 40 | " " | 0.6 | 6.6 |
| 60 | " " | 0.3 | 3.3 |
| 80 | stunted, yellow | nil | 0 |
| 100 | " " | nil | 0 |

Phosphorus appeared to produce symptoms of nitrogen deficiency above 20 mgms. per week. Concentrations of above 60 mgms. per week produced stunted plants.

The disease reaction with phosphorus was very marked. Absence of phosphorus resulted in a very high index of severity. The addition of phosphorus brought the value to less than one third of that without phosphorus. At higher levels of phosphorus, disease severity fell off. No attack occurred at the 80 and 100 mgm. phosphorus levels. Phosphorus was therefore an important factor governing the susceptibility of tobacco to *A. longipes*. The effect of phosphorus is illustrated graphically in Fig. 2.

Potassium Series.

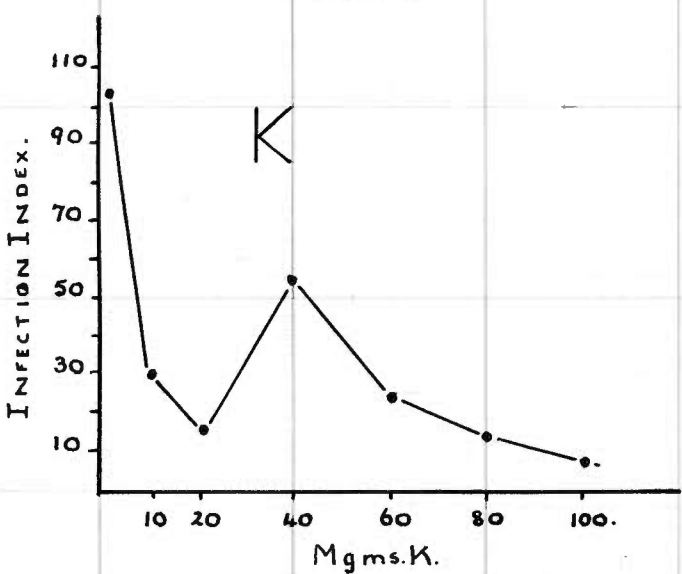
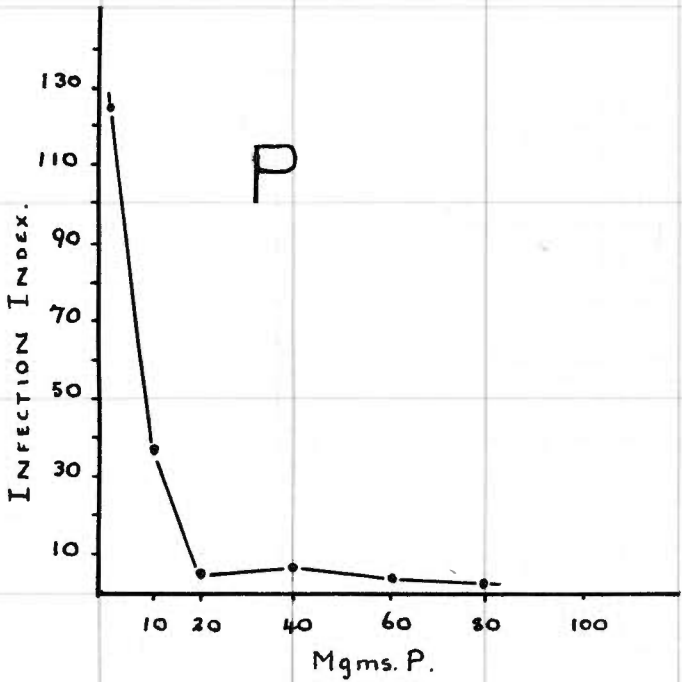
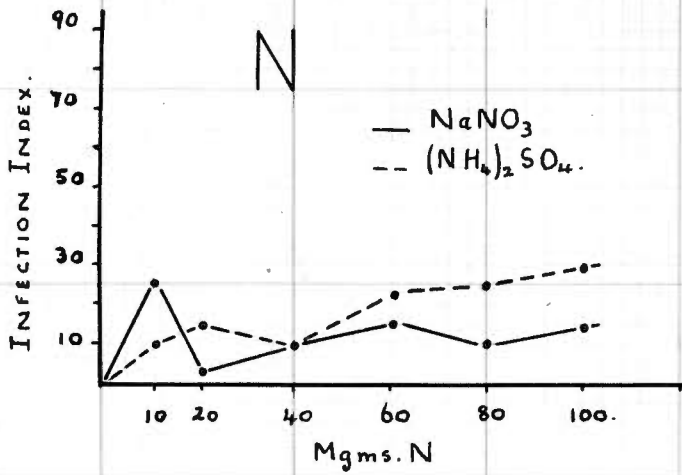
Basic nutrients are given in Table I. Potassium was added as K_2SO_4 in doses of 0 - 10 mgms. per week or multiples of these figures.

Table V. Influence of K on the growth and susceptibility of tobacco to A. lengipes.

| K added mgms. | Appearance plants | Mean No. leaves infected/plant. | Severity index. |
|---------------|--|---------------------------------|-----------------|
| 0 | Severe K defic. Crinkling & firing of leaves. | 3.6 | 103.3 |
| 10 | slightly yellow & crinkling. | 1.6 | 30.0 |
| 20 | slightly yellow. | 1.6 | 16.6 |
| 40 | " " | 2.6 | 53.3 |
| 60 | normal | 2.3 | 23.3 |
| 80 | " | 1.0 | 10.0 |
| 100 | " | 0.6 | 6.6 |

Severe symptoms of potassium deficiency showed up only in the pots receiving no potassium. All the other plants grew free of potash deficiency symptoms, though some were slightly yellow.

Disease reaction was high in plants receiving no potassium. Severity fell off with increasing potassium to 20 mgms., then rose to another high level at 40 mgms., thereafter falling off again to a low figure at 100 mgms. The effect of potassium is shown graphically in Fig. 3.



Figs. 1, 2, 3 Showing influence of N, P, K on susceptibility.

Discussion of results.

The index of severity used here should give a satisfactory indication of the extent of infection by A. longipes. The assessment of damage due to leaf-spotting diseases does not present an easy problem. Counting the number of lesions is not without danger, since it is difficult to control the number of spores applied per plant in the inoculum. Again, lesions run together so that it is not always possible to count discrete spots. The use of an arbitrary index obviates some of these sources of error.

There was good agreement between replicates, so that an analysis of variance was not necessary to establish the effect of the three principal elements on the susceptibility of tobacco to brown spot.

The main feature apparent was the marked effect of phosphorus on the incidence and severity of attack. Potassium, while resulting in a high disease index when absent, did not reduce the disease to the same extent at the higher levels as did phosphorus.

The effect of nitrogen was not marked, except in so far as NaNO_3 produced less susceptible plants than $(\text{NH}_4)_2\text{SO}_4$ as mentioned previously.

The experiment was conducted at an all-round low level of nutrition. This was to endeavour to promote the maximum amount of disease and to show up the effects more clearly. The writer (17) has shown that high levels of nutrition result in a decrease in disease severity.

At the commencement, the rate of nutrient application was considered sufficient. Later, however, when demands became greater, the quantities were increased as stated earlier. Nevertheless, plants were near starvation conditions from the point of view of the principal elements.

Overall, results agree closely with those obtained by the writer in England (17). i.e. phosphorus and potassium are the most important elements governing susceptibility to A. longipes but that phosphorus is relatively more important than potassium. Nitrogen, while influencing the severity of attack by its presence or absence, does not cause any striking increase in disease with increase in concentration provided it is balanced by phosphorus and potassium.

2. Field fertilizer trials.

As mentioned previously by the writer (17) reports of increased or decreased susceptibility to A. longipes were based originally on observations of infected crops. No information is available from experimental lay-outs. The data available relate principally to the effect of the proportions of N, P and K on susceptibility.

Greenhouse experiments in sand culture have shown that very significant results may be expected in this medium from increase in the phosphorus and potash content of the nutrient solutions.

Observations on two adjoining crops during the 1948/49 season, suggested that boron (as borax) might considerably influence the susceptibility of tobacco to brown spot. The two crops were sited on essentially the same type of soil and were planted at the same time. The one crop treated with some 4 lbs. borax to the acre was lightly infected by the fungus while the other was badly damaged. Numerous reports from farmers suggest that borax reduces disease intensity but in these cases there have been no adequate controls. It is of interest to note here, however, that Neergaard (16) states that Gigante recorded an increase in resistance of potato to Alternaria solani by the use of borax.

The experiments described here were made to determine whether significant control of brown spot could be achieved by supplementing standard fertilizers with extra phosphorus and potash and with borax.

Experimental details.

Experiments in each case consisted of a uniform area of 55 1/40 - acre plots of Bonanza tobacco spaced 3' x 3'. The designs were randomized blocks with five replications of eleven treatments. Details of treatments are given in Table VI. .

Table VI. Fertilizer treatments.

| No. | N | : | P | : | K ^x | No. | N | : | P | : | K |
|-----|---|---|----|---|----------------|-----|-------|---|----|---|--------------------------|
| 1. | 6 | : | 12 | : | 8 | 7. | 10 | : | 12 | : | 8 |
| 2. | 3 | : | 13 | : | 8 | 8. | 3 | : | 13 | : | 8 + 1 lb. borax/acre. |
| 3. | 3 | : | 13 | : | 15 | 9. | ditto | | | | + 2 lb. " |
| 4. | 3 | : | 20 | : | 8 | 10. | ditto | | | | + 4 lb. " |
| 5. | 3 | : | 20 | : | 15 | 11. | ditto | | | | + 6 lb. " |
| 6. | 0 | : | 13 | : | 8 | | | | | | |

^x N as NaNO_3 , P as superphosphate, K as K_2SO_4 .

Natural inoculum was relied upon to infect the experimental areas. In each case the previous crop had been badly infected with brown spot and the infected stalks were harrowed over the surface to provide the inoculum.

Disease estimation.

As it was only possible to visit the experiments fortnightly, the exact date of the appearance of brown spot is not known. It appears to have been 7 - 8 weeks after planting out.

The first estimates of the percentage infection by Alternaria longines were made on the 27th. and 28th. February 1950 about 10 - 11 weeks after planting out, and the second estimates two weeks later.

A weighted percentage of infection was obtained according to intensity of attack. The method used was to place each plant into one of four arbitrary classes as follows: 0 no infection, 1 light spotting of lower leaves, 2 heavy spotting of lower leaves; light on upper leaves, 3 heavy spotting on entire plant. For convenience, the data were reduced to a single figure for each plot - an infection index after the method of McKinney (15) as follows:

$$\text{Infection Index} = \frac{\text{Sum of class numbers} \times \text{No. plants/class} \times 100}{\text{Total No. plants} \times \text{highest class number}}$$

Experiment 1. Romsey Farm, Sinoia.

Soil: Heavy grey sand.
 Rotation: Tobacco, Maize, Maize, Fallow, Tobacco, Tobacco (Expt.)
 Seed beds sown: 13/10/49.
 Planted to land: 13/12/49.
 Fertilizers: Pre-fertilized 75 lb. cups 22/11/49.
 Post-fertilized 75 lb. cups 17/1/50.

Table VII. Mean infection indices - Romsey.

| Treatment | Mean Infection Index % | |
|-------------|------------------------|---------|
| | 27/2/50 | 13/3/50 |
| 6 : 12 : 8 | 24.4 | 48.2 |
| 3 : 13 : 8 | 28.9 | 50.2 |
| 3 : 13 : 15 | 26.6 | 50.9 |
| 3 : 20 : 8 | 29.4 | 51.0 |
| 3 : 20 : 15 | 22.8 | 49.4 |
| 0 : 13 : 8 | 25.8 | 48.5 |

contd. overleaf.

Table VII. contd.

| Treatment | Mean Infection Index % | |
|------------------|------------------------|---------|
| | 27/2/50 | 13/3/50 |
| 10 : 12 : 8 | 25.6 | 49.7 |
| 1 lb. borax/acre | 30.9 | 51.6 |
| 2 lb. " " | 28.2 | 52.6 |
| 4 lb. " " | 25.0 | 49.7 |
| 6 lb. " " | 24.2 | 48.3 |
| Standard errors: | 4.94 | 3.54 |

Experiment 2. Kyogle Farm, Karoi.

Soil: Brown-red sandy loam.
 Rotation: Tobacco, Tobacco (Expt.)

Seed beds sown: 3/10/49.
 Planted to land: 5/12/49.

Fertilizer: Prefertilized 75 lb. cups 23/11/49.
 Post-fertilized 75 lb. cups 17/1/50.

Table VIII. Mean infection indices - Kyogle.

| Treatment | Mean Infection Index % | |
|------------|------------------------|---------|
| | 28/2/50 | 14/3/50 |
| 6 : 12 : 8 | 37.1 | 75.5 |
| 3 : 13 : 8 | 37.4 | 70.2 |

contd. overleaf.

Table VIII. contd.

| Treatment | Mean Infection Index % | |
|------------------|------------------------|---------|
| | 28/2/50 | 14/3/50 |
| Ø : 13 : 15 | 32.1 | 74.4 |
| 3 : 20 : 8 | 34.9 | 72.3 |
| 3 : 20 : 15 | 48.6 | 76.6 |
| 0 : 13 : 8 | 35.0 | 61.8 |
| 10 : 12 : 8 | 39.3 | 78.2 |
| 1 lb. borax/acre | 32.6 | 75.0 |
| 2 lb. " " | 28.8 | 66.7 |
| 4 lb. " " | 38.2 | 69.5 |
| 6 lb. " " | 38.5 | 76.3 |
| Standard errors: | 7.82 | 7.15 |

Discussion of results.

Analyses of variance of both estimates and in both experiments showed no significant differences for disease data between treatments (appendix 2a.). The effect of fertilizers on yield is not known: obvious difficulties presented themselves in the recording of yields on farms visited infrequently. Visual effects of treatments were only noticeable in Experiment 2, where it was possible to pick out the plots receiving no nitrogen by their colour. The tobacco in Experiment 1 was of a uniform dark green colour irrespective of treatment.

Coupled with the lighter green colour in the non-nitrated

plots in Experiment 2, there was a lower but non-significant figure for the percentage infection.

The results were in marked contrast to the very definite results obtained in sand culture: they agree, however, with a previous experiment conducted by the writer using soil in pots (17) where four fertilizer ratios showed no significant differences in percentage infection, by brown spot.

It can be concluded that those elements responsible for reduction of disease in sand culture, namely phosphorus and potassium, are in some way 'inactivated' in soils. It is well known that phosphorus readily becomes unavailable on some soils. Increased or decreased nitrogen in no way affected the percentage infection so as to afford practical control.

Borax had no effect on the susceptibility of tobacco to brown spot at the rates of application used here.

Some remarks regarding the experimental design of the trials are relevant here. The effect of a plot on its immediate neighbours must be considerable. Once a plot has become infected, it raises the potential inoculum for bordering plots. A strong case exists therefore for isolated treatments. This would enable any differences between treatments to be accentuated. Close planted maize or other non-susceptible crop planted round the experimental plots is suggested.

III. THE SUSCEPTIBILITY OF TOBACCO VARIETIES.

A certain measure of resistance was found to exist in the variety White Stem Orinoco when four varieties of tobacco were tested for susceptibility to brown spot under greenhouse conditions in England (17). The four varieties could be arranged in order of susceptibility as follows: Bonanza, Willow Leaf, Jamaica Wrapper, and White Stem Orinoco. Statistically, only White Stem Orinoco was less attacked than the other three varieties.

It was considered to be of interest to test a large number of varieties for susceptibility, particularly those suited to heavy soils. A number of samples of seed of varieties suitable for culture on heavy soils was obtained from New Zealand and the U.S.A. by Dr. J.C.F. Hopkins of the S. Rhodesia Dept. of Agriculture.

Eleven varieties, including the four standard varieties were chosen for the experiments. These were tested both in the greenhouse under more or less controlled conditions, and in the field in districts where brown spot had become a problem.

1. Greenhouse experiment.

Five potted plants of each variety were grown under approximately uniform conditions in the greenhouse. Each plant was supplied with a 200 lb. cup of 6:12:8 fertilizer mixture three days after potting. Four weeks after the fertilizer application, the plants were sprayed with a

spore suspension of Alternaria longipes from a 6 week -old culture. Plants were kept moist subsequently by atomizing with water as required. The experiment was arranged as a randomized block.

Results.

Disease data were recorded three weeks after inoculation. The reaction was slow due largely to low night temperatures and low humidity (May - June). The total diameters of spots on each plant were obtained and these figures were then divided by the number of affected leaves. The data were treated statistically by the analysis of variance (appendix 2b.) Means for varieties are given in Table IX.

Table IX. Mean infection data - greenhouse variety expt.

| Variety | Mean Disease Data mm. | % of Mean. |
|-----------------------------|--------------------------|------------|
| Bonanza | 23.20 | 150.8 |
| White Stem Orinoco | 14.50 | 94.3 |
| Willow Leaf | 19.94 | 129.6 |
| Jamaica Wrapper | 12.56 | 82.1 |
| C.7.(Jamaica Wr. x Bonanza) | 17.40 | 113.7 |
| Oxford 26. | 12.10 | 79.1 |
| Harrison's Special | 9.04 | 59.1 |
| Yellow Mammoth | 16.28 | 106.4 |
| Gold Dollar | 15.30 | 100.0 |
| Broad Leaf | 19.66 | 128.4 |
| Special 400 | 9.21 | 60.1 |

contd. overleaf.

Table IX contd.

| | | |
|--------------|-------|-------|
| Variety Mean | 15.38 | 100.0 |
|--------------|-------|-------|

Standard error: 4.23

Significant differences: 5% : 8.54

1% : 11.42

Two varieties were outstanding in showing tolerance to attack by Alternaria longipes, namely Harrison's Special and Special 400. These varieties were significantly less attacked than Bonanza at the 1% point and less than Broad and Willow Leaf at the 5% point. Oxford 26, Jamaica Wrapper and White Stem Orinoco were less affected than Bonanza at the 5% point. Bonanza is taken here as a reference standard: it is normally a highly susceptible variety. No significant differences existed between Bonanza, Willow Leaf, Broad Leaf, C.7., Yellow Mammoth and Gold Dollar.

The four varieties commonly grown in Rhodesia can be arranged in order of susceptibility as follows: Bonanza, Willow Leaf, White Stem Orinoco and Jamaica Wrapper. These figures agree well with those obtained by the writer in a previous experiment (17) except for a reversal of the order of Jamaica Wrapper and White Stem Orinoco. Jamaica Wrapper was significantly less diseased than Bonanza here: this was not found previously.

2. Field trials.

The same eleven varieties used in the greenhouse experiment were tried under field conditions. As it was necessary to carry out these experiments at a distance from headquarters and to rely on lay assistance for planting, a randomized lay-out was not practicable. The varieties were planted in parallel strips, 11 rows wide x 44 rows long i.e. 1/10 acre on a planting 3' x 3'.

The experiments were sited on land which had carried one or more crops of tobacco infected with brown spot. Tobacco stalks from the previous season were harrowed over the soil surface to provide inoculum.

Details of planting, fertilizing etc. are given with each of the four experiments carried out.

Disease estimation.

Two series of estimates were made of the percentage brown spot infection, a) soon after the lower leaves had ripened, b) approximately one month later when the leaves were overripe. Estimates were made by counting the number of plants falling into four arbitrary classes as in the fertilizer experiments (page 15) and the results recorded as an index of infection. Bonanza is taken as the reference standard. Final observations were made on the experiments when the majority of the varieties under test were completely destroyed.

Experiment 1. Sinoia Citrus Estate.

Soil: Heavy red loam.
 Rotation: Tobacco, Tobacco, Tobacco (Expt.)
 Seed beds sown: 27/9/49
 Planted to land: 14/12/49
 Fertilizer: Pre-fertilized 22/11/49 75 lb. cups 3:13:8
 Post fertilized 18/1/50 mixture.

**Table X. Infection Indices & final observations,
Sinoia Citrus Estate.**

| Variety | Infection Indices | | Final Observations. |
|------------------|-------------------|---------|--|
| | 13/2/50 | 13/3/50 | |
| Bonanza | 38.3 | 94.6 | 100% destruction. |
| White Stem Or. | 33.9 | 88.5 | " " |
| Willow Leaf | 36.8 | 88.7 | " " |
| Jamaica Wr. | 34.7 | 89.3 | " " |
| C.7. | 27.7 | 59.4 | " " |
| Oxford 26 | 24.0 | 79.2 | " " |
| Harrison's Spec. | 30.2 | 75.0 | 3-4 spotted leaves left on 50% plants. |
| Yellow Mammoth | 27.1 | 79.6 | 100% destruction. |
| Gold Dollar | 31.9 | 84.5 | " " |
| Broad Leaf | 7.9 | 70.0 | Up to 12 leaves left on 47% plants. |
| Special 400 | 23.4 | 72.8 | ditto on 26%. |

Bonanza was the most severely affected variety. Tolerance to infection was shown by Broad Leaf, Special 400, Oxford 26, Yellow Mammoth, C.7 and Harrison's Special early in the ripening period: a month later, C.7, Broad Leaf, Special 400

and Harrison's Special still remained tolerant. Three varieties only, retained a proportion of their leaves two months after the first counts were made, namely, Broad Leaf, Special 400 and Harrison's Special.

Experiment 2. Romsey Farm, Sinoia.

Soil: Heavy grey sand.
 Rotation: Tobacco, Maize, Maize, Fallow, Tobacco, Tobacco (Expt.)

Seed beds sown: 13/10/49
 Planted to land: 13/12/49

Fertilizer: Pre-fertilized 22/11/49 75 lb. cups 3:13:8 mixture.
 Post-fertilized 17/1/50

Table XI. Infection indices & final observations,
Romsey, Sinoia.

| Variety | Infection Indices | | Final Observations. |
|------------------|-------------------|---------|--------------------------|
| | 13/2/50 | 13/3/50 | |
| Bonanza | 8.4 | 39.2 | Tops retained, v.s:otted |
| White Stem Or. | 2.7 | 44.3 | 100% destruction. |
| Willow Leaf | 4.8 | 77.6 | " " |
| Jamaica Wr. | 4.6 | 78.6 | " " |
| C.7. | 5.4 | 79.1 | " " |
| Oxford 26 | 14.4 | 86.0 | " " |
| Harrison's Spec. | 3.3 | 52.6 | Tops retained, v.spotted |
| Yellow Mammoth | 8.7 | 90.4 | 100% destruction. |
| Gold Dollar | 10.4 | 91.4 | " " |
| Broad Leaf | 11.1 | 77.2 | " " |
| Special 400 | 6.7 | 75.2 | " " |

Bonanza was the least affected variety at Romsey. This was the only experiment where Bonanza fell into the tolerant category. White Stem Orinoco, Harrison's Special, Jamaica Wrapper, Willow Leaf, C.7 and Special 400 were tolerant at the ripening stage. A month later, Bonanza, White Stem Orinoco and Harrison's Special remained tolerant. Six weeks after the first counts, only Bonanza and Harrison's Special remained tolerant.

Experiment 3. Kyogle Farm, Karoi.

Soil: Heavy pink sand.
Rotation: Tobacco, Tobacco (Expt.)
Seed beds sown: 3/10/49.
Planted to land: 5/12/49.
Fertilizer: Pre-fertilized 28/11/49 75 lb. cups 3:13:8
Post-fertilized 17/1/50 mixture.

**Table XII. Infection indices & final observations,
Kyogle, Karoi.**

| Variety | Infection Indices 14/2/50 % 14/3/50 | | Final Observations. 28/3/50 |
|----------------|--|------|--------------------------------|
| Bonanza | 29.8 | 79.6 | Few spotted tops retained. |
| White Stem Or. | 33.9 | 73.3 | " " |
| Willow Leaf | 46.1 | 77.6 | " " |
| Jamaica Wr. | 29.9 | 76.6 | " " |
| C.7. | 31.8 | 77.7 | " " |
| Oxford 26 | 31.1 | 75.4 | " " |

contd. overleaf.

Table XII. contd.

| Variety | Infection Indices | | Final Observations |
|------------------|-------------------|---------|--------------------------------------|
| | 14/2/50 | 14/3/50 | |
| Harrison's Spec. | 33.9 | 72.4 | Spotted tops retained on 55% plants. |
| Yellow Mammoth | 33.4 | 86.6 | 100% destruction. |
| Gold Dollar | 35.6 | 86.6 | " " |
| Broad Leaf | 29.3 | 71.6 | Few spotted tops retained. |
| Special 400 | 25.3 | 74.2 | " " |

No variety was markedly tolerant at Kyogle in the early stages of infection, except Special 400. This variety, however, did not remain tolerant. Harrison's Special showed tolerance six weeks after the first counts.

Experiment 4. Dundrennan Farm, Sinoia.

Soil: Heavy grey-brown sand.
 Rotation: Tobacco, Tobacco, Tobacco (Expt.)
 Seed beds sown: 29/10/49.
 Planted to land: 30/12/49.
 Fertilizer: Post-fertilized 3/1/50 75 lb. cups 3:13:8
 Post-fertilized 30/1/50 mixture.

Table XIII. overleaf.

Table XIII. Infection indices and final observations,
Dundrennan, Sineia.

| Variety | Infection Indices | | Final Observations. |
|------------------|-------------------|---------|--|
| | 27/3/50 | 12/4/50 | |
| Bonanza | 27.2 | 56.1 | Few spotted tops retained. |
| White Stem Or. | 10.8 | 41.9 | " " |
| Willow Leaf | 17.4 | 66.8 | " " |
| Jamaica Wr. | 21.8 | 52.8 | " " |
| C.7. | 28.3 | 66.8 | " " |
| Oxford 26 | 51.3 | 83.7 | 100% destruction. |
| Harrison's Spec. | 17.6 | 63.5 | Few spotted tops retained. |
| Yellow Mammoth | 14.3 | 62.2 | " " |
| Gold Dollar | 33.3 | 64.6 | " " |
| Broad Leaf | 12.4 | 49.4 | 50% plants with heavy spot on middle leaf. |
| Special 400 | 11.2 | 35.1 | Remainder -tops retained |

White Stem Orinoco, Special 400 and Broad Leaf were the least affected varieties at the first count, and remained tolerant two weeks later. Only the latter two varieties remained tolerant one month after the first count.

Discussion of results.

None of the varieties tested showed absolute resistance. Certain varieties, however, showed tolerance towards attack by A. longipes. Tolerance appeared to vary within a variety according to the physiological condition of the leaf. The two series of infection estimates and the final observations

served to show how varieties react a) at the ripening stage, b) when overripe, c) in the stage of decline.

Tolerance to infection i.e. absence of serious leaf wilt and collapse of the infected area was more marked in the first stage. At the stage of overripeness, varieties were not so readily distinguishable from one another, all being highly susceptible to the fungus at this time. This is in accord with Hopkins (12) observations of the change in susceptibility of tobacco to A. longipes with age.

Varieties showing the maximum tolerance were most noticeable in the stage of decline. Tolerance was exhibited here by the retention of the tops and in some cases the middle leaf. These leaves were all badly spotted but had not been completely invaded as in the case of the highly susceptible varieties.

Results of the four experiments are represented graphically in Figs. 4 a, b, c, d where changes of susceptibility with age are more readily discernible.

Effect of soil types.

The behaviour of varieties was not always found to be consistent from one experiment to another. Differences in the order of susceptibility or of tolerance were attributed to soil differences. The prevailing wind might also have had the effect of increasing the inoculum on some

strips and reducing it on others thus putting a bias on certain varieties. This explanation was ruled out as the inoculum was well distributed over the soil surface on all variety strips.

Change in the order of susceptibility was particularly marked at Romsey. Bonanza was found to be tolerant in this experiment, while it was moderately to highly susceptible in the other three experiments and was highly susceptible in the greenhouse.

Harrison's Special was the most consistent variety over the four experiments. This variety exhibited the greatest degree of tolerance at three farms, while at the fourth it was moderately tolerant.

It is of interest to compare here the four commonly-grown varieties in their order of susceptibility over the four farms. The figures obtained in the greenhouse experiment are also included (Table XIV.). The order of susceptibility was found to be relatively constant and was broken only by the results from Kyogle and minor shifts.

Table XIV. overleaf.

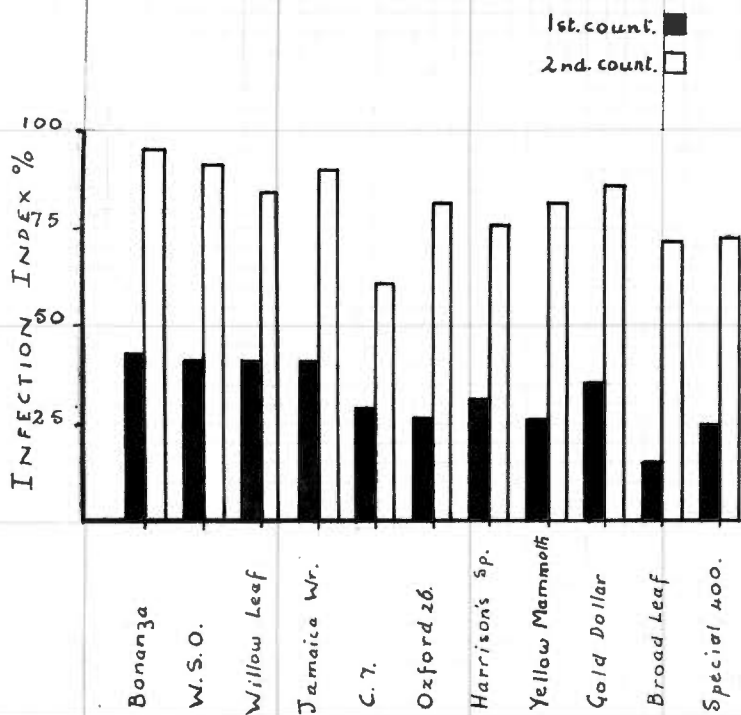


Fig. 4a. Showing susceptibility of varieties,
Sinoia Citrus Estate.

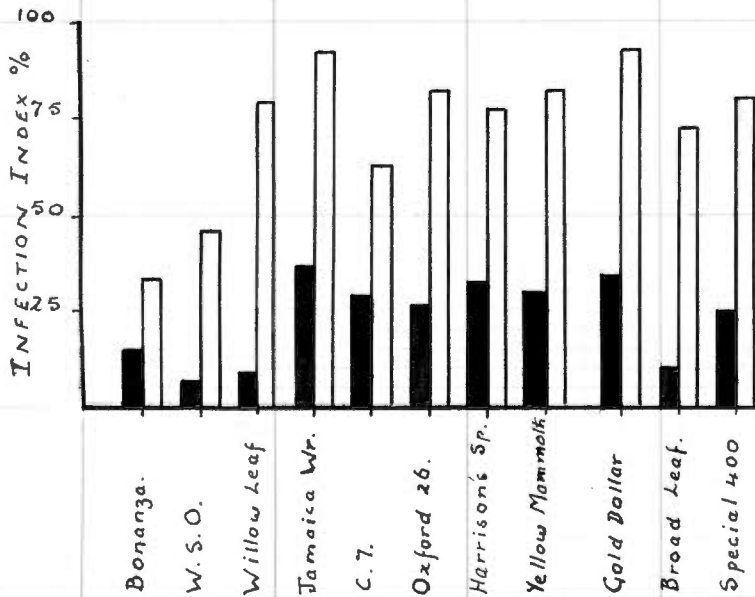
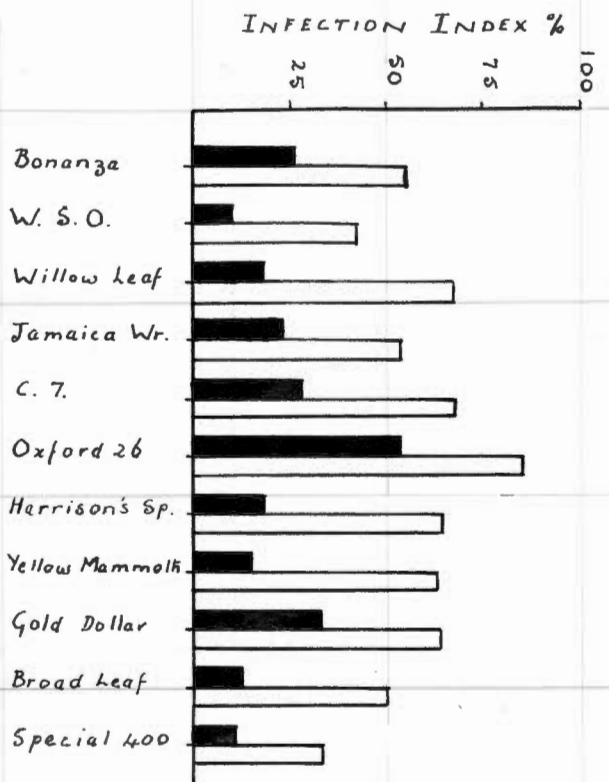


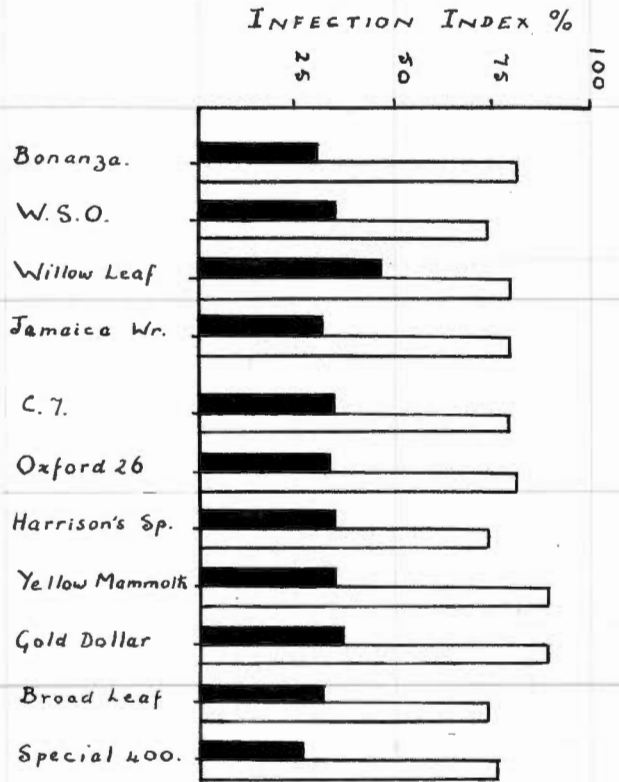
Fig. 4b. Showing susceptibility of varieties,
Romsey.

FIG. 4d. Showing susceptibility of varieties,



Dundrennen.

FIG. 4c. Showing susceptibility of varieties,



Kyojle.

Table XIV. Order of susceptibility of standard tobacco varieties. (1st. counts)

| Greenhouse | Sin.Cit.Est. | Romsey | Kyogle | Dundrennan |
|-------------|----------------|-------------|-----------|----------------|
| Jamaica Wr. | White Stem Or. | White Stem | Bonanza | White Stem Or. |
| White Stem | Jamaica Wr. | Jamaica Wr. | Jamaica | Willow Leaf |
| Willow Leaf | Willow Leaf | Willow L. | White St. | Jamaica Wr. |
| Bonanza | Bonanza | Bonanza | Willow L. | Bonanza. |

To eliminate to a certain extent the differences over the four soil types, the arithmetic means of the first and second counts were determined for the four experiments. Figures are given in Table XV.

Table XV. Infection indices - means of 4 experiments.

| Variety | Means | |
|--------------------|-----------|-----------|
| | 1st.count | 2nd.count |
| Bonanza | 25.92 | 67.37 |
| White Stem Orinoco | 20.32 | 62.00 |
| Willow Leaf | 26.27 | 77.67 |
| Jamaica Wrapper | 22.75 | 74.32 |
| G.7. | 23.20 | 70.75 |
| Oxford 26 | 30.20 | 81.07 |
| Harrison's Special | 21.25 | 65.87 |
| Yellow Mammoth | 20.87 | 79.70 |
| Gold Dollar | 27.80 | 81.77 |
| Broad Leaf | 15.17 | 67.05 |
| Special 400 | 16.66 | 64.32 |

The effect of age on the infection index is very clear here. All varieties were highly susceptible at the time of the second counts.

Tolerant varieties over all farms were: White Stem Orinoco, Special 400, Broad Leaf and Harrison's Special.

Practical implications of varieties tolerant to A. longipes.

From a practical point of view, it would suffice for varieties to exhibit tolerance as far as the ripening stage, since reaping progresses from this time forward. This would allow of the use of a wider range of varieties than if they were considered on the basis of tolerance in the stage of decline. Those varieties tolerant in the stage of decline are naturally the more resistant types.

Varieties considered here may be broadly divided into two groups, a) narrow to medium leaf types, and b) broad leaf types. The former group comprises the four standard varieties, Yellow Mammoth, C.7 and Gold Dollar. The remaining varieties fall in the latter group, although Oxford 26 is of a complex nature and does not properly fit here.

Of the narrow leaf group, C.7 showed a certain measure of tolerance at three farms in the ripening stage. Over all farms, however, the only variety in this group that showed tolerance in the overripe stage was White Stem Orinoco.

The broad leaf group contains the three varieties that

exhibited marked tolerance to A. longipes even in the stage of decline, namely Harrison's Special, Broad Leaf and Special 400.

The broad leaf types are characterised by the width of leaf, upright habit of the leaves and the decidedly yellow cast in the leaf colour. The dark green colour indicative of excess nitrogen on the heavy soils showed readily on the standard varieties but not on the broad leaf types. The nature of the resistance shown by some of these varieties is not known but they are clearly better suited to the nutrient of heavy soils than are the standard types.

IV. THE OVERWINTERING OF ALTERNARIA LONGIPES.

An important factor in the control of brown spot is the occurrence of the disease in successive seasons.

There are four possibilities to be considered here:

a) the fungus survives in the soil as a saprophyte during the winter, b) it exists in some form of resting stage on tobacco debris from an infected crop, c) wild host plants are present on which the fungus continues existence overwinter, d) it overwinters on tobacco seed. All four aspects of the problem are considered here, the former two being treated jointly.

1. Survival in the soil on tobacco debris, and in compost.

a) 1948/49 season.

Diseased leaf, stalks and seed capsules were placed in wire gauze baskets and buried at 12 ins., 6 ins., and 3 ins., in six types of soil. Along with the buried material, baskets containing similar material were left on the soil surface.

The material was put down on the 9th. and 10th. May 1949 and recovered on the 30th. September 1949 before the commencement of the rainy season.

In order to determine the survival or otherwise of Alternaria spores or mycelium in compost, a heap was made on the 6th. July 1949 and diseased stalks were placed in the centre. The heap was watered and turned as required and the stalks were recovered in part on the 30th. September along with the buried material.

Examination of material.

On recovery, the state of decomposition of the buried material was recorded and is shown in Table XVI. Part of the decomposition was in some cases due to termites. In no case was the material completely destroyed.

Pieces of obviously diseased material were placed on corn-meal agar to which potassium thiocyanate^x had been added to suppress bacterial development: Alternaria colonies are readily recognised on corn-meal agar by virtue of their colour and sporulation.

^x0.1 gms. per 5 cc. agar medium

Isolations were replicated nine times, i.e. 3 isolations per plate, and 3 plates per sample. Results are recorded in Table XVII.

To avoid any confusion that might arise from a direct interpretation of the Alternaria colonies appearing on the agar, it was decided to place pieces of infected debris on portions of tobacco leaf in damp chambers. Three pieces of leaf were used for each depth of burial. The numbers of infections resulting after 7 days at 28° C. were counted; results are given in Table XVIII.

Table XVI. Decomposition of material (portions remaining)

| Farm. | Surface | 3" | 6" | 12" |
|-------------------------|----------------|--------------|----------------|-----------------------|
| Sinoia Citrus Estate. | all intact | stalks | stalks | stalks & caps. |
| Dundrennan | stalks & caps. | stalks | stalks | stalks & caps. + leaf |
| Romsey | all intact | leaf & caps. | stalks | stalks & caps. |
| Govt. Farm, Karoi | stalks & caps. | stalks | stalks | stalks |
| Kyogle | all intact | stalks | stalks & caps. | stalks |
| Tob.Res.Stn., Trelawney | all intact | stalks | stalks | stalks. |

Table XVII. Alternaria isolations from debris, 1948/49

| Replicates | Surface | 3" | 6" | 12" |
|---|---------|----|----|-----|
| <u>a. Sinoia Citrus Estate.</u> | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1/3 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 |
| <u>b. Dundrennan</u> | | | | |
| 1 | 3/3 | 0 | 0 | 0 |
| 2 | 3/3 | 0 | 0 | 0 |
| 3 | 2/3 | 0 | 0 | 0 |
| <u>c. Romsey</u> | | | | |
| 1 | 2/3 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 |
| <u>d. Government Farm, Karoi.</u> | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 1/3 | 0 | 0 | 0 |
| 3 | 2/3 | 0 | 0 | 0 |
| <u>e. Kyogle</u> | | | | |
| 1 | 1/3 | 0 | 0 | 0 |
| 2 | 3/3 | 0 | 0 | 0 |
| 3 | 2/3 | 0 | 0 | 0 |
| <u>f. Tobacco Res. Stn., Trelawney.</u> | | | | |
| 1 | 0 | 0 | 0 | 0 |
| 2 | 3/3 | 0 | 0 | 0 |
| 3 | 1/3 | 0 | 0 | 0 |

There were no isolations from stalks buried in compost.

Table XVIII. Check inoculations to tobacco.

| Farm | Surface | 3" | 6" | 12" |
|-------------------------------|---------|------|-----|-----|
| Sinoia Citrus Estate. | ++3/3 | +3/3 | nil | nil |
| Dundrennan | +1/3 | nil | nil | nil |
| Romsey | +++3/3 | nil | nil | nil |
| Govt. Farm, Karoi. | +1/3 | nil | nil | nil |
| Kyogle | +1/3 | nil | nil | nil |
| Tobacco Res. Stn., Trelawney. | +1/3 | nil | nil | nil |

Discussion of results.

Alternaria longipes did not survive burial in the soil or composting. Isolations from material left on the soil surface, however, were positive in a high percentage of cases. Results of positive isolations were confirmed in their pathogenicity to tobacco.

The soils, though differing widely in texture and appearance, did not give rise to any differences in their capacity for elimination of Alternaria.

Theoretically, it would seem safe to plough in infected stalks and leaves, provided that none of the material was left on the surface.

Compost could safely be made from infected stalks, precautions being taken to ensure thorough decomposition.

Results confirm those of Tisdale & Watkins (19) who found the fungus was eliminated by burial in the soil but remained viable suspended above the ground.

b) 1949/50 season.

In view of the close agreement between the various experiments in the 1948/49 season, it was decided to reduce the number of experiments to three, and to change the depths of burial. As there was complete elimination of the fungus at 3" depth in the 1948/49 season, material was buried in wire gauze baskets at 1", 2" and 3". Only stalks were used. Material was also left on the soil surface as in previous experiments.

Sufficient samples were buried to enable them to be taken monthly to determine the time necessary for the elimination of Alternaria. Material was buried on the 5th. and 6th. May 1950 and recovered monthly until September.

Examination was carried out as previously described. Results are given in Table XIX.

Table XIX. Alternaria isolations from Webris 1949/50

| <u>Time of burial & place.</u> | <u>Surface</u> | <u>1"</u> | <u>2"</u> | <u>3"</u> |
|--|----------------|-----------|-----------|-----------|
| <u>1 month</u> | | | | |
| Sinoia Citrus Est. | 3/3 | 3/3 | 3/3 | 3/3 |
| Romsey | 3/3 | 3/3 | 1/3 | 1/3 |
| Kyogle | 3/3 | 3/3 | 3/3 | 2/3 |
| <u>2 months</u> | | | | |
| Sinoia Citrus Est. | 3/3 | 3/3 | 2/3 | 0 |
| Romsey | 3/3 | 2/3 | 0 | 0 |
| Kyogle | 3/3 | 3/3 | 2/3 | 0 |
| <u>3 months.</u> | | | | |
| Sinoia Citrus Est. | 3/3 | 3/3 | 1/3 | 1/3 |
| Romsey | 2/3 | 1/3 | 0 | 0 |
| Kyogle | 3/3 | 2/3 | 3/3 | 3/3 |
| <u>4 months</u> | | | | |
| Sinoia Citrus Est. | 3/3 | 3/3 | 0 | 0 |
| Romsey | 3/3 | 0 | 0 | 0 |
| Kyogle | 3/3 | 0 | 0 | 0 |

All colonies were interpreted directly on the appearance of the spores: no inoculations were made as in the previous experiments.

Results confirmed those of the previous season in that infected material left on the soil surface retained the fungus in viable form overwinter.

Burial at a depth of 3" seemed to be necessary to eliminate the fungus; even at this depth, however, occasional isolations were made in the third month after burial.

Fewer isolations were made with increasing time of burial, thus potential inoculum would be reduced by early burial (ploughing under) and late preparation of lands which had carried an infected crop.

There was a suggestion of soil difference influencing the rate of elimination of Alternaria at Romsey where burial at a depth of 2" eliminated the fungus after two months.

c) Greenhouse experiment, 1949.

The chances of survival of Alternaria longipes in the field, under the dry conditions of winter, might be considerably greater than under moist conditions. It was therefore decided to determine the survival of the fungus in three widely different types of soil under various moisture conditions.

Badly infected material was left on the surface of pots containing the soils and also buried in the soils. Pots were treated as follows: 1) not watered, 2) watered weekly, 3) watered every 14 days. The total number of treatments was 18 i.e. 3 soils, 3 rates of moisture, and either left on the surface or buried. Treatments were continued for 4 months.

Isolations were made on corn-meal agar as in the field experiments. Results of plate inoculations are shown in Table XX. There were nine replications per treatments.

Table XX. Survival of A. longipes under different moisture conditions.

| <u>No water</u> | | | <u>Water, 7 days</u> | | | <u>Water, 14 days.</u> | |
|-----------------|--------|---|----------------------|--------|---|------------------------|--------|
| Surface | Buried | | Surface | Buried | | Surface | Buried |
| 2/3 | 0 | | 2/3 | 0 | | 2/3 | 0 |
| 0 | 0 | T | 3/3 | 0 | T | 1/3 | 0 |
| 0 | 0 | | 2/3 | 0 | | 1/3 | 0 |
| 1/3 | 1/3 | | 1/3 | 0 | | 2/3 | 1/3 |
| 0 | 0 | R | 0 | 0 | R | 0 | 0 |
| 0 | 0 | | 0 | 0 | | 0 | 0 |
| 0 | 0 | | 1/3 | 1/3 | | 3/3 | 1/3 |
| 0 | 0 | K | 2/3 | 0 | K | 1/3 | 0 |
| 0 | 0 | | 0 | 0 | | 1/3 | 0 |

Soils from: T - Tobacco Research Station, Trelawney.
 R - Romsey Farm, Sinoia.
 K - Kyogle Farm, Karoi.

In accordance with expectation, watered pots with buried material did not show a higher number of isolations than unwatered. Except in one case, the buried material in those pots receiving no water gave negative results, similarly in the pots watered weekly. It would be expected that antagonistic organisms would be more active

under moist conditions but there appeared to be little difference in their activity under the conditions of the experiment.

Watering of infected material lying on the soil surface in pots produced an increase in the number of isolations over non-watered pots. The fungus, therefore, continued to grow saprophytically under these conditions.

As in the field burial experiments, there was no marked difference in the behaviour of the soil types.

2. The occurrence of wild host plants.

At least four weeds belonging to the Solanaceae and common in the tobacco-growing areas of Rhodesia have spotting of a type similar to that caused by Alternaria longipes on tobacco. Whether A. longipes can infect these plants is the subject of the present study. The writer (17) found that potato, tomato, pepper (Capsicum sp.), Datura stramonium and Nicandra physaloides were all slightly attacked under greenhouse conditions in England. The latter two plants are common weeds in Rhodesia.

During the course of this work, it was found possible to examine a large number of infected weeds. In the majority of the lesions examined, Alternaria spores were found. The spores closely resembled A. longipes with one exception; Alternaria spores found on Datura stramonium were invariably large and long-beaked and are referred to

A. crassa Rands.

Sources of material and infection experiments.

Collections of infected material were obtained from the Sinoia and Karoi areas where brown spot is a serious disease. Spore measurements from these collections and a comparison with A. longipes (Ell. & Ev.) Mason are referred to in Section VII.

Laboratory pathogenicity tests were carried out with agar inocula. Small pieces of culture were placed on pieces of washed leaf in damp chambers. Experiments were replicated two or three times.

Greenhouse inoculations to the four weeds were made with a virulent culture from tobacco (T.R.S. 25).

Uninoculated controls were included with all experiments.

Table XXI. Inoculation of weeds with their respective isolates and a tobacco isolate, 9 days at 28° C.

| Weed | Isolate No. | Result of inoculation |
|-----------------------------|---------------|-----------------------|
| <u>Nicandra physaloides</u> | T.R.S. 7 | +1/3 |
| | 11 | +2/3 |
| | 21 | +2/3 |
| | 23 | +3/3 |
| | 25 ex tobacco | nil |

contd. overleaf.

Table XXI. contd.

| Weed | Isolate No. | Result of inoculation |
|-----------------------------|---------------|-----------------------|
| <u>Solanum incanum</u> | T.R.S. 13 | +3/3 |
| | 17 | +1/3 |
| | 20 | +3/3 |
| | 30 | +3/3 |
| | 38 | +1/3 |
| | 25 ex tobacco | +2/3 |
| <u>Solanum panduriforme</u> | T.R.S. 27 | ++2/3 |
| | 29 | +2/3 |
| | 37 | ++3/3 |
| | 25 ex tobacco | +3/3 |

Results in Table XXI show that isolates from the weeds Nicandra physaloides, Solanum incanum and Solanum panduriforme were actually pathogenic to these plants. Owing to non-availability of material, it was not possible to include Datura stramonium in these inoculations.

These experiments were carried out in damp chambers. Inoculations with the tobacco isolate (T.R.S. 25) were positive on the two Solanum species only. However, inoculations to growing plants in the greenhouse were positive to all four weeds. Isolation from the lesions and reinoculation to tobacco was positive in every case.

Results of greenhouse inoculations are given in Table XXII and notes on the appearance of lesions produced by A. longipes on the weeds are appended below.

Table XXII. Inoculation of weeds in the greenhouse with a tobacco isolate. 18° - 27° C. - 10 days.

| Weed ^x | Inoculated | Control |
|-----------------------------|------------|-----------------------------|
| <u>Nicandra physaloides</u> | +3/4 | 3 nil |
| <u>Solanum incanum</u> | +6/8 | 6 nil |
| <u>Solanum panduriforme</u> | +2/5 | 6 nil |
| <u>Datura stramonium</u> | +3/3 | + 2/3 bordering inoculated. |
| Tobacco check | +1/1 | |

^x Plants raised from seed treated with 1/1000 silver nitrate.

Notes on the appearance of lesions on inoculated weeds.

- N. physaloides Spots nearly circular, light brown at centre with dark border. On mature leaves only. Yellow halo produced around some lesions. No stem attack.
- S. incanum Spots dark brown, irregular in outline due to venation of leaves. Yellow halo intense around lesions. No stem attack.
- S. panduriforme Spots light brown, circular, confined to declining leaves. Yellow halo not pronounced and present around some lesions only. No stem attack.
- D. stramonium Spots nearly circular, very pale, almost white at centre with darker margin; large and numerous. Comparing lesions with those produced by A. crassa in spontaneous attacks, the latter appeared to be much darker and more zonate.

Table XXIII. Inoculation of detached tobacco leaves with isolates from weeds.

| Weed | Isolate No. | Inoculations. | | | |
|------------------------|-------------|------------------|---------------------|------------------|-----------------|
| | | In lab. | Incubator at 28° C. | | |
| | | 19/7- 25/7/49 | 25/7- 4/8/49 | 10/1- 19/1/50 | 22/2- 1/3/50 |
| <u>N. physaloides</u> | T.R.S 7 | nil | nil | nil | +1/2 |
| | 11 | nil | +1/2 | +1/3 | nil |
| | 21 | nil | +1/2 | +3/3 | +1/2 |
| | 23 | nil | nil | nil | nil |
| <u>S. incanum</u> | 13 | nil | +2/2 | +3/3 | nil |
| | 17 | nil | nil | nil | nil |
| | 20 | nil | nil | +2/3 | +1/3 |
| | 30 | nil | nil | +1/3 | nil |
| | 38 | nil | +2/2 | +2/3 | nil |
| <u>S. panduræforme</u> | 27 | +1/3 | nil | nil | nil |
| | 29 | nil | nil | +2/3 | +3/3 |
| | 37 | nil | +2/2 | nil | +3/3 |
| <u>D. stramonium</u> | 28 | nil | nil | nil | no expt. |
| | 33 | +1/3 | nil | nil | no expt. |

The first isolations from weeds were made late in the 1949 season and it was not possible to carry out inoculations until July/August. It is evident from a comparison of columns 3 and 4 in the table, i.e. lab. temperatures and incubator at 28° C. that temperature effects were operative here. The very slightly positive results with S. panduræforme and D. stramonium in

column 1 were inexplicable.

Incubator inoculations were inconsistent but this can be at least partly accounted for by the type of leaf used. Owing to non-availability, it was not possible to use leaf of the same stage of ripeness for all inoculations, and it is well-known that physiological conditions in the leaf can profoundly affect the pathogenicity of A. longipes for tobacco.

Results suggest that Alternaria spp. from three Solanaceous weeds, namely N. physaloides, S. incanum and S. panduriforme can attack tobacco in a mild degree. Under optimal conditions, e.g. temperature and humidity and ripeness of leaf, attack would probably be more virulent.

Experimental conditions were more satisfactory in the series of inoculations with A. longipes to the weeds. Thus it can be concluded that A. longipes can attack four species in addition to tobacco. Pathogenicity of the fungus to N. physaloides and D. stramonium reported by the writer (17) was confirmed.

3. Survival overwinter on the seed.

Gulyás (8) reports that in Rumania, brown spot is a seed-borne disease. Seed capsules are readily attacked in Rhodesia but seed heads are bagged early in the season, thus minimizing chances of infection. All seed, too, is sterilised before sowing.

The presence of the fungus on seed from infected capsules was checked by placing seeds on tobacco leaf in damp chambers. Typical brown spot lesions developed after 5 days at 28° C.

To test the efficiency of the standard treatment for seed against Alternaria longipes, two batches of infected seed stored for one year were plated out on cornmeal-agar. One batch was treated before plating by soaking for 15 mins. in 1/1000 silver nitrate solution and washing in three changes of sterile water. Results are given in Table XXIV and show that seed treatment with silver nitrate was highly effective against A. longipes.

Table XXIV. Seed treatment against Alternaria.

| Replicate | Treated. colonies per plate | Untreated. colonies per plate |
|-----------|--------------------------------|----------------------------------|
| 1 | nil | 4 |
| 2 | nil | 6 |
| 3 | nil | 6 |
| 4 | 1 | 4 |
| 5 | nil | 3 |

Table XXV. Effect of removal of leaf hairs on spore germination.

| Seedlings | Days. | | |
|-------------------|---------|---------|---------|
| | 1. % | 2. % | 3. % |
| <u>2 week-old</u> | | | |
| Scraped | 36.8 | 32.8 | 32.1 |
| Singed | 45.1 | 52.8 | 56.1 |
| Control | 47.0 | 29.6 | 22.2 |
| <u>4 week-old</u> | | | |
| Scraped | 76.0 | 37.8 | 40.0 |
| Singed | 87.5 | 68.6 | 60.7 |
| Control | 30.1 | 36.6 | 27.4 |
| <u>8 week-old</u> | | | |
| Scraped | 55.5 | 63.2 | 56.6 |
| Singed | 92.5 | 86.2 | 51.2 |
| Control | 44.4 | 32.7 | 25.9 |
| Water controls | 96.7 | 89.6 | 93.1 |

Scraping and singeing the leaves appreciably increased the percentage spore germination. Singeing was more effective than scraping. Glandular hairs therefore inhibited the germination of spores.

Percentage germination fell off with time of diffusion from the leaves; this was presumably due to the accumulation of the inhibitory substances with time. The same effect was observed in the control and treated leaves, though the former was more pronounced than the latter. It was

concluded that in the treated leaves this effect was due to diffusion of inhibitory substances from glandular hairs not removed by the treatments.

The inhibitory effect of the leaf diffusates on spore germination fell off with the age of the seedlings. This would be accounted for by the relatively smaller concentration of hairs per unit area on the older seedlings, but reduced or changed activity of the glands may also have been responsible. Bentley & Wolf (3) state that the period of maximum activity of the glands of oriental tobaccos is at present a controversial matter. They themselves, maintain that maximum activity coincides with the ripening stage of the leaf. If this is the case, it would seem that either the content of the excretion becomes changed or that the density of the hairs on mature leaf outweighs the capacity for excretion: it is now well established that tobacco becomes increasingly susceptible to A. longipes with age.

Effect of removal of glandular hairs on the susceptibility of seedlings to infection.

Drops of spore suspension of A. longipes were placed on marked areas of seedling leaves of three ages treated as previously. There were six replications of each treatment. Controls consisted of leaves not scraped or singed. The leaves were incubated in damp chambers for two days at 28° C. and subsequently examined for penetration by germ-tubes of the fungus. Preparation of

it was in singed leaves that maximum infection occurred. It can be concluded that the glandular hairs were partly, at least, responsible for resistance in seedlings.

2. Seedling resistance in 11 varieties.

Spore germination tests were carried out in the manner previously described. Drops of water were taken from the leaves of 6 week-old seedlings after 3 days in situ. The experiment was replicated three times. Results are shown in Table XXVII.

Table XXVII. Seedling resistance in 11 varieties.

| Variety | % germination. |
|----------------------|----------------|
| Bonenza | 30.8 |
| White Stem Orinoco | 29.2 |
| Willow Leaf | 30.1 |
| Jamaica Wrapper | 50.0 |
| C.7. | 50.0 |
| Oxford 26 | 31.2 |
| Harrison's Special | 37.1 |
| Yellow Mammoth | 40.3 |
| Gold Dollar | 35.4 |
| Broad Leaf | 36.3 |
| Special 400 | 39.3 |
| <u>Water control</u> | <u>76.5</u> |

Varieties tested reacted very similarly. White Stem Orinoco produced the lowest percentage spore germination and Jamaica Wrapper and C.7 the highest.

Results were not related in any way to the order of susceptibility of the 11 varieties in the mature stage.

Various factors might be responsible for the differences observed here; among other things, differences in density of the leaf hairs in different varieties. No hair counts were made, however: Bentley & Wolf have found that for oriental tobaccos varieties differ widely in the density of the hair population, (3).

VI. TOXIC FACTORS PRODUCED BY A. LONGIPES IN CULTURE.

The effects of A. longipes on tobacco leaf as shown by the production of a yellow halo around the lesions are presumably due to the formation of a toxin. The toxic substance or substances may result from the growth of the fungus on the substrate, from the release of some toxic material by the host it-self or from the breakdown of fungal tissue. The following is an account of experiments relating to the production of a toxin in artificial culture and its possible significance in the etiology of brown spot.

1. Formation of a toxin in artificial culture.

A virulent isolate of A. longipes was grown on the following media for two weeks at 28° C. :

Richard's solution^x filtrate produced the strongest reaction, both when roots were immersed in the filtrate and when leaves were punctured. Rubbing the filtrates into the leaf did not produce much reaction. All the controls immersed in uninoculated media wilted slightly, presumably due to osmosis. No reaction was noted in the controls immersed in water.

It was thought that a stronger leaf reaction might be obtained by introducing filtrates into the vascular system of the seedlings by means of a hypodermic syringe. The needle was inserted into the midrib of a leaf in each case and a small amount of the filtrate injected. Controls consisted of a leaf on the same seedling injected with sterile distilled water. There were five replications of each treatment. Results were read after two days at room temperature and appear in Table XXIX.

Table XXIX. Injection of seedlings with filtrates.

| Medium | Injected | Control |
|--------------------|-------------------------------------|--------------|
| Richard's solution | slight necrosis & marked yellowing. | no reaction. |
| Tobacco extract | very faint yellowing. | no reaction. |

^x Richard's solution has the following composition:
Sucrose 50 gms., KNO_3 10 gms., KH_2PO_4 5 gms.,
 $MgSO_4$ 2.5 gms., $FeCl_3$ trace, Water 1000 cc.

Reactions were not markedly more intense when filtrates were injected than reactions produced by other methods described. The most satisfactory reaction, and one permitting of expression in figures was the wilt obtained by immersion of seedling roots. All further experiments were carried out using this method. Seedlings were scored for wilt intensity as follows: 0, no wilting, 10, wilting at leaf tips, 20, wilting half-way down leaf, 30, wilting of all leaves except those at the bud, 40, total collapse of seedlings. The period of immersion was two days.

2. Effect of time on toxin production.

Twenty-four flasks containing 50 cc. Richard's solution each were inoculated with a small plug of A. longipes culture from corn-meal agar and incubated at 28° C. Every two days, the mycelium was filtered from three flasks, dried and weighed, while the filtrates were assayed for toxin production with well-washed 8 week-old seedlings by the method described. Results are given in Table XXX. and illustrated graphically in Fig. 5.

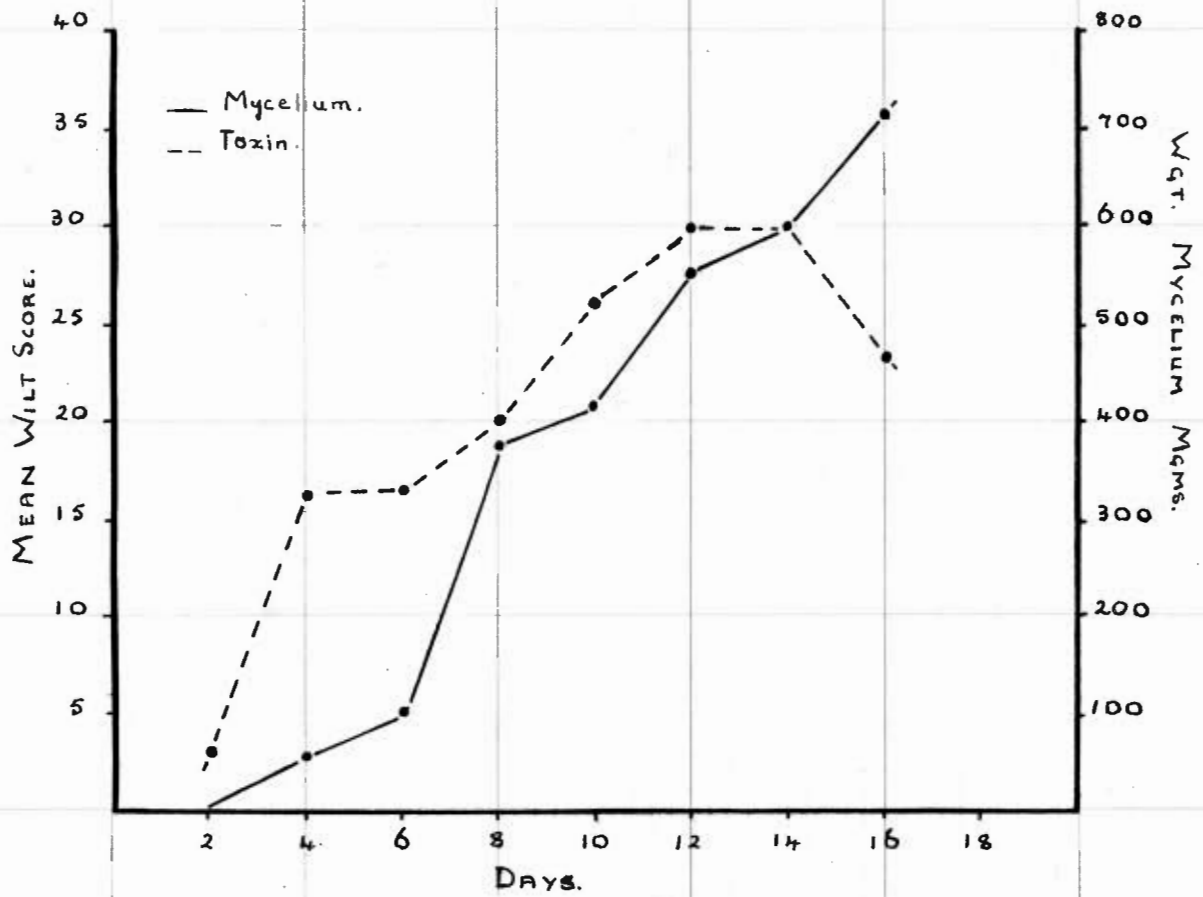


Fig. 5 Showing toxin production and wgt. mycelium.

inoculated with a culture of A. longipes and incubated for 14 days at 28° C., room temperature (18° - 20°C.), and 4° C. The filtrates were assayed for toxin production using 8 week-old seedlings. There were three replicates at each temperature. Results are given in Table XXXI.

Table XXXI. Effect of 3 temperatures on toxin production.

| Temperature | Mean Wilt Score. |
|-----------------------------------|------------------|
| 28° C. | 40.0 |
| 18°- 20° C. | 33.3 |
| 4° C. | 0 |
| controls (uninoculated medium) | 0 |

Toxin was formed in greatest concentration at the high temperature. Although a certain amount of mycelial growth occurred at 4° C., no toxin was formed at this temperature.

pH

Flasks containing 20 cc. Richard's solution were autoclaved, titrated to pH's 3, 4, 5, 6, 7, 8, 9, using the indicator method, and re-autoclaved. After inoculation with A. longipes, they were incubated at 28° C. for 14 days. There were three replications of each pH. Mean wilt scores of the filtrates were determined using 8 week-old seedlings, and appear in Table XXXII.

Table XXXII. Effect of pH of the medium on toxin production.

| pH. | Mean Wilt Score. |
|--|------------------|
| 3 | 13.3 |
| 4 | 13.3 |
| 5 | 13.3 |
| 6 | 20.0 |
| 7 | 16.6 |
| 8 | 13.3 |
| 9 | 3.3 |
| controls (uninoculated medium at each pH) | 0 |

Toxin was formed over a wide range of pH. The greatest concentration was formed at pH 6. Alkalinity reduced the amount of toxin formed.

Constituents of the medium.

Richard's solution was prepared omitting each of the following in turn: sugar, nitrogen, phosphorus, potassium. Controls consisted of the complete medium. Media were inoculated with A. longipes and incubated for 14 days at 28° C. There were nine replicates of each treatment. Filtrates were assayed for toxin production with 8 week-old seedlings. Results are given in Table XXXIII.

Table XXVIII. Effect of omission of the principal elements on toxin production.

| Solution | Mean Wilt Score. |
|----------------------------|------------------|
| Richard's solution | 30.0 |
| Richard's solution - sugar | 10.0 |
| " " - N | 3.3 |
| " " - P | 5.5 |
| " " - K | 10.0 |

Omission of any one of the principal elements reduced the amount of toxin formed. Absence of nitrogen and phosphorus reduced concentration more than did absence of sugar or potassium.

Concentration of main constituents of the medium.

Modified Richard's solution was prepared with varying contents of nitrogen, phosphorus, potassium and sugar. In the nitrogen series, KNO_3 was omitted and replaced by $NaNO_3$ to avoid changing the K concentration. Similarly in the potassium series, $NaNO_3$ was substituted for KNO_3 and varying amounts of K_2SO_4 were added. The phosphorus series was made up with $NaNO_3$ and K_2SO_4 plus varying amounts of Na_2HPO_4 . There were five concentrations in each series, each concentration being replicated three times. Media were inoculated with A. longipes and incubated for 14 days at 28° C. Filtrates were assayed for toxin production with 8 week-old seedlings. Mean wilt scores are given in Table XXXIV.

Table XXXIV. Effect of concentration of main constituents of medium.

| Medium | Mean Wilt Score | Controls (uninoc.medium) |
|---|-----------------|-----------------------------|
| Modified R.S. + NaNO_3 | | |
| 1.0% | 30.0 | |
| 2.0% | 10.0 | |
| 4.0% | 26.6 | |
| 8.0% | 16.6 | slight wilt |
| 10.0% | 13.3 | slight wilt |
| Modified R.S. + Na_2HPO_4 | | |
| 0.5% | 40.0 | |
| 1.0% | 13.3 | |
| 2.0% | 30.0 | |
| 4.0% | 26.6 | slight wilt |
| 8.0% | 30.0 | slight wilt |
| Modified R.S. + K_2SO_4 | | |
| 0.5% | 40.0 | |
| 1.0% | 36.6 | |
| 2.0% | 26.6 | |
| 4.0% | 26.6 | |
| 8.0% | 10.0 | slight wilt |
| Modified R.S. + sucrose | | |
| 5.0% | 26.6 | |
| 10.0% | 13.3 | |
| 15.0% | 20.0 | |
| 20.0% | 20.0 | |
| 25.0% | 20.0 | |

Toxin was formed at all concentrations of the chemicals used. Potency was reduced, a) by increase in nitrogen concentration, b) by increase in potassium concentration. Potency of the filtrate was largely unaffected by the concentration of phosphorus and sucrose. A result not so far explained was the drop in potency observed at 1.0% Na_2HPO_4 , 2.0% NaNO_3 and 10.0% sucrose, with a subsequent rise at higher concentrations.

4. Effect of heat on toxin.

Three portions of filtrate from 14-day cultures on Richard's solution were brought to boiling point for 5 mins and then cooled. Assay of toxin potency was carried out with 8 week-old seedlings. Controls consisted of unheated filtrate.

The heated filtrate gave a mean wilt score of 13.3, while the unheated gave a score of 20.0. Boiling resulted in only a slight reduction in potency, therefore it was concluded that the toxin is not an enzyme.

5. Source of toxin.

Having established that a toxin is formed by A. longipes and diffused into the culture medium, it was decided to test the mycelium itself for the presence of toxic substances.

The mat formed after 14 days growth of the fungus on 250 cc. Richard's solution was thoroughly washed in sterile distilled water to remove adhering culture fluid, and macerated in a mortar. Extract was made with sterile

distilled water and filtered through sterile cotton wool. The resulting fluid was tested for toxin at three dilutions with 8 week-old seedlings. There were five replicates of each dilution. Mean wilt scores are given in Table XXXV.

Table XXXV. Effect of mycelial extract on tobacco seedlings.

| Extract | Mean Wilt Score. |
|---------|------------------|
| 1 in 1 | 10.0 |
| 1 in 2 | nil |
| 1 in 4 | nil |

Wilt was almost negligible in two replicates of the undiluted extracts and moderate in the other three. Dilution of the extracts resulted in no wilt. It is probable that wilting in the undiluted extract was due to osmotic causes and that no toxic principle was present in the mycelium.

6. Attempts to antidote toxin.

There are numerous references in the literature to attempts to antidote fungal toxins. Zentmyer (20) used a number of organic chemicals against the toxin formed by Ceratostomella ulmi causing the Dutch Elm disease. Amongst these, he tried hydroquinone and 8-hydroxy-quinoline sulphate.

Hydroquinone, hydroxyquinoline and salicylic acid were tried by the writer for antidoting properties to Alternaria longipes toxin. Seedling roots were immersed in 5 cc. Richard's solution culture filtrates to which 2 cc. of 2% solutions of the chemicals were added. Controls consisted of 5 cc. sterile water plus 2 cc. of the solutions and Richard's solution filtrate to which there was no addition. The solutions were assayed for toxin potency with 8 week-old seedlings. Results are given in Table XXXVI and are the means of five replicates.

Table XXXVI. Effect of organic chemicals on toxin potency.

| Solution | Mean Wilt Score & Remarks. |
|-----------------------------|--------------------------------------|
| R.S. + hydroxyquinoline | 14.0 |
| Water + " | 0 |
| R.S. + hydroquinone | Seedlings badly damaged by chemical. |
| Water + " | " " " " |
| R.S. + salicylic acid | 36.0 |
| Water + " " | Seedlings slightly scorched |
| Richard's solution control. | 36.0 |

Both hydroquinone and salicylic acid were toxic to the seedlings. Neither appeared to depress the potency of the toxin. Hydroxyquinoline was non-toxic and gave a considerable reduction in the potency of toxin as compared with the Richard's solution control.

Discussion of results.

There is no precise way of determining the relationship that exists, if any, between toxin produced in culture and the effects 'at a distance' in the host.

It is of interest to compare the effect of some of the environmental factors on toxin production with similar effects on the disease in tobacco. Both the toxin and the disease are more 'active' at high temperatures (28° - 30° C.) Both are inhibited by high N and high K concentrations. In contrast to this, the disease is reduced in intensity by high P concentrations whereas P had little effect on toxin production in culture.

Toxins have been reported in other Alternaria species, particularly A. solani. Thomas (18) states that Whipple has reported the production of a toxin in tomato seedlings attacked by A. solani. The effects 'at a distance' described by Whipple are very similar to those resulting from infection of tobacco by A. longipes, and it is a reasonable conclusion that a related toxin is formed by A. longipes. Brian et al. (5) have isolated an antifungal and phytotoxic principle from A. solani and suggest that it may be significant in the etiology of early blight of tomato.

VII. THE MORPHOLOGY, VARIABILITY & TAXONOMY OF A. LONGIPES.

As has been mentioned previously, the causal fungus of brown spot in Rhodesia was originally described by Hopkins (9) as Alternaria tabacina (Ell.& Ev.) Hori. Hopkins in 1931, (10) also described a second species of Alternaria parasitic to tobacco which Mason (14) determined as A. longipes (Ell.& Ev.) comb. nov. Mason says of this fungus that it is not obviously distinct from A. solani. Hopkins failed to infect either potato, tomato or Datura stramonium with this isolate and therefore concluded that it was neither A. solani nor A. crassa.

The type of spore resembling A. solani has been encountered by Hopkins (12) from time to time since 1931 and he suggests that as the genus Alternaria is so variable as to size and shape of spores, the possibility exists that one species may cover the range of what has been described as two species. Hopkins stresses the importance of knowing whether one or two species of Alternaria parasitic on tobacco exist as the spores of the A. solani type are indistinguishable from that species on potato, and from A. crassa on Datura stramonium; these plants may therefore be additional hosts.

During the course of the last two years, it has been possible to examine a large number of isolates of Alternaria from tobacco and other Solanaceous plants

together with infected leaf material. The following account is an attempt to clarify the taxonomic and pathogenic relations of A. longipes in the light of these examinations.

Methods.

Spore measurements were made in lacto-phenol under a 45:1 objective and a x6 micrometer eyepiece with divisions of 3.3 μ . The tube length was set at 170 mm. A mechanical stage enabled the field to be changed so as to bring fresh spores within view.

Portions of leaf tissue were mounted in lacto-phenol, covered with a glass slip and warmed gently over a spirit lamp to expel air bubbles. The leaf was then examined directly. Fifty spores were measured for each sample.

Cultures were grown for two months from hyphal-tip transfers at 28° C. on Difco corn-meal agar slopes. Portions of cultures were removed for spore measurement and mounted under a cover slip in lacto-phenol. One hundred spores were measured for each sample.

Four measurements were taken on every spore; length of the spore body, length of the beak, if any, width, and the number and direction of septations.

As a general rule, the spore body could be quite easily differentiated from the beak, as the former was of a dark brown colour while the latter was invariably hyaline. Septation was easily determined, except in old

spores where secondary walls obscured the primary septations.

1. Morphology and variations.

Morphology of spores - tobacco isolates.

Six of eleven isolates of A. longipes from tobacco were chosen as showing maximum variability in size. Tables XXXVII, XXXVIII, and XXXIX show the percentage distribution of spores from the host and cultures as to length, width and septation. Figures in the tables are given for convenience in micrometer divisions, each division being equal to 3.3 μ .

Overall variation was much greater on the host than in culture; spore size was generally smaller in culture.

Percentage distribution curves for six isolates combined are shown in Fig. 6, a, b, c, d, e.

The width of the spores was a very constant factor, particularly on the host, being between 9.9 and 16.5 μ . As opposed to length, width was more variable in culture, being between 6.6 and 19.8 μ .

The length of the spore body, excluding the beak, was found to be more constant than when the beak was included. Figures in the tables are given for spore body and beak separately.

Table XXXVII. Percentage distribution A. longipes spores as to length, width, and length of beak - on the host.

| Micrometer divs. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | |
|------------------|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| <u>T.R.S.4</u> | | | | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | 2 | 6 | 12 | 8 | 14 | 10 | 17 | 8 | 4 | 17 | | | | 5 | | | | |
| width | | | | 32 | 62 | 4 | | | | | | | | | | | | | | | | | | |
| length beak | | | | 6 | 16 | 6 | 18 | 20 | 14 | 8 | | | 2 | 2 | 4 | 2 | 2 | | | | | | | |
| <u>T.R.S. 25</u> | | | | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | | 2 | 2 | 4 | 42 | 2 | 22 | 8 | 4 | 14 | | | | | | | | |
| width | | | | 92 | 8 | | | | | | | | | | | | | | | | | | | |
| length beak | 2 | | 2 | 2 | 2 | 6 | 8 | 14 | 20 | 6 | 12 | 6 | 10 | 4 | | 2 | | | | 2 | | | | 2 |
| <u>T.R.S.26</u> | | | | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | 2 | 2 | 2 | 10 | 44 | 20 | 8 | 4 | 2 | 6 | | | | | | | | |
| width | | | | 60 | 38 | 2 | | | | | | | | | | | | | | | | | | |
| length beak | | | | | | 6 | 2 | 8 | 10 | 6 | 14 | 6 | 22 | 6 | 6 | 10 | 4 | | | | | | | |
| <u>T.R.S.31</u> | | | | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | 4 | | | | 2 | 6 | 28 | 2 | 36 | 8 | 8 | 4 | 2 | | | | | | | |
| width | | | | 30 | 66 | 4 | | | | | | | | | | | | | | | | | | |
| length beak | | | | | | | 8 | 22 | 10 | 14 | 12 | 4 | 10 | 12 | 6 | 2 | | | | | | | | |
| <u>T.R.S32</u> | | | | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | | | | | 24 | 4 | 16 | 10 | 14 | 28 | 2 | 2 | | | | | | |
| width | | | | 24 | 66 | 10 | | | | | | | | | | | | | | | | | | |
| length beak | | | | | | 4 | 2 | 2 | 10 | 10 | 8 | 6 | 8 | 18 | 4 | 6 | 4 | 10 | 2 | | | 6 | | |

contd. overleaf

| Micrometer divs. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------|---|---|---|---|----|----|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <u>T.R.S. 36</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | | | | 2 | 14 | 8 | 24 | 20 | 8 | 18 | 4 | 2 | | | |
| width | | | | | 70 | 30 | | | | | | | | | | | | | | | |
| length beak | | | | | 2 | 8 | 8 | 10 | 26 | 10 | 14 | 8 | 6 | | 2 | 6 | | | | | |

Table XXXVIII. Percentage distribution of A. longipes spores as to length, width, and length of beak - cultures.

| Micrometer divs. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------|----|----|----|----|----|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <u>T.R.S. 4</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | 4 | 24 | 25 | 19 | 15 | 12 | 1 | | | | | | | | | |
| width | | 1 | 4 | 52 | 38 | 4 | 1 | | | | | | | | | | | | | | |
| length beak | 24 | 59 | 10 | 4 | 1 | 2 | | | | | | | | | | | | | | | |
| <u>T.R.S. 25</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | 2 | 14 | 39 | 32 | 6 | 7 | | | | | | | | | | |
| width | | | | 39 | 56 | 5 | | | | | | | | | | | | | | | |
| length beak | 4 | 57 | 25 | 6 | 4 | 4 | | | | | | | | | | | | | | | |
| <u>T.R.S. 26</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | 6 | 13 | 38 | 19 | 15 | 6 | 2 | 1 | | | | | | | | |
| width | | | 1 | 30 | 63 | 5 | 1 | | | | | | | | | | | | | | |
| length beak | 48 | 33 | 11 | 5 | 2 | | 1 | | | | | | | | | | | | | | |

Table XXXVIII. contd.

| Micrometer divs. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <u>T.R.S. 31.</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | | | | 1 | 12 | 8 | 29 | 13 | 12 | 11 | 6 | 7 | | 1 | | |
| width | | | | 12 | 40 | 36 | 4 | | | | | | | | | | | | | | |
| length beak | 19 | | 19 | 11 | 20 | 4 | 8 | 5 | 4 | 4 | 4 | 1 | | | | | 1 | | | | |
| <u>T.R.S. 32</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | 4 | 15 | 40 | 23 | 11 | 4 | 2 | 1 | | | | | | | | | |
| width | | | | 33 | 62 | 5 | | | | | | | | | | | | | | | |
| length beak | 40 | 38 | 12 | 8 | 1 | 1 | | | | | | | | | | | | | | | |
| <u>T.R.S. 36</u> | | | | | | | | | | | | | | | | | | | | | |
| length | | | | | | 3 | 8 | 30 | 32 | 18 | 7 | 1 | 1 | | | | | | | | |
| width | | | 2 | 46 | 50 | 2 | | | | | | | | | | | | | | | |
| length beak | 31 | 41 | 18 | 6 | 2 | | 1 | | | 1 | | | | | | | | | | | |

Table XXXIX. Percentage distribution of spores as to septation.

| No. Septations. | Host | | | | | | | | | | | No. Septations. | Culture | | | | | | | | | | |
|-------------------|------|----|----|----|----|----|----|----|---|---|----|-------------------|---------|----|----|----|----|----|----|---|---|---|--|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| <u>TR.S. 4.</u> | | | | | | | | | | | | <u>T.R.S. 4</u> | | | | | | | | | | | |
| Trans. Longit. | 48 | 42 | 10 | 18 | 22 | 36 | 24 | | | | | Trans. Longit. | 58 | 1 | 14 | 69 | 9 | 7 | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| <u>T.R.S. 25</u> | | | | | | | | | | | | <u>T.R.S. 25</u> | | | | | | | | | | | |
| Trans. Longit. | 24 | 28 | 42 | 22 | 24 | 30 | 16 | 6 | 2 | | | Trans. Longit. | 31 | 43 | 26 | 67 | 7 | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| <u>T.R.S. 26</u> | | | | | | | | | | | | <u>T.R.S. 26</u> | | | | | | | | | | | |
| Trans. Longit. | | 24 | 2 | 4 | 8 | 22 | 34 | 22 | 8 | | | Trans. Longit. | 23 | 42 | 12 | 78 | 10 | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| <u>T.R.S. 31</u> | | | | | | | | | | | | <u>T.R.S. 31</u> | | | | | | | | | | | |
| Trans. Longit. | 28 | 52 | 18 | 4 | 26 | 28 | 36 | 6 | | | | Trans. Longit. | 29 | 28 | 10 | 32 | 25 | 20 | 12 | 1 | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| <u>T.R.S. 32</u> | | | | | | | | | | | | <u>T.R.S. 32</u> | | | | | | | | | | | |
| Trans. Longit. | 36 | 42 | 20 | 2 | 10 | 24 | 40 | 20 | 4 | | | Trans. Longit. | 21 | 52 | 14 | 80 | 4 | 1 | 1 | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |
| <u>T.R.S. 36</u> | | | | | | | | | | | | <u>T.R.S. 36</u> | | | | | | | | | | | |
| Trans. Longit. | 24 | 38 | 26 | 8 | 18 | 26 | 32 | 16 | | | | Trans. Longit. | 15 | 50 | 4 | 80 | 8 | 6 | 2 | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |

Fig. 6a.

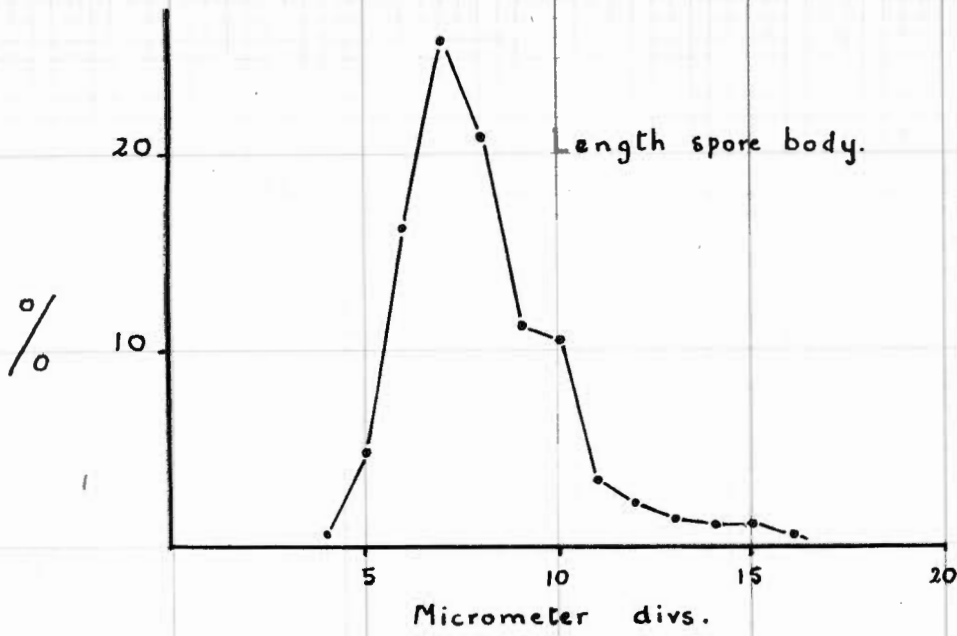
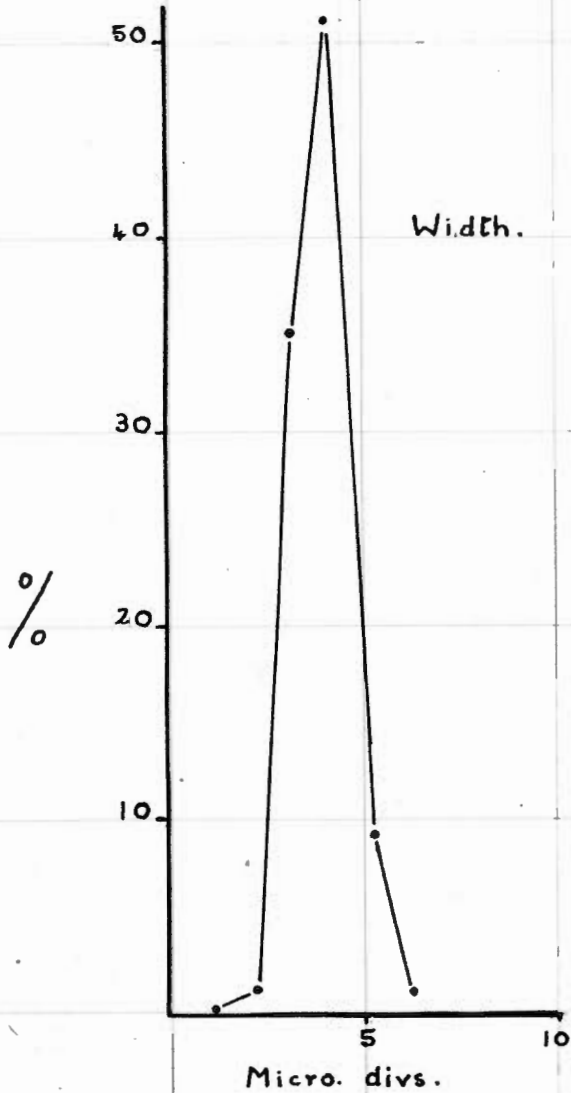
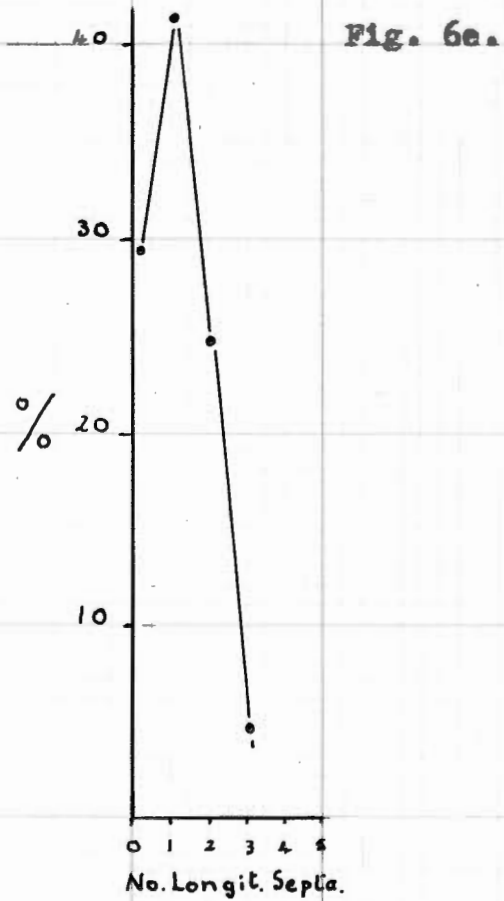
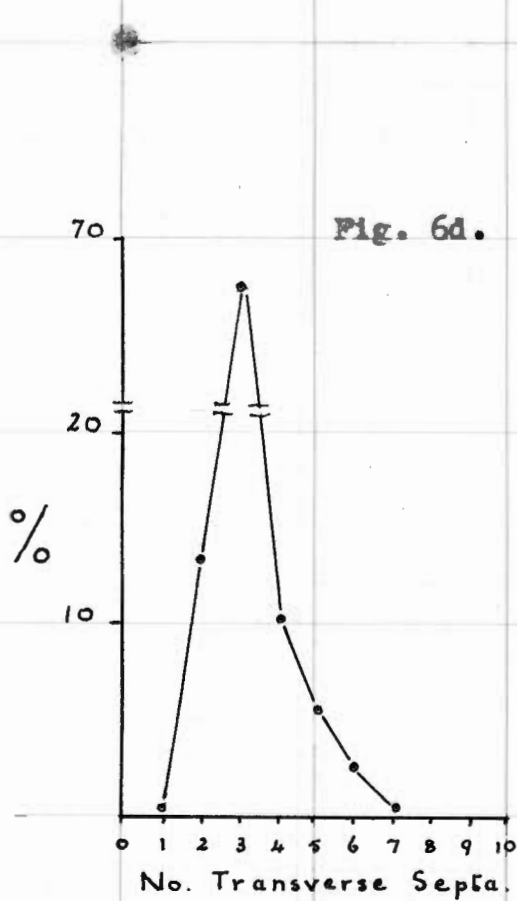
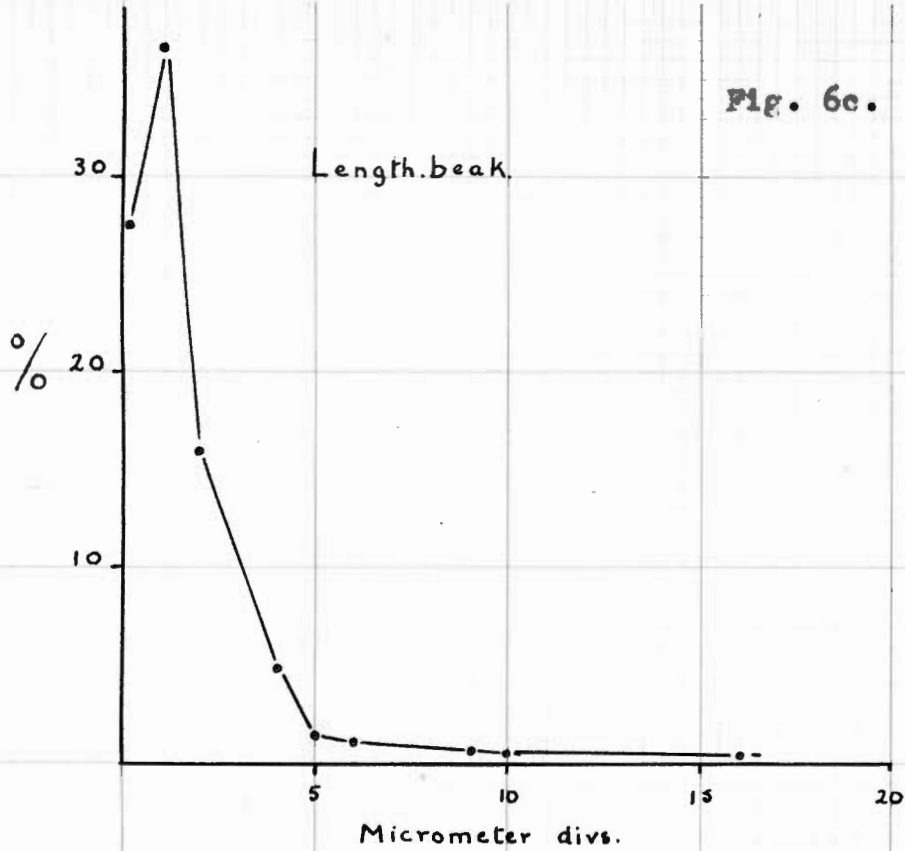


Fig. 6b.



Figs. 6a & b Showing percentage distribution of spores of A. longines as to length and width.



Figs. 6 c, d, e Showing percentage distribution of spores of *A. longipes* as to length of beak & septation.

Variation

Table XL shows the range of variation of six isolates of A. longipes.

Table XL. Range of variation of A. longipes.

| No. | Culture | Host. |
|-----------|-----------------------------------|-----------------------|
| T.R.S. 4. | 16.5-36.3 [†] x 3.3-19.8 | 19.8-59.5 x 9.9-16.5 |
| 25. | 16.5-33.0 x 6.6-16.5 | 23.1-49.5 x 9.9-13.2 |
| 26. | 16.5-39.6 x 6.6-19.8 | 19.8-49.5 x 9.9-16.5 |
| 31. | 23.1-49.5 x 9.9-19.8 | 13.2-52.8 x 9.9-16.5 |
| 32. | 16.5-39.6 x 9.9-16.5 | 33.0-56.1 x 9.9-16.5 |
| 36. | 16.5-39.6 x 6.6-16.5 | 29.7-56.1 x 13.2-16.5 |

[†] spore body only.

The variation in spore size observed in leaf samples can as such have little significance, the size and shape of the spores being largely dependent on the type of leaf on which the fungus was growing. The range of variation in cultures maintained under similar conditions should, however, indicate actual and real differences between isolates. Isolate T.R.S. 31 had a range in length and width of 23.1-49.5 x 9.9-19.8 μ in culture, the length being substantially greater than for other isolates. 73% of the spores from T.R.S. 31 fell between 33.0 and 42.9 μ , while only 3% to 13% of the spores from the other five isolates fell within this

range. A corresponding increase in beak length also occurred in this isolate. 50% of the spores had a beak range of 6.6-13.2 μ , while in the other isolates only 15% to 35% fell within this range. The resemblance of this isolate to A. solani was quite striking, particularly as to shape of spores and the presence of a long beak. Even the largest spores fell outside the range of A. solani, however.

Septation of the spores on the host and in culture was fairly constant. The commonest degree of transverse septation on the host was from 3 to 6; the extreme range was 2 to 8. Longitudinally, 0 to 3 septa were the most frequent: the extreme range was 0 to 7. From 24% to 48% of the spores in five samples were without longitudinal septa. However, in T.R.S. 25 all the spores were longitudinally septate. In culture, the most frequent transverse septation was 2 to 5, the extreme range being 1 to 7. The most frequent longitudinal septations were 0 to 2, the extreme range being 0 to 3; isolates had 15% to 58% of the spores with no septations.

The position regarding beaks was interesting. Of the six samples and isolates studied, 2% in one isolate only were without beaks on the host: in culture, 4% to 40% were without beaks. Five of the isolates had from 19% to 40% of non-beaked spores.

Both in culture and on the host, the length of the

beak covered a wide range. In culture, beaks were generally shorter than on the host, 3.3 to 13.2 μ being the commonest range of variation: the extreme range was 0 to 56 μ . Beaks of the extreme length were, however, unusual. No particular beak lengths were the most frequent on the host. The range was very wide, being from 0 to 72.6 μ .

Comparison of *A. longipes*, *A. solani*, *A. crassa* and *A. tenuis*.

The range of form shown by *A. longipes* extends from the *A. solani* type to that of *A. tenuis*. Hopkins (9) states that the fungus exhibits great polymorphism. It was considered to be of interest to compare these species and also *A. crassa* on a morphological and taxonomical basis, with a view to determining the position of *A. longipes* in the genus *Alternaria*. Details of spore measurements of the four fungi are given in Table XLI.

Table XLI. *A. longipes*, *A. solani*, *A. crassa* & *A. tenuis* compared.

| Spore measurement. | <u><i>A. longipes</i></u> | <u><i>A. solani</i></u> ^x | <u><i>A. crassa</i></u> ^x | <u><i>A. tenuis</i></u> ^x |
|-----------------------------|---------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| | μ | μ | μ | μ |
| Length: | | | | |
| Range with beak | 19.8-95.7 | 118.5-297.0 | 128-448 | 13.2-33.0 |
| " without beak | 13.2-26.4 | 36.0-87.0 | - | 9.9-33.0 |
| " beak | 3.3-52.8 | 67.5-234.0 | - | 3.3-9.9 |
| ⁺ Mean with beak | 31.5 | 219.3 | 261 | 21.5 |
| Mean without beak | 26.3 | 60.0 | - | 18.5 |
| Mean beak | 5.2 | 159.2 | - | 4.3 |
| | | contd. overleaf. | | |

Table XLI. contd.

| Spore measurement | <u>A.longipes</u> | <u>A.solani</u> ^x | <u>A.crassa</u> ^x | <u>A.tenuis</u> ^x |
|-------------------|-------------------|------------------------------|------------------------------|------------------------------|
| Width: | | | | |
| Range | 3.3-19.8 | 13.5-30.0 | 16-40 | 3.3-16.5 |
| Mean | 11.9 | 19.8 | 23 | 8.3 |

^x A. solani = A. porri, f.sp. solani ex tomato (Neergaard (16))
A. crassa Rands ex Neergaard (16)
A. tenuis - culture from Branch of Botany & Plant Pathology,
 Salisbury, W.R. 9164 (4).

+ Beaked spores only.

The spore measurements of A. longipes fall between those of A. solani and A. tenuis. Those of cultures such as T.R.S. 25 compared with A. tenuis, while those of T.R.S. 31 approached A. solani (Table XL). None of the isolates discussed here attained the size of A. solani, nor have spores as large as those described by Hopkins (9) been seen by the writer. Nevertheless, the capacity to produce larger forms such as T.R.S. 31 suggests the possibility that on occasion spores may reach the size of A. solani. Neergaard (16) reports 95% to 100% beaked spores in A. solani cultures and 60% to 98% in A. tenuis cultures. A. longipes falls nearer A. tenuis in this respect, having 60% to 96% beaked spores in culture.

A. crassa falls well outside the range of the three species already discussed, and shows well-defined differences to support the species. Neergaard (16)

summarises these differences as follows: a) A. crassa has a longer, thicker beak than A. solani, and the beak never branches, b) the spores are considerably larger than in A. solani, c) A. crassa is non-chromogenic in culture as opposed to A. solani, d) A. crassa is pathogenic to Datura but not to potato.

The range of form shown by cultures of the four fungi is shown in Table XLII. Six isolates of A. longipes are entered for comparison. Cultures were grown on Difco corn-meal agar for 7 days at 28° C.

Table XLII. Cultures of four Alternaria species compared.

| Culture | Growth Characters. |
|--------------------|--|
| <hr/> | |
| <u>A. longipes</u> | |
| T.R.S.4 | Slow-growing. Central portion of colony olive-grey to black. Broad margin of white submerged mycelium around central portion. Chromogenic. |
| T.R.S.25 | Fast-growing. Central portion of colony mouse-grey. Broad margin of white submerged mycelium. Non-chromogenic. |
| T.R.S.26 | Slow-growing. Central portion of colony brown-black surrounded by a band of olive-grey. Broad margin of white submerged mycelium. Chromogenic. |
| T.R.S.31 | Moderately fast-growing. Small central portion, olive-grey surmounted by white aerial mycelium. Broad margin of white submerged mycelium. Non-chromogenic. |
| T.R.S.32 | Slow-growing. Central portion ill-defined, grey-black. Broad margin of white submerged mycelium. Chromogenic. |
| T.R.S.36 | Very slow-growing. Colony compact, mouse-grey. Narrow white margin. Chromogenic. |

contd. overleaf

Table XLIII. contd.

| Culture | Growth Characters. |
|------------------|--|
| <u>A. solani</u> | Fast-growing. Central portion of colony light brown, surmounted by tuft of white aerial mycelium. Broad margin of white submerged mycelium. Chromogenic. |
| <u>A. crassa</u> | Fast-growing. Largest portion of colony mouse-grey with narrow band of white submerged mycelium. Non-chromogenic. |
| <u>A. tenuis</u> | Fast-growing. Central portion of colony olive-brown. Narrow band of white submerged mycelium. Non-chromogenic. |

The cultural characters of A. longipes vary widely. The growth rates of different isolates, in particular, are characteristic of this variation as also are the various colours of the mycelium.

Isolates of A. solani and A. crassa were quite distinct from those of A. longipes principally in respect of the type of mycelial growth.

The phenomenon of the diffusion of a red-carmine pigment in those cultures designated chromogenic has been discussed by Angell (1) and Bonde (4) with reference to A. solani. Neergaard (16) suggests that Macrosporium porri Ell., Macrosporium solani Ell. & Hart. (A. solani) and Alternaria brassicae v. dauci (Kuhn) Lindau be grouped together as one species - A. porri (Ell.). Neergaard on the basis of pigment production among other things. As a basis for classification this would not be satisfactory since Bonde (4) has shown

that the capacity for pigment production varies with the strain. Also, pigment production is not confined to these forms alone, e.g. A. longipes is also chromogenic.

In A. longipes, pigment production varies with the isolate. Certain isolates are non-chromogenic (Table XLII.) It has been found by the writer that potato-dextrose agar provides a more suitable medium for production of pigment than do other synthetic media. The intensity of pigment is also proportional to the temperature of incubation (17). The same applies to the pigment formed by A. solani.

The production of pigment by A. longipes suggests a strong affinity with the A. solani group, (incl. A. anagallidis Raabe & A. linicola sp. n. Neergaard, which are both chromogenic.), as opposed to the A. tenuis group which is non-chromogenic.

Pathogenicity, growth rate and chromogenicity.

Angell (1) states that in A. solani, pigment production is proportional to the growth rate on potato-dextrose agar. In A. longipes, this does not appear to be the case. In isolates examined by the writer, non-chromogenic strains have been found to be more vigorous than chromogenic and vice-versa. Bonds (4) found no correlation between pathogenicity of A. solani for potato and pigment production. In an experiment to determine whether this was the case with A. longipes, portions of

tobacco leaf in damp chambers were inoculated with six isolates of the fungus. Results shown as mean lesion diameters after 18 days at 28° C. are given in Table XLIII.

Table XLIII. Pathogenicity & Chromogenicity of A. longipes.

| Isolate | Pigment production | Mean lesion diam. mm. |
|----------|--------------------|--------------------------|
| T.R.S. 4 | chromogenic | 1.7 |
| 25 | non-chromogenic | 6.3 |
| 26 | chromogenic | 11.0 |
| 31 | non-chromogenic | 1.8 |
| 32 | chromogenic | 10.0 |
| 36 | chromogenic | 2.0 |

Results showed that there was no correlation between pathogenicity and chromogenicity of isolates. Different isolates varied widely in their intensity of attack.

Morphology of Alternaria isolates from wild species of Solanaceae.

In view of the positive results obtained in pathogenicity tests with a number of Alternaria isolates from wild species of Solanaceae (Sect. IV.), it was decided to compare the fungi with A. longipes from tobacco on the basis of morphology. Table XLIV. shows the range of form in culture and on the host of eight isolates from four weeds.

Table XLIV. Range of variation of Alternaria spp. from weeds.

| Weed. | Culture μ | Host μ |
|-------------------------------|-----------------------|----------------------|
| <u>Nicandra physaloides</u> | 16.5-36.3 x 9.9-23.1 | 26.4-59.4 x 9.9-16.5 |
| | 26.4-46.2 x 9.9-23.1 | 36.3-89.1 x 9.9-16.5 |
| <u>Solanum incanum</u> | 19.8-46.2 x 9.9-19.8 | 26.4-49.5 x 9.9-16.5 |
| | 13.2-33.0 x 9.9-16.5 | 23.1-42.9 x 9.9-16.5 |
| <u>Solanum panduraciforme</u> | 19.8-39.6 x 9.9-19.8 | 23.1-39.6 x 9.9-16.5 |
| | 19.8-36.3 x 19.8-26.4 | no spores. |
| <u>Datura stramonium</u> | 33.0-82.5 x 13.2-19.8 | 33.0-75.9 x 9.9-16.5 |
| | 23.1-59.4 x 9.9-19.8 | 13.2-82.5 x 6.6-19.8 |

The two D. stramonium isolates were undoubtedly strains of A. crassa though the spore dimensions were somewhat smaller than those given by Neergaard (16). The second of these two isolates approached A. longipes, in culture, but beaks, where present, were very long, being up to 99 μ. Neither of these isolates was pathogenic to tobacco (Sect. IV.). The second S. incanum isolate seems to have been a member of the A. tenuis group, the spores being rather smaller than those of A. longipes and having a shorter beak.

Mean spore dimensions of the remaining five isolates are given in Table XLV., together with those of A. longipes derived from six isolates. Figures are for spores from

corn-meal agar cultures.

Table XLV. Weed isolates and A. longipes compared

| Spore measurement | <u>A. longipes</u> | <u>Alternaria</u> ex <u>S. inc.</u> | <u>Alternaria</u> ex <u>S. pand.</u> | <u>Alternaria</u> ex <u>N. phys.</u> |
|-------------------|--------------------|--|---|---|
| | μ | μ | μ | μ |
| Length: | | | | |
| Range with beak | 19.8-95.7 | 23.1-52.8 | 19.8-42.9 | 19.8-62.7 |
| " without beak | 13.2-26.4 | 19.8-46.2 | 19.8-39.6 | 16.5-46.2 |
| Range beak | 3.3-52.8 | 3.3-16.5 | 9.9-19.8 | 3.3-19.8 |
| * Mean with beak | 31.5 | 32.0 | 32.4 | 34.6 |
| Mean without beak | 26.3 | 27.2 | 27.8 | 28.1 |
| Mean beak | 5.2 | 4.8 | 4.6 | 6.5 |
| Width: | | | | |
| Range | 3.3-19.8 | 9.9-19.8 | 9.9-13.2 | 9.9-23.1 |
| Mean | 11.9 | 13.0 | 13.5 | 13.5 |

* Beaked spores only.

Mean spore measurements were found to agree very well with those of A. longipes. Differences that were apparent could readily be accounted for through natural variation of isolates. If mean spore measurements can be accepted as being diagnostic, it is reasonable to advance the statement that 'A. longipes has a host range covering at least four species of Solanaceae, including tobacco, and that spontaneous attack may occur on Solanum incanum, Solanum panduraeforme and Nicandra physaloides.'

Spontaneous attack, as indicated by spore measurements, has not been observed on Datura stramonium but artificial

infection has been achieved in the greenhouse (Sect.IV.)
 The occurrence of smaller than usual spores in the
D. stramonium isolates cannot preclude the possibility
 of a mixed infection of A. longipes and A. crassa.

2. Taxonomy.

Alternaria longipes has been reported under a
 number of names since it was first recorded, and it
 is important to be clear as to the correct designation.
 Records of the fungus under various names and fungi
 reported as A. longipes are given in Table XLVI, together
 with their spore dimensions.

Table XLVI. Alternaria longipes and synonyms.

| Fungus | Author | Spores μ | Septation | |
|---|-------------------------------|----------------------|-----------|------------|
| | | | Trans. | Long. |
| <u>Macrosporium longipes</u> Ell.& Ev. n.sp. | Ellis & Everhart, 1892. | 40-50 x 15-20 | 3-7 | 1-2 |
| <u>M. tabacinum</u> Ell.& Ev.1894. | | 50-90 x 10-15 | - | - |
| <u>Alternaria longipes</u> (Ell.& Ev.) n.comb. | Tisdale & Wadkins 1931 | 30-50 x 10-13 | 3-7 | 1-2 |
| <u>A. tabacina</u> (Ell.& Ev.) Hori. | Hopkins 1931 | 35-50(90) x 8-15 | 3-7 | - |
| <u>A. longipes</u> (Ell.& Ev.) Mason | " | 80-275 x 9-20 | 5-10 | - |
| <u>A. longipes</u> (Ell.& Ev.) Tisd.& Wadk. | Hopkins 1939 | 35-50(90) x 8-15 | 3-7 | - |
| <u>A. longipes</u> (Ell.& Ev.) Mason. | Riley 1949 | 19.8-59.4 x 9.9-16.5 | 2-8 | (7) 0-4 |

Ellis & Everhart (6) originally described two species of Macrosporium from tobacco in 1892; M. tabacinum (white speck) with spores 15-25 x 10-12 μ , and larger spores 35-45 μ , and M. longipes (brown spot) with spores 40-50 x 15-20 μ . The latter agrees fairly closely with that of Tisdale & Wadkins (19) for their species of Alternaria associated with brown spot in Florida.

In 1894, Ellis & Everhart (7) again described an Alternaria from tobacco as M. tabacinum with spores 50-90 x 10-15 μ . In 1908, Hori (13) recognised this fungus, but since the spores were formed in chains, referred to it as Alternaria tabacina (Ell.& Ev.) n.comb.

In 1931, Hopkins (9) described two species of Alternaria on tobacco, namely A. tabacina with spore dimensions agreeing with those of Hori - 35-90 x 8-15 μ , and A. longipes with spores 80-275 x 9-20 μ . Mason (14), three years previously, had determined this latter fungus as A. longipes (Ell.& Ev.) n.comb. Mason says of the fungus that it is not obviously distinct from A. solani from potato.

Coincidentally with the publication of Hopkins measurements, there appeared two papers by Tisdale & Wadkins (19) and Gulyás (8) on A. longipes and A. tabacina respectively. Hopkins (10) in 1939, stated that there was no doubt that these two fungi were identical with that which attacks tobacco in Rhodesia. Tisdale & Wadkins

referred their fungus to A. longipes (Ell.& Ev.) n. comb. basing their diagnosis on that of Ellis & Everhart (6) for brown spot, namely M. longipes n.sp. Since the spores were formed in chains, it was, however, necessary to change the name to Alternaria. Gulyás referred to his fungus as A. tabacinum (Ell.& Ev.) n. comb. basing the diagnosis on that of Ellis & Everhart (7) of 1894, namely M. tabacinum n. sp. Hopkins (10), taking into account the paper of Tisdale & Wadkins decided to adopt the binomial A. longipes (Ell.& Ev.) Tisd. & Wadk. and proposed to drop the name A. longipes (Ell.& Ev.) Mason as he had not encountered the latter fungus again between 1931 and 1939. The combination of Mason, however, was published in 1928 and therefore has precedence over that of Tisdale & Wadkins of 1931; hence, the correct name for the fungus causing brown spot of tobacco is Alternaria longipes (Ell.& Ev.) Mason.

Conclusions.

From the foregoing account of the morphology and variability of A. longipes, it is clear that the species shows a wide range of form. This variation is due, in part, to the environment and the substrate e.g. tobacco of different types. The fungus is more constant in form in culture, spore dimensions falling over a wide, but fairly well-defined range. Apart from the variation due to the above causes, there is an inherent variation

between isolates. Mention may be made here of the frequent occurrence of saltations in A. longipes cultures.

Isolates of A. longipes show some to be of the A. tenuis type and some approach the A. solani type. The affinity of the species with the A. solani group is quite marked, particularly in respect of the type of disease produced by the two fungi, i.e. zonate leaf-spotting with varying degrees of toxic activity associated with the spotting. The production of pigment in cultures also suggests relationships with A. solani.

Probably the only criterion that can be used to distinguish the extreme forms is pathogenicity, though even here certain difficulties arise. Experiments by the writer have pointed to a wider host range of A. longipes than has been previously been recorded, and potato and tomato may be attacked under artificial conditions (17). It is true that no virulent, natural attacks have been observed on these hosts, nor have isolates from them been strikingly pathogenic to tobacco. Passage through tobacco may be necessary to establish virulence. Neergaard (16) has noted that passage of A. solani from culture through tomato seedlings resulted in increased virulence.

VIII. SUMMARY.

1. Susceptibility of tobacco in sand culture was conditioned by the P and K concentrations of the nutrients applied. High P was more effective than high K in reducing susceptibility. N did not markedly alter susceptibility, but plants receiving $(\text{NH}_4)_2\text{SO}_4$ were more susceptible than those receiving NaNO_3 .

Field trials with fertilizers of various N:P:K ratios did not alter susceptibility. Borax applied at four rates had no effect on the disease.

2. Varieties tested for susceptibility in the greenhouse showed Harrison's Special, and Special 400 to be some-what resistant. Bonanza was most severely attacked.

Field trials indicated that soils influence the order of susceptibility of different varieties: results were not consistent from farm to farm.

Over all farms, White Stem Orinoco, Harrison's Special, Broad Leaf and Special 400 were the least susceptible varieties.

3. Alternaria longipes can overwinter on the soil surface on tobacco debris. Infected stalks and leaves buried in the soil gave negative results when isolations were attempted after nearly five months.

Composting also destroyed the fungus.

Different amounts of moisture applied to infected material buried in soil, in pots, did not alter the number of isolations from those in unwatered soil.

A. longipes was pathogenic to at least four wild Solanaceous species, and Alternarias isolated from three species were pathogenic to detached tobacco leaves in damp-chambers. This suggests a further means of overwintering.

Seed treatment, as practised, in Rhodesia, was effective in destroying the fungus on infected seed.

4. Pre-penetration resistance in seedlings appears to be due to the glandular leaf-hairs. Removal of hairs increased the susceptibility of seedling leaves. Eleven varieties in the seedling stage, showed the same order of susceptibility to the fungus. Varietal differences were not marked and there was no correlation with susceptibility of mature leaf.
5. A. longipes produced a toxic factor in culture which wilted tobacco seedlings when the roots were immersed in the culture filtrate. Maximum production of toxin occurred after 12 to 14 days at 28° C. on Richard's solution. High N and K concentrations reduced potency. Increase in sugar and P concentrations did not materially affect potency, nor did heating

the filtrate. Toxin was formed in the absence of N, P, K, and sugar, but in reduced amount; N and P when absent were more effective in reducing potency than K and sugar.

Toxicity appeared to be due to the excretions of the fungus, not to the mycelium.

Toxin was antidoted to some extent by hydroxyquinoline.

6. The morphology and taxonomic relations of A. longipes are discussed. Spores of the fungus fall between the A. tenuis type and the A. solani type: pathogenically and physiologically, the fungus is related to A. solani.

Spore measurements of Alternaria isolates from suspected wild hosts showed close similarity with A. longipes.

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PART II.

FROG EYE CAUSED BY CERCOSPORA NICOTIANAE KIL. & EV.

PART II.

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I. HISTORICAL SURVEY

There does not appear to be any mention in the literature as to when frog-eye was first reported. It is known in practically every country where tobacco is grown, varying in intensity with the locality. Thus in the U.S.A., the disease is reported to be of minor importance, whereas in Southern Rhodesia and in parts of Queensland, Australia it causes serious damage.

Plants are first susceptible in the seedling stage, particularly if at all nitrogen deficient. Regular spraying with a fungicide such as Bordeaux mixture will keep the disease in check at this stage.

The control of the disease in the lands presents a more difficult problem. No varieties have yet been observed to be resistant to frog-eye, though McLean (4) has found certain varieties to escape infection through earlier ripening. The host range in Rhodesia is not known, but in the light of evidence from the U.S.A. it is not confined to tobacco. Johnson & Valleeu (2) obtained frog-eye infection in tobacco with isolates from 16 species in 11 plant families.

Present control measures take the form of priming off the lower leaves of plants before these become infected, and to a limited extent, field spraying.

II. PRIMING EXPERIMENTS.

1. The theory and practice of priming.

'Priming' consists in the removal of the lower declining leaves of the tobacco plant. In America, the term is used synonymously with reaping, but in Rhodesia it usually refers to disease control operations.

Priming for disease control is based on theoretical considerations which have been borne out in practice.

The essential part of the operation is the early removal of the lower leaves, including the seedling leaves, before these decline and become subject to leaf-spotting diseases, in particular frog-eye. It has been established, both by observation and experiment, that frog-eye more readily attacks nitrogen deficient leaves, and as there is an upward translocation of nitrogen from the lower leaves with increasing plant age, these leaves are the first to become attacked.

Once the lower leaves have become infected with frog-eye and the lesions bear spores, rapid infection of the upper leaves ensues. Very often this is not obvious in the land unless the leaves are held up to the light when the light green areas of incipient infection may be seen.

On incubation in the warm atmosphere of the curing

barns, however, severe black spotting develops which ruins the leaf.

Priming can take various forms and is very largely a matter of particular preference. At least three methods are in common usage: a) to remove the first three or four leaves from the plant and destroy them by burning or burial in the soil, b) to draw up the soil on the ridges to cover the leaves which then decay in the soil, c) to take the first reaping before the leaf is properly ripe and cure as best as possible.

The present experiments attempt to answer two questions in relation to priming; a) where does the initial infection originate which makes priming necessary, and b) what effect has priming on the yield and quality of tobacco.

2. Experiments relating to the invasion of lands by frog-eye.

The precise way in which frog-eye infects a land is not at present completely understood. The disease can affect lands isolated from growing tobacco as shown by Norval (5). It would seem therefore that frog-eye primary infection is from some source external to tobacco lands.

It is of considerable interest to know, a) whether frog-eye is definitely windborne and that primary infection results externally, and b) whether secondary infection results from frog-eye setting in on unprimed plants and thence spreading to primed.

Field experiment - Trelawney 1948/49.

The experimental area consisted of some four acres of Bonanza tobacco on land used for the third time running. Three 1/40 acre plots were marked out at each corner as shown in the diagram (Fig.1) These plots were left unprimed while the rest of the land was primed.

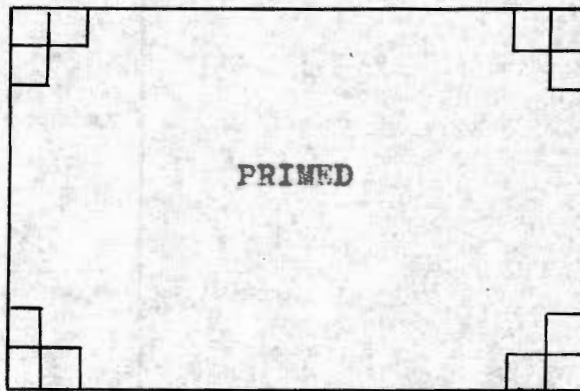


Fig. 1. Showing lay-out of Trelawney field experiment.

The treatment given the tobacco was as follows:

Planted: 20/11/49

Pre-fertilized: 15/11/48 150 lb. cups 6:12:8 mixture.

Post-fertilized: 8/1/49 100 lb. " " "

Primed: 20/12/48
3/1/49.

Sampling for estimation of disease spread.

Whole plants were taken at random in the primed portion and their positions marked relative to the corner plots. Leaves were stripped from the stems and incubated between sheets of absorbent paper for five days. Within this period any incipient frog-eye lesions will appear as black spots similar to those developing in the curing barns.

Four sets of samples were taken between 12/1/49 and 21/2/49. No spots developed on these samples during the incubation period. These results were consistent with the non-appearance of frog-eye in the corner plots at this time.

Spore trapping.

Attempts to trap Cercospora spores falling within the experimental area were made, using 3" x 1" microscope slides coated with glycerine jelly and supported horizontally under shields. Six traps were placed diagonally and six horizontally in the land.

The first exposures were made for a day at a time, but as no spores were trapped after this period the time was increased to two and finally three days. Slides were examined for spores microscopically at 250 diameters and low light intensity since Cercospora spores are hyaline.

Results of spore trapping were consistent with the very low incidence of frog-eye which appeared late in the season in the corner plots. The maximum number of spores trapped at any one time on all 12 traps was 10. This amounts to less than one spore per slide. In view of the small number of spores caught, it is doubtful whether any significance can be attached to the results. However, there was no evidence to suggest that there were more spores in the vicinity of the corner plots: spores appeared at random. It is therefore probable that the primary infection resulted from outside sources. These may take the form of infected tobacco debris or wild host plants.

Field experiment - Trelarney 1949/50

The lay-out of the 1948/49 experiment was repeated with modification during the next season. The whole experimental area was divided into 40 1/20 acre plots (3'6" x 2' planting), three plots being left unprimed in each corner while the remainder of the land was primed. Details of the experiment are given below.

Land: 2nd year tobacco.
Planted: 24/11/49
Pre-fertilized: 12/11/49 100 lb. cups 6:12:8 mixture.
Post-fertilized: 14/12/49 150 lb. cups " "
28/12/49 " " " " "
6/1/50 " " " " "
Primed: 24/12/49
14/1/50

Sampling for estimation of disease spread.

One plant from each plot was taken at random on 6/1/50 and 21/1/50. Samples were incubated as previously described: only corner plots showed spotting, the remainder of the plots being quite clear. Two plants from each plot were taken on 4/2/50 and 17/2/50 with similar results.

Reaping and grading.

The tobacco was taken from the experiment in five reapings and cured. Reapings 1 and 2, 3 and 4, and 5 were graded separately. Grading was so arranged as to provide two classes of spotted tobacco, light and heavy, while the remainder was classed as clean.

Figures for spotted leaf were reduced to a percentage by multiplying class 1 spot by one and class 2 spot by two, dividing the sum by the total weight per plot x 2 and multiplying by 100.

All three reapings (combined data, 1st. & 2nd., 3rd.& 4th. and 5th.) showed there to be a higher percentage of barn spot in the vicinity of unprimed plots: percentage spot fell off away from these plots. Results are exemplified by the diagrams showing percentage spot in the 3rd.& 4th. and 5th. reapings. (Figs 2 and 3).

| | | | | | | | |
|------|------|------|------|------|------|------|------|
| 75.9 | 33.8 | 22.6 | 30.7 | 17.6 | 21.4 | 89.7 | 90.9 |
| 78.6 | 50.0 | 35.1 | 39.4 | 26.8 | 30.3 | 28.6 | 86.7 |
| 88.7 | 39.6 | 45.4 | 34.2 | 34.5 | 40.2 | 24.1 | 32.4 |
| 84.6 | 41.7 | 41.0 | 24.3 | 12.2 | 37.3 | 30.7 | 90.8 |
| 79.6 | 93.5 | 44.1 | 32.2 | 37.5 | 38.1 | 89.7 | 94.5 |

Fig. 2 Showing percentage spot in each plot, 3rd. & 4th. reapings combined.

| | | | | | | | |
|------|------|------|------|------|------|-------|------|
| 83.6 | 94.6 | 97.4 | 95.1 | 79.6 | 85.5 | 95.4 | 94.7 |
| 94.2 | 83.6 | 81.5 | 63.9 | 77.5 | 76.4 | 85.6 | 68.7 |
| 76.7 | 64.0 | 93.3 | 74.2 | 88.4 | 78.1 | 75.6 | 88.1 |
| 95.6 | 93.4 | 83.5 | 82.9 | 76.6 | 85.1 | 86.1 | 87.9 |
| 96.5 | 93.2 | 90.6 | 85.7 | 90.0 | 98.4 | 100.0 | 93.5 |

Fig. 3 Showing percentage spot in each plot,
5th reaping.

Spore trapping.

Four stationary traps were placed one at each corner in the angle of the unprimed plots. A moving trap was situated external to the land on the side of the prevailing wind. Glycerine jelly slides were used as in the 1948/49 experiment, but the jelly was coloured with safranin to facilitate counting the spores.

Six exposures of the slides were made between 31/12/49 and 22/2/50, two days at a time.

The maximum number of spores caught at any time was six. A higher number of spores was always caught in the moving trap, thus a certain amount of infection was entering the land from outside.

There was no consistency in the number of spores caught in the stationary traps, the number varying from 0 to 4.

Discussion of results.

Clear-cut results would not be expected with a wind-borne disease, thus if frog-eye is wind-borne inconsistency in experimental results might be anticipated.

Results presented here suggest that Cercospora spores are borne by the wind from sources external to the land, and that primary infection is established on susceptible leaf in this way. The evidence presented is not clear-cut e.g. results of spore trapping: this is consistent with wind-borne invasion.

Secondary infection appears to be the result of spread within the land from primarily infected plants. Again, results are not well-defined but examination of Figs. 2 and 3 shows that a gradient of intensity does exist between the high percentage of spot in the corner plots and across the primed plots.

3. Effect of priming on yield and quality of tobacco.

The value of priming in field practice is still a matter of dispute. The value to be gained from priming can be resolved into two parts: a) the gain in good quality clean leaf and b) the enhanced price resulting from the sale of such leaf. Priming need not necessarily result in loss in weight per acre. An increased supply of nutrients becomes available to a smaller number of leaves in primed plants, thus increasing their weight.

The present experiments were designed to study the effect of priming on a) disease incidence - in particular frog-eye, b) yield and c) quality in terms of price per pound, percentage bright leaf, percentage heavy leaf etc. The prices used were fixed and arbitrary and bear no relation to present day market values.

a) 1948/49 Experiment - Government farm, Karoi.

The experiment consisted of a randomized block design for two treatments, primed and unprimed and each replicated 7 times. The variety used was Bonanza and the experiment was planted on land which had carried one previous crop of tobacco. Experimental plots were 1/20 acre in extent (3' x 3' planting) and each plot was surrounded by two guard rows of primed tobacco and also separated from other experimental plots by primed plots. The design is illustrated in Fig. 4. Previous experiments on effects of priming have been complicated by the junction of primed and unprimed tobacco. The presence of unprimed in the immediate vicinity of primed must increase the possibility of infection of primed tobacco from any diseased leaves on unprimed. Guard rows and plots reduce this possibility.

| | | | |
|-----------|---|--------------|---|
| P | P | P | P |
| Exp. P | P | Exp. U.P. | P |
| P | P | P | P |

Fig. 4. Showing design of priming experiment - Karoi.

The treatment given the tobacco was as follows:

Planted: 24/12/48
Pre-fertilized: 1/12/48 100 lb. cups 3:13:8 mixture.
Post-fertilized: 9/2/49 " " " " "
Primed: 7/2/49
18/2/49
3/3/49

Sampling for estimation of disease intensity.

Samples consisting of 2 - 5 plants per plot were taken at random at intervals during the growing period. Unprimed plants were stripped of the first three leaves and these were discarded to make leaf numbers comparable with primed plants. Remaining leaves on unprimed, and leaves from primed plants were incubated between sheets of absorbent paper for five days to develop barn-type spotting as previously described. The average number of spots in an arbitrary circle of 38.5 sq. cms. was calculated by counting spots within the circle at three points on each leaf. The results are illustrated graphically in Fig. 5.

Two points showed clearly from the graphs, a) the total amount of spotting on unprimed was greater than that on primed tobacco. (This was true of all 5 sets of samples taken), and b) spotting occurred up the plants, one to two leaves higher on unprimed tobacco than on primed.

Comparison of sampling results with rainfall previous to sampling by one week did not show any correlation between amount of spot and precipitation.

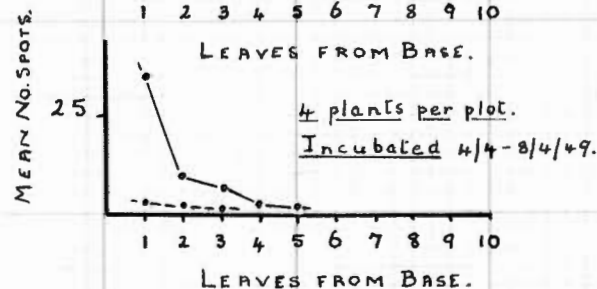
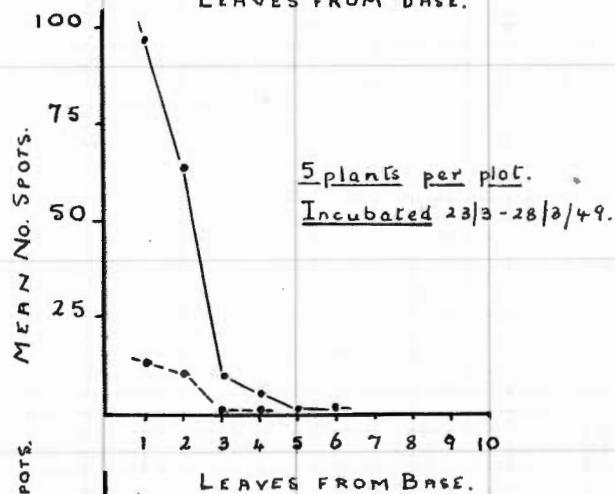
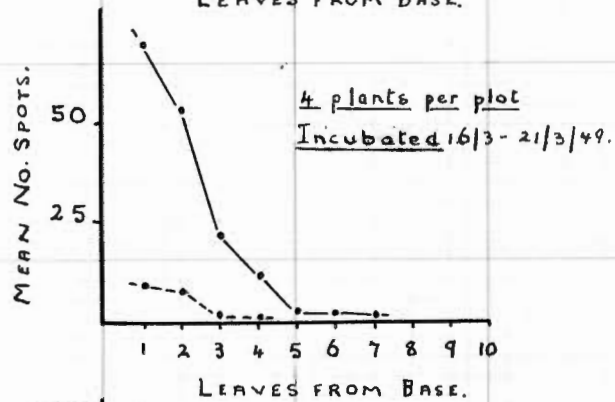
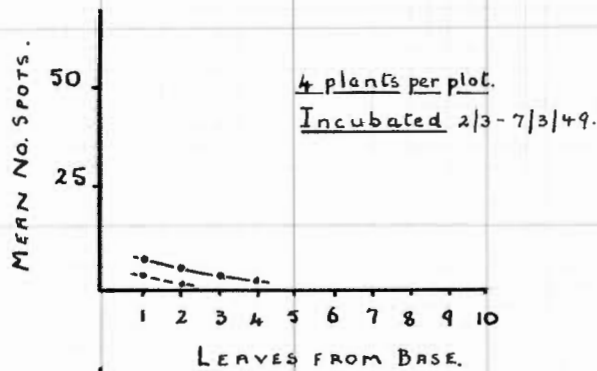
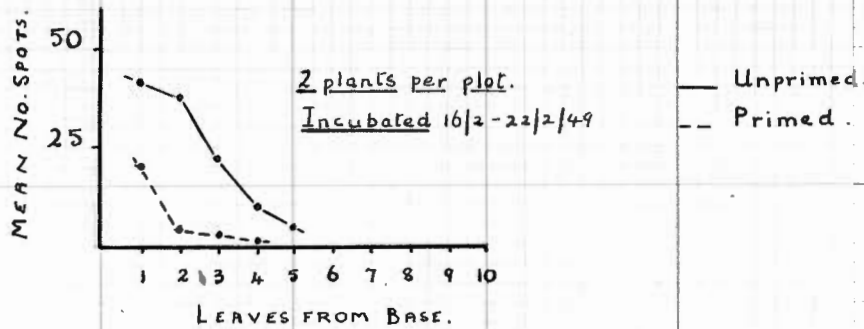


Fig. 5 Showing intensity of spot, Kerol, 1948/49.

Graded tobacco.

After curing, the tobacco from the experiment was graded to supply the following information: % barn-spotted leaf, % bright leaf, % short leaf, and % heavy leaf. These results, together with yields, price per lb. and value per acre appear in Tables I, II, and III. Results were treated statistically by the analysis of variance (Appendix 2c) and significant differences are included where appropriate.

Table I. Percentage barn-spotted leaf.

| Primed | | Unprimed | |
|----------|--------|----------|---------|
| Plot No. | % Spot | Plot No. | % Spot. |
| 124 | 6.39 | 125 | 59.12 |
| 139 | 11.91 | 126 | 41.85 |
| 151 | 28.33 | 137 | 22.06 |
| 152 | 1.46 | 138 | 59.09 |
| 163 | 11.10 | 150 | 12.65 |
| 164 | 14.44 | 165 | 39.11 |
| 176 | 4.69 | 177 | 35.74 |
| Mean | 11.19 | Mean | 38.51 |

Standard error: 7.35

Significant differences: 5% : 16.01

1% : 22.45

Table II. Percentage brights, shorts, and heavies. (Means)

| Treatment | % Brights | % Shorts | % Heavy-bodied leaf. |
|------------------|-----------|----------|----------------------|
| Primed | 21.47 | 5.82 | 5.47 |
| Unprimed | 16.12 | 5.38 | 7.68 |
| Standard errors: | 6.11 | 1.75 | 3.73 |

Table III. Yield per acre, value per acre, and price per lb.
(Means)

| Treatment | Yield/acre lbs. | Yield/acre adjusted to 100% stand. | Value/acre (adjusted value) | Price/lb. pence |
|------------------|--------------------|--|-----------------------------------|--------------------|
| Primed | 491.1 | 690.7 | £17:5:9d. | 6.03 |
| Unprimed | 540.8 | 745.7 | £17:19:9d. | 5.70 |
| Standard errors: | 57.87 | 90.83 | - | 0.37 |

The only significant difference found was for percentage spotted leaf. There was a greater percentage of spotted leaf from unprimed than from primed tobacco, significant at the 1% point.

Although differences were apparent for yield per acre and price per lb., these were not significant. In this particular experiment therefore, satisfactory control of barn-spot was obtained by priming but no financial gain accrued from the practice.

b) 1949/50 Experiment - Government farm, Karol.

This experiment was a repetition of that of the previous year. The lay-out was unchanged except that the number of replications was increased from 7 to 10 and the planting changed to 3'6" x 2'. The tobacco was treated as follows:

| | |
|------------------|-------------------------------------|
| Planted: | 24/11/49 |
| Post-fertilized: | 1/12/49 50 lb. cups 3:13:8 mixture. |
| | 16/ 1/50 " " " " " |
| Primed: | 29/12/49 |
| | 12/ 1/50 |
| | 26/ 1/50 |

Sampling for estimation of disease intensity.

Samples of two plants per plot were taken at random during the growing period and treated as described previously. Four sets of samples were taken in all. Results are illustrated graphically in Fig. 6.

Less frog-eye spot appeared than in the previous season but results of incubation confirmed the observations of the 1948/49 season, i.e. a greater amount of spotting on unprimed than on primed tobacco and a greater number of leaves affected per plant on unprimed than on primed.

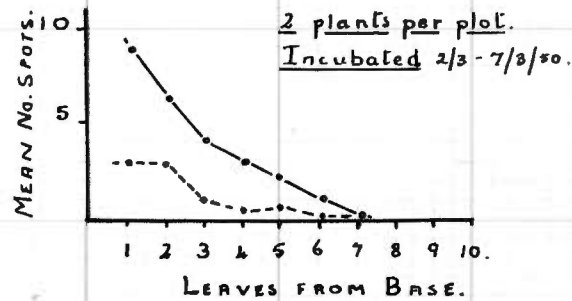
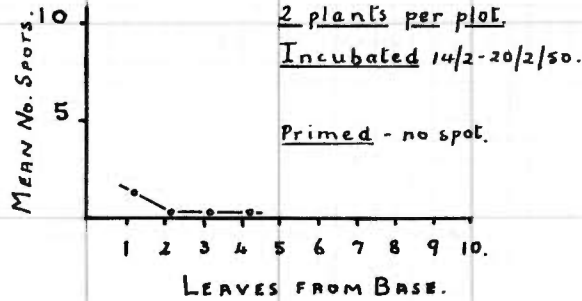
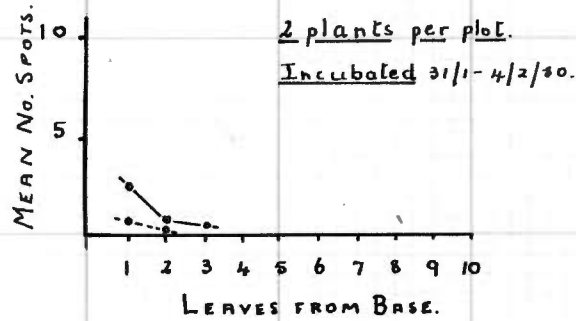
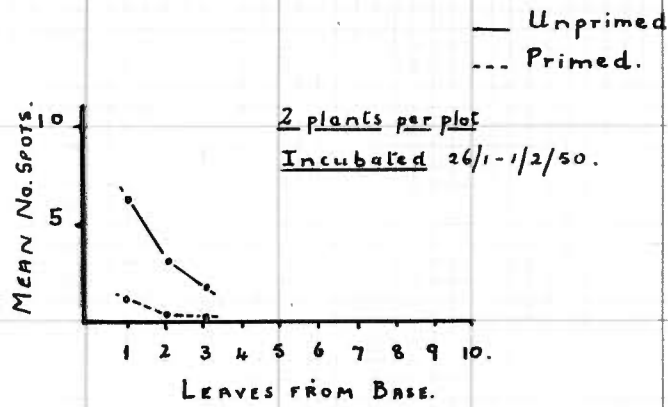


Fig. 6 Showing intensity of spot, Karoi, 1949/50.

Graded tobacco.

Information as to % barnspot, % brights, % shorts and % heavy-bodied leaf was obtained as previously. Results, together with those of yield per acre, price per lb. and value per acre are given in Tables IV, V and VI.

Table IV. Percentage barn-spotted leaf.

| Primed | | Unprimed | |
|----------|--------|----------|---------|
| Plot No. | % Spot | Plot No. | % Spot. |
| 2 | 49.5 | 1 | 45.3 |
| 3 | 44.4 | 5 | 26.8 |
| 4 | 40.3 | 6 | 31.5 |
| 7 | 21.7 | 9 | 40.1 |
| 8 | 36.5 | 10 | 36.5 |
| 13 | 28.6 | 11 | 44.2 |
| 14 | 30.3 | 12 | 27.0 |
| 16 | 42.5 | 15 | 29.3 |
| 19 | 16.8 | 17 | 34.5 |
| 20 | 20.8 | 18 | 31.5 |
| Mean | 33.14 | Mean | 34.67 |

Table V. Percentage brights, shorts, and heavies. (Mean)

| Treatment | % Brights | % Shorts | % Heavy-bodied leaf. |
|-------------------------|-----------|----------|----------------------|
| Primed | 34.62 | 4.70 | 8.71 |
| Unprimed | 42.91 | 3.95 | 3.24 |
| Standard errors: | 7.21 | 1.61 | 2.28 |
| Significant difference: | - | - | 4.76 (5%) |

Table VI. Yield per acre, value per acre, and price per lb.
(Means)

| Treatment | Yield/acre lbs. | Yield/acre adjusted to 100% stand. | Value/acre (adjusted value) | Price/lb pence |
|------------------|--------------------|--|-----------------------------------|-------------------|
| Primed | 1213.0 | 1359.6 | £36:18:5d. | 6.42 |
| Unprimed | 1147.4 | 1322.2 | £37: 1:4d. | 6.72 |
| Standard errors: | 54.73 | 48.38 | - | 0.02 |

No analysis of variance was required to show the non-significance of percentage spot. The only significant difference found in the experiment was for percentage heavy-bodied leaf, a higher proportion of which occurred in the primed plots. As in the 1948/49 experiment, yield per acre and price per lb. were not significant.

Comparing the two years, the results of yield and price per lb. were consistent in being non-significant as also were those of $\frac{1}{4}$ brights and $\frac{1}{4}$ shorts. In the 1948/49 experiment, the larger amount of heavy-bodied leaf was in the unprimed plots though the difference was not significant from primed: the reverse was found in the 1949/50 experiment with a significant difference.

In this particular experiment therefore, priming neither altered the disease picture in so far as weight of barn-spotted leaf was concerned, nor did it alter the value of the crop.

III. FUNGICIDE TRIALS.

1. Field trial 1948/49

This trial consisted of a Latin square arrangement for five treatments (incl. control) and was designed primarily for testing three recent sprays against Bordeaux mixture for control of frog-eye spot. Each treatment plot was divided into three $\frac{1}{40}$ acre sub-plots (3' x 3' planting) for time of spray application. The land used had had previously three crops of tobacco and had been fallow one year. The variety planted was Bonanza. Treatment of the tobacco was as follows:

| | | | | | |
|------------------|----------|---------------------------------|-----|-----|-----|
| Planted: | 21/1/49 | | | | |
| Pre-fertilized: | 26/11/48 | 150 lb. cups of 6:12:8 mixture. | | | |
| Post-fertilized: | 5/ 2/49 | " " | " " | " " | " " |
| | 28/ 2/49 | " " | " " | " " | " " |

Spray application.

The sprays used in this trial were as follows:

- a) Ky-Bordeaux, 4-4-50 strength.
- b) Rainfast Blitox, 4 lbs. per 100 gals.
- c) Boots copper oxychloride white oil emulsion, 1 pint per 10 gals.
- d) Dithane Z-78, 3 lbs. per 100 gals. plus trace Lissapol as spreader.

The control was unsprayed.

All sprays were applied at the rate of 60 gals. per acre (1½ gals. per sub-plot). Time intervals between spray applications were: 1 week, 10 days, and 14 days. Spraying was commenced on the 25th. Jan. 1949 and continued at the appropriate time intervals over the sub-plots till the 8th. March 1949. Sprays were applied with knapsack sprayers throughout.

Sampling for disease estimation.

Plants were sampled from the plots at intervals during the growing period up to the time of reaping. These were examined for spot development by the absorbent paper technique used in the priming experiments (page 4). At no time did any spot develop in the papers. A small amount of frog-eye spotting appeared in the experiment late in the season and it was decided to count the number of spotted plants per plot and calculate the percentage. Virtually no barn-spot appeared during the curing, hence no records were kept of graded weights from experimental plots.

Results.

Mean number of spotted plants for each treatment irrespective of time of spraying is given in Table VII, while spray-time interactions are shown in Table VIII. Significant differences are included where appropriate. (Analysis of variance - Appendix 2d.)

Table VII. Treatment means - % spot, Latin square (means of 3 sub-plots)

| Ky-Bordeaux | Blitox | Copper oxychloride | Dithane | Control |
|-------------|--------|--------------------|---------|---------|
| 40.49 | 63.68 | 61.48 | 62.53 | 68.21 |

Standard error: 7.89
Significant difference: 17.19 (5%)

The sprays were placed in the following order of efficiency:

Ky-Bordeaux better than control at the 5% point.

Ky-Bordeaux better than Blitox, Copper oxychloride and Dithane at the 5% point.

There were no significant differences between means for the control, Blitox, Copper oxychloride and Dithane.

Table VIII. Latin square re-arranged as randomized block.
Time-spray interaction, % spotted plants.

| Ky-Bordeaux | | | Rainfast Blitox | | | Copper oxychloride | | | Dithane | | | Control | | |
|-------------|-------|-------|-----------------|-------|-------|--------------------|-------|-------|---------|-------|-------|---------|-------|-------|
| 1w. | 10d. | 14d. | 1w. | 10d. | 14d. | 1w. | 10d. | 14d. | 1w. | 10d. | 14d. | 1w. | 10d. | 14d. |
| 24.45 | 36.19 | 63.79 | 59.00 | 67.10 | 64.95 | 53.87 | 62.47 | 68.09 | 54.54 | 61.98 | 67.07 | 51.40 | 71.73 | 81.48 |

Mean control: 68.20

Standard error: 9.60

Significant differences: 5% : 19.20
 1% : 25.54

The spray-time interaction figures showed significant differences to exist between Bordeaux applied weekly and at 10-day intervals, and the mean control at the 1% point, also between Bordeaux at weekly and 10-day intervals, and Bordeaux at 14-day intervals (1% point). The other three sprays were not significantly better than the controls at any time intervals.

An interesting result was the appearance of a significant difference between the controls for time of spraying(between 1 week and 10 days at the 5% point, and between 1 week and 14 days at the 1% point.) This appeared to be due to the depressant effect of the sprays on spot development where controls bordered sprayed plots. The highest amount of spot in the controls was found in those plots bordered above and below by plots sprayed at 14-day intervals, and the least in those bordered by weekly sprayed plots; the 10-day interval plots occupied an intermediate position. Correspondingly, the most spot developed in the 14-day sprayed plots and the least in weekly-sprayed plots for all the sprays, although only Bordeaux mixture showed a significant difference. Hopkins (1) found similar depressant effects in field spraying experiments in the 1946/47 season.

2. Greenhouse and field fungicide trials 1949/50

A large number of fungicides, hitherto untried on tobacco, became available for test during the 1949/50 season. Quantities available were less than 1 lb. in most cases so that no large-scale field trials were possible. Accordingly, it was decided to test these fungicides, a) in the greenhouse, and b) on a small plot scale in the field. Nineteen different fungicides, including those in general usage, were tried in the greenhouse but, owing to limitations imposed by some of the materials, the field trial contained only fifteen.

a) Greenhouse fungicide experiment.

The experiment consisted of a randomized block design for 19 treatments and a control, with 5 replications. Experimental units were Bonanza tobacco plants grown for six weeks in 5" clay pots. Each plant received the equivalent of 300 lbs. per acre of 6:12:8 fertilizer mixture two days after the seedlings were potted. In order to minimize light effects, pots were moved round the greenhouse according to a fixed plan every week.

Application of fungicides.

All sprays were applied with a Vermorel pneumatic hand sprayer. Linsapol was used as a spreader throughout. The one dust in the experiment was applied with a small hand bellows.

Plants were thoroughly coated with the fungicides individually and left to dry before inoculation.

Inoculation.

Inoculum was prepared by crushing dried tobacco leaf which had been badly infected with frog-eye. The inoculum was sieved through two thicknesses of fine muslin to give a uniform powder; this powder was dusted over the plants immediately after the fungicides had dried. Plants were lightly atomized with water as required to keep them moist.

Results.

Very few frog-eye lesions developed after 11 days. After this time, leaves were stripped from the plants and incubated in absorbent paper for three days. Incubation produced a considerable increase in the number of lesions. The data were recorded by counting the number of lesions per plant and the number of leaves affected. Figures for analysis were obtained by the division of the former figure by the latter. Means for the five replications are given in Table IX, together with details of spray concentrations and significant differences. (Analysis of variance - Appendix 2d.)

Table IX. Effectiveness of 19 fungicides against frog-eye in the greenhouse.

| Fungicide & Rate of Application per 5 plants. | | <u>Lesions per plant</u> No. leaves affected |
|--|-------------------|---|
| | | Mean. |
| Ky-Bordeaux | 3.2 gms./150 cc. | 26.8 |
| Burgundy mixture | 0.94 gms./150 cc. | 11.0 |
| 20% copper dust | 11 gms. | 16.0 |
| 50% dispersible copper. | 0.8 gms./150 cc. | 30.5 |
| Yellow cuprocide | 0.4 gms./150 cc. | 15.2 |
| B.S. potato fungicide | 0.8 gms./150 cc. | 84.0 |
| Crag (Cu.Zn. chromate) | 0.4 gms./150 cc. | 28.7 |
| Mycol | 0.5 cc. /150 cc. | 32.6 |
| Pesco Gwaai Spray | 0.5 gms./150 cc. | 47.5 |
| Bloquin | 0.4 gms./150 cc. | 50.8 |
| Bloquin 100 | 0.4 gms./150 cc. | 21.7 |
| Goodrite Z.A.C. | 0.8 gms./150 cc. | 28.8 |
| Dithene Z-78 | 0.8 gms./150 cc. | 18.4 |
| Fermate | 0.4 gms./150 cc. | 45.7 |
| Parzate | 0.4 gms./150 cc. | 73.6 |
| Zerlate | 0.4 gms./150 cc. | 77.0 |
| Ethyl mercury phosph. | 0.01 gms./150 cc. | 29.8 |
| Ethyl mercury chlor. | 0.01 gms./150 cc. | 37.9 |
| Phenyl mercury chlor. | 0.01 gms./150 cc. | 29.4 |
| Control untreated | | 68.0 |

Standard error: 4.57

Significant differences: 5% : 9.14, 1% : 12.16.

All the fungicides, except Parzate, Zerlate and B.S. potato fungicide gave significantly lower disease counts than the control at the 1% point. B.S. potato fungicide gave a significantly higher count than the control at the 1% point. The cause of this is not known: all five replicates were in fairly close agreement. Yarwood (6) has recorded that Bordeaux mixture frequently showed a stimulatory effect on the development of powdery mildews on beans, under certain conditions of illumination. It is possible that similar conditions were operative here.

Burgundy mixture, Yellow cuproside, 20% copper dust and Dithane Z-78 were the most effective fungicides against frog-eye. The first fungicide gave better control than Bordeaux at the 1% point, and the second and third at the 5% point.

None of the fungicides caused any visible signs of damage to the plants during the course of the experiment.

b) Field fungicide trial - Trelawney 1949/50.

In addition to testing new fungicides under field conditions, this trial was by way of being an experiment in itself to determine the suitability or otherwise of the small plot technique.

Plots consisted of 15 Bonanza plants on a 3'6" x 2' spacing. There were fifteen fungicidal treatments and an untreated control with 5 replications of each arranged as a randomized block.

On the experience of the 1948/49 spray trial, where results showed that adjoining plots exerted a considerable influence over one another, it was decided to isolate the experimental units in the present trial by means of guard plots as in the Karoi priming experiment (page 10). Guard plots were of the same size and shape as the experimental plots and consisted of unsprayed tobacco.

Details of treatment given the tobacco were as follows:

| | | | | | |
|------------------|----------|---------|------|--------|----------|
| Planted: | 16/12/49 | | | | |
| Pre-fertilized: | 15/12/49 | 100 lb. | cups | 6:12:8 | mixture. |
| Post-fertilized: | 11/ 1/50 | 150 lb. | " | " | " |
| | 19/ 1/50 | " | " | " | " |
| | 26/ 1/50 | " | " | " | " |

Application of fungicides.

Sprays were applied with a Vermorel hand sprayer as in the greenhouse experiment. The dust was applied with a Cooper-Pegler hand bellows. The rate of spray application was 3 pints over five plots (approx. 30 gals. per acre). This was found to give adequate coverage without runoff. Lissapol was used as a spreader with all the sprays. Fungicide application was commenced on the 25th. Jan. 1950 and continued at weekly intervals till the 23rd. Feb. Notes on the applications are given in Table X.

Table X. Weather conditions on days of fungicide application.

| | |
|------------------------|---|
| 1st. spraying 25/1/50. | Clear, hot day. No wind. All fungicides applied between 7.0 a.m. & 1.0 p.m. |
| 2nd. spraying 2/2/50 | Clear, very hot day. No wind. All fungicides applied between 7.0 a.m. and 11.0 a.m. |
| 3rd. spraying 9/2/50 | Clear, cool day. Windy. All sprays applied between 7.0 a.m. and 11.0 a.m. |
| 4th. spraying 16/2/50 | Clear, hot day. No wind. All sprays applied between 8.0 a.m. & 12 noon. |
| 5th. spraying 23/2/50 | Clear, hot day. No wind. All sprays applied between 7.0 a.m. and 11.0 a.m. |

Disease estimation.

An estimate of the amount of frog-eye spotting was made in the land on the 3rd. March 1950. Plants were arbitrarily classed into three infection groups and the results were reduced to a single figure by the McKinney method (3) (page 15, part I). An analysis of variance showed there to be no significant differences between the means for treatments.

Figures for comparison of the effectiveness of the fungicides were obtained from graded leaf. Tobacco was taken from the trial in two reapings only, one on the 4th. March and the second on the 22nd. March 1950. Leaf from each reaping was graded into three barn-spotted classes and a clean grade. The three spotted classes were reduced to a single figure by the McKinney method (3). Data from each reaping and for the two reapings combined

appear in Table XI. together with details of the fungicides and significant differences (Analysis variance - Appendix 2d.)

Table XI. Effectiveness of 15 fungicides against frog-eye in the field.

| Fungicide & Rate of Application | | Mean Disease Data (% spot) | | |
|---------------------------------|----------------------|----------------------------|-----------|----------|
| | | 1st.reap. | 2nd.reap. | Combined |
| Ky-Bordeaux | 4-4-50 | 48.48 | 43.48 | 45.98 |
| Burgundy mixture | 1-1½-50 | 52.42 | 37.56 | 44.99 |
| 20% copper dust | 33 gms./plot | 61.28 | 45.42 | 53.35 |
| Yellow cuprocide | 1½ lbs./100 gals. | 65.52 | 36.70 | 51.10 |
| B.S. potato fungicide | 4 lbs./100 gals. | 59.48 | 42.86 | 51.17 |
| Greg (Cu.Zn. chromate) | 2 lbs./100 gals. | 65.10 | 57.26 | 61.18 |
| Mycol | 2 pints/100 gals | 69.92 | 59.14 | 64.53 |
| Pesco Gweal Spray | 5 ozs./10 gals. | 54.72 | 54.32 | 54.62 |
| Bioquin | 1½ lbs./100 gals. | 62.20 | 66.08 | 64.14 |
| Bioquin 100 | 1½ lbs./100 gals. | 68.20 | 55.02 | 61.60 |
| Goodrite Z.A.G. | 2 lbs./100 gals. | 66.72 | 50.10 | 58.41 |
| Dithane Z-78 | 2 lbs./100 gals. | 54.64 | 48.44 | 51.54 |
| Fermate | 2 lbs./100 gals. | 61.88 | 66.18 | 64.03 |
| Parzate | 2 lbs./100 gals. | 63.34 | 51.66 | 57.50 |
| Zerlate | 2 lbs./100 gals. | 65.62 | 44.64 | 55.13 |
| Control untreated. | | 69.86 | 58.94 | 64.40 |

Standard error: 6.79

Significant difference: 5% : 13.58.

There were no significant differences in percentage barn-spot in the first and second reapings taken separately. Combined data to which significant differences refer, showed Burgundy mixture and Bordeaux mixture to be better than the control at the 5% point. No other fungicide was better than the control.

Phytotoxic effects.

Plants were examined regularly during the course of the experiment for any damage caused by the fungicides. No signs of damage were noticeable after the first spraying. After the second spraying, brown necrotic spotting was found on the lower leaves of the Bordeaux and Burgundy treated plants. All five replicates were affected. Effects were noticed two days after spraying and it was concluded that they were due to the high temperature prevailing on the day of fungicide application, (Table X.).

After the third spraying, there was a faint brown necrotic spotting present on the plants treated with B.S. potato fungicide, and several plants in two replicates of the Yellow cuprocide plots had slight spotting on the lower leaves.

Necrotic spotting as described above, is consistent with damage due to copper.

Very distinctive toxic effects, very different from those already mentioned, appeared after the third spraying in the plots treated with Bioquin: no phytotoxic

effects were noted in the Bioquin 100 plots. The injury took the form of interveinal chlorotic patches, principally on the tops of plants, with an accompanying distortion and puckering of the leaves. Later, the chlorotic areas were killed and became brown. The effects were very similar to those caused by some viruses. Damage appears to have been due to accumulation of the fungicide as no effects had been noted previously.

Retentive properties of fungicides.

No chemical analyses were made on leaf coated with fungicide, hence no absolute figures are available for spray retention. However, most of the fungicides were readily seen on the leaf surface after application and their relative retentive properties could be estimated visually.

Plots were examined after the last spraying and again after a fall of 2.71 ins. of rain. There was good retention of Bordeaux, Burgundy, Yellow cuprocide and Crag. No other fungicides were visible on the leaves after the same fall of rain.

Greenhouse and field results compared.

The superior fungicidal properties of Burgundy mixture were evident in both trials. Yellow cuprocide, 20% copper dust and Dithane Z-78 which were effective in the greenhouse did not give significant control in the field. Bordeaux mixture, however, showed the same order of efficiency as Burgundy in the field though it did not

show up as well in the greenhouse.

There are several explanations that can be offered to account for the differences between greenhouse and field results. The factor having the biggest influence is climate. It was noticed that with 20% copper dust the fungicide did not remain very long on dry leaf presumably being removed by wind and shaking of the plants. This probably accounted for the considerably greater fungicidal power of the dust under the moist, still atmospheric conditions of the greenhouse. The nature of the leaf surface is another factor to be taken into account when considering plants grown under different conditions of humidity and illumination: the adhesiveness of fungicides is largely dependent on the nature of the leaf surface.

Results of the two trials serve to show that the true criterion of fungicidal power is to be found by testing the fungicides under field conditions, and that at any rate under local conditions, greenhouse trials have limited applicability only.

Small plots versus large.

Small plots have the advantage of compactness and easy handling. They have the disadvantage, however, of being subject to greater error than large plots due to the smallness of the population. This can be overcome to a certain extent by increased replication of treatments.

In order to compare the relative efficiency of the large and small plots in the 1948/49 and 1949/50 seasons respectively, the standard errors of the means of these trials were expressed as percentages of their general means. Contrary to expectation, the large plot experiment (121 plants on a 3' x 3' spacing) gave a higher percentage error than the small plot experiment (15 plants on a 3'6" x 2' spacing). The figures obtained were 9.30% and 6.71% respectively. A satisfactory explanation of this result may be advanced if the methods of fungicide application are considered. Fungicides were applied to large plots by means of knapsack sprayers. It is difficult with this equipment to secure complete and uniform coverage of the plants, whereas in the small plot trial, the hand sprayer used enabled thorough coverage of the leaves. Disease counts under the latter conditions would give a more uniform result than under the former, with a consequent reduction in the error due to variation. It is essential, however, with small plots that the percentage stand be at or near 100% as reduction of the stand by even two or three plants constitutes a large percentage of the plot population.

IV. BARN SPOT.

1. Relation of frog-eye to barn-spot.

Barn-spot is the jet black spotting which develops in the curing barns on leaf infected with frog-eye. This type of spot constitutes the most serious aspect of frog-eye and control measures in the field are aimed primarily at reducing the barn-spot phase of frog-eye.

Barn-spotting results, not only from visible frog-eye lesions on the leaf, but also from incipient lesions and from spores lying on the leaf surface.

The exact nature of barn-spot is not known. The black spotting may be the result of the fungus entirely, or it may be due to death of the leaf tissue around the lesions. Examination of a large number of barn-spot lesions microscopically has invariably shown the hyphae of the fungus to extend up to the edge of the lesions, thus it seems likely that barn-spot is due to luxuriant growth of the fungus itself under the favourable conditions prevailing in the barns. Preliminary experiments with frog-eye infected leaf to determine, a) the effect of temperature on barn-spot and b) the effect of delimiting frog-eye lesions on the subsequent development of barn-spot, are reported below.

2. Effect of temperature on barn-spot.

The usual method of controlling barn-spot is to commence the curing procedure at 100° F, instead of 90° F in a saturated atmosphere. This has the effect of inhibiting the fungus and results in very much smaller lesions. It was thought to be of interest to compare the development of barn-spot at various temperatures and to combine the experiment with the delimitation of frog-eye lesions.

Three sets of leaves were taken from the land as follows:

- a) Apparently clean leaf.
- b) Leaf with incipient lesions (light green areas on leaf)
- c) Leaf with obvious lesions.

The areas covered by obvious and incipient lesions were carefully cut out, using a scalpel or cork-borer. Four leaves from each set were placed in barns at 90°, 100°, 120° and 150°. Four control leaves for treatments (b) and (c) i.e. with lesions not delimited were included with each set. Leaves were left in the barns for 16 hrs. Results are given in Table XII.

Table XII. Barn-spot and temperature.

| Temperature | Treatment | Observations. |
|-------------|--------------------|--|
| 90° F. | Clean leaf. | No spotting. |
| | Spot delimited. | Leaves spotted in areas not delimited. No damage beyond delimitations. |
| | Spot control. | Severe spotting. |
| | Incipient delim. | Leaves spotted apart from those delimited. |
| | Incipient control. | Light spotting. |
| 100° F. | Clean leaf. | No spotting. |
| | Spot delimited | No spotting outside delimited areas nor damage beyond delimitations. |
| | Spot control | Spotting present, but only light. |
| | Incipient delim. | No spotting outside delimited areas nor damage beyond delimitations. |
| | Incipient control. | Very light spotting. |
| 120° F. | | |
| 150° F. | All treatments | No spotting additional to that already on leaf. |

Results showed that frog-eye spots and latent barn-spots were inhibited from further development at 100° F.

Delimitation of lesions indicated that barn-spots were due entirely to the growth of the fungus and not to death of the leaf in advance of the fungus. Barn-spotting due to germination of spores on the leaf was confirmed by the

appearance of spots at 90° on leaves with incipient lesions delimited: spots developed on apparently clean areas of the leaf and it was concluded that this was due to the germination of spores.

V. SUMMARY.

1. Frog-eye appears to originate from sources external to lands, and sets up primary infection on susceptible leaf. Secondary infection results from spread from primary infection e.g. from unprimed to primed plants. Yield and quality of tobacco were found to be unaffected by priming. In 1948/49, priming resulted in significantly less barn-spotted leaf but not in 1949/50. Field samples of leaf incubated in absorbent papers showed less spot on primed than unprimed leaf in both seasons.
2. Field fungicide trials in 1948/49 and 1949/50 showed that Bordeaux and Burgundy mixtures were the most effective fungicides against frog-eye of a large number tested. A greenhouse experiment with the same fungicides showed some to be effective which did not show up well in the field.
3. Barn-spot was found to be due to the luxuriant growth of the frog-eye fungus and not to death of the host tissue in advance of the fungus. Spotting was reduced by placing the leaf at a temperature of 100° F. in the curing barns as against 90° F. normally.

VI. LITERATURE CITED.

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APPENDIX.1. Notes on field culture of tobacco.

Some brief notes are appended below to indicate the general cultural operations adopted for field experiments with tobacco in Rhodesia.

- a) Seed-beds are sown from early September till early November. Seedlings are planted to the lands on the advent of suitable rain ($\frac{1}{2}$ to 1" fall required depending on time of season) when approximately 8 weeks old.
- b) Lands are prepared by hilling or ridging at the desired spacing. The old method was to hill at 3' x 3", but ridges spaced 3'6" apart are in general use now, plants being placed 2' apart on the ridges.
- c) Fertilizer is applied with small cups (sizes range from those supplying 50 lbs., to 200 lbs. fertilizer per acre on a 3' x 3 $\frac{1}{2}$ " planting.) The fertilizer is applied either before planting, in which case a cupful is placed at the base of the straws marking planting stations and covered by ridging, or after planting by placement in a small hole 3-4" from the stems of the plants. The rate of fertilizer is entirely dependent on the type of soil and the weather conditions prevailing.
- d) Reaping is commenced 8 to 9 weeks after planting out (again this is very much dependent on weather). Reaping consists of taking 2 to 3 leaves per plant by hand. Selection of the leaves is by judgement of the correct colour.
- e) After tying the leaves in 'hands' on sticks, they are then transferred to the curing barns. Curing takes 6 to 8 days, and consists of three stages, 1) colouring, 90 - 100° F. in a moist atmosphere, 2) fixing the colour, 110 - 125° F. with moisture decreased, 3) drying the leaf, 130 - 160° F. with no moisture, and ventilators open to release moisture from the leaf.
- f) Leaf is ready for grading subsequent to curing, but is generally 'bulked' or baled until field operations cease and labour becomes available for grading.

2. Statistical data.a) Analyses of variance - Alternaria - field fertilizer trials.Romsey, Sinoia.
1st. count

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|-------------------|-----|----------|---------|---------|
| Treatments | 322.3 | 10 | 32.23 | 0.52 | 2.00 5% |
| Replicates | 91.8 | 4 | 22.90 | 0.37 | 2.62 " |
| Error | 2455.9 | 40 | 61.40 | | |
| Total | 2870.0 | 54 | | | |

2nd. count.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|--------|-----|----------|---------|---------|
| Treatments | 101.5 | 10 | 10.15 | 0.32 | 2.00 5% |
| Replicates | 513.3 | 4 | 128.30 | 4.07 | 2.62 " |
| Error | 1260.7 | 40 | 31.52 | | |
| Total | 1875.5 | 54 | | | |

Kyogle, Karoi.
1st. count.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|--------|-----|----------|---------|---------|
| Treatments | 1307.1 | 10 | 130.71 | 0.85 | 2.00 5% |
| Replicates | 345.8 | 4 | 86.45 | 0.56 | 2.62 " |
| Error | 6122.4 | 40 | 153.06 | | |
| Total | 7775.3 | 54 | | | |

2nd. count.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|--------|-----|----------|---------|---------|
| Treatments | 1219.3 | 10 | 121.93 | 0.93 | 2.00 5% |
| Replicates | 508.7 | 4 | 127.18 | 0.97 | 2.62 " |
| Error | 5215.6 | 40 | 130.39 | | |
| Total | 6943.6 | 54 | | | |

b) Analysis of variance - Alternaria -greenhouse variety expt.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|--------|-----|----------|---------|--------------------|
| Varieties | 1228.1 | 10 | 122.81 | 2.70 | 2.00 5% 2.66 1% |
| Replicates | 241.1 | 4 | 60.27 | 1.32 | 2.61 5% |
| Error | 1817.1 | 40 | 45.42 | | |
| Total | 3286.3 | 54 | | | |

c) Analyses of variance - Priming experiments, Karol.1948/49

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-------------------------------|-----------|-----|----------|---------|---------|
| <u>Yield/acre</u> | | | | | |
| Treatments | 18,000.2 | 1 | 18,000.2 | 1.53 | 4.75 5% |
| Error | 140,701.8 | 12 | 11,725.1 | | |
| Total | 258,702.0 | 13 | | | |
| <u>Yield/acre (corrected)</u> | | | | | |
| Treatments | 10,587.5 | 1 | 10,587.5 | 0.37 | " |
| Error | 346,560.9 | 12 | 28,880.1 | | |
| Total | 357,148.4 | 13 | | | |
| <u>% spot</u> | | | | | |
| Treatments | 2613.98 | 1 | 2613.98 | 13.81 | " |
| Error | 2271.47 | 12 | 189.28 | | 9.33 1% |
| Total | 4885.45 | 13 | | | |
| <u>% brights</u> | | | | | |
| Treatments | 100.2 | 1 | 100.2 | 0.77 | 4.75 5% |
| Error | 1568.0 | 12 | 130.7 | | |
| Total | 1668.2 | 13 | | | |

contd. overleaf.

Priming expts. contd.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|------------------|--------|-----|----------|---------|---------|
| <u>% shorts.</u> | | | | | |
| Treatments | 0.7 | 1 | 0.7 | 0.065 | 4.75 5% |
| Error | 129.4 | 12 | 10.8 | | |
| Total | 130.1 | 13 | | | |
| <u>% heavy</u> | | | | | |
| Treatments | 11.75 | 1 | 11.75 | 0.20 | " |
| Error | 584.50 | 12 | 48.70 | | |
| Total | 596.25 | 13 | | | |
| <u>Price/lb.</u> | | | | | |
| Treatments | 0.43 | 1 | 0.43 | 0.79 | " |
| Error | 6.48 | 12 | 0.54 | | |
| Total | 6.91 | 13 | | | |

1949/50

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------------------------|---------|-----|----------|---------|---------|
| <u>Yield/acre</u> | | | | | |
| Treatments | 21,516 | 1 | 21,516 | 1.43 | 4.41 5% |
| Error | 269,699 | 18 | 14,983 | | |
| Total | 291,215 | 19 | | | |
| <u>Yield/acre (corrected)</u> | | | | | |
| Treatments | 7004 | 1 | 7004 | 0.59 | " |
| Error | 21,067 | 18 | 11,704 | | |
| Total | 21,768 | 19 | | | |
| <u>% brights</u> | | | | | |
| Treatments | 343.6 | 1 | 343.6 | 1.31 | " |
| Error | 4729.0 | 18 | 262.7 | | |
| Total | 5072.6 | 19 | | | |

contd. overleaf.

Priming expts. contd.

| Source variance | SS | D/F | Variance | V.ratio | f. |
|------------------|-------|-----|----------|---------|---------|
| <u>% shorts</u> | | | | | |
| Treatments | 2.8 | 1 | 2.8 | 0.21 | 4.41 5% |
| Error | 237.8 | 18 | 13.1 | | |
| Total | 240.6 | 19 | | | |
| <u>% heavy</u> | | | | | |
| Treatments | 149.6 | 1 | 149.6 | 5.68 | " |
| Error | 474.0 | 18 | 26.3 | | |
| Total | 623.6 | 19 | | | |
| <u>Price/lb.</u> | | | | | |
| Treatments | 0.44 | 1 | 0.44 | 3.38 | " |
| Error | 2.36 | 18 | 0.13 | | |
| Total | 2.80 | 19 | | | |

d) Analyses of variance - Fungicide trials1948/49 Latin square (combined sub-plots)

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|-----------|-----|----------|---------|---------|
| Rows | 1,428.90 | 4 | 376.30 | 0.27 | 3.26 5% |
| Columns | 2,067.70 | 4 | 689.23 | 0.49 | |
| Treatments | 13,968.74 | 4 | 3492.18 | 3.28 | |
| Error | 16,838.06 | 12 | 1403.17 | | |
| Total | 38,844.22 | 24 | | | |

1948/49 Randomized block (sub-plots)

| Source variance | SS | D/F | Variance | V.ratio | f. |
|-----------------|-----------|-----|----------|---------|------------|
| Blocks | 1,905.29 | 4 | 476.32 | 2.06 | 1% 3.65 5% |
| Treatments | 13,867.40 | 14 | 990.52 | 4.29 | 2.50 1.92 |
| Error | 12,917.76 | 56 | 230.67 | | |
| Total | 28,690.45 | 74 | | | |

Greenhouse trial 1949/50

| Source variance | SS | D/F | Variance | V.ratio | f. | |
|-----------------|--------|-----|----------|---------|------|------|
| Treatments | 2608.2 | 19 | 137.2 | 2.59 | 1% | 5% |
| Replicates | 50.8 | 4 | 12.7 | 0.24 | 2.12 | 1.70 |
| Error | 4015.8 | 76 | 52.8 | | | |
| Total | 6674.8 | 99 | | | | |

Field trial 1949/50 (1st. & 2nd. reapings combined.)

| Source variance | SS | D/F | Variance | V.ratio | f. | |
|-----------------|----------|-----|----------|---------|------|----|
| Treatments | 12,703.3 | 15 | 846.9 | 1.83 | 1.81 | 5% |
| Replicates | 1,017.6 | 4 | 254.4 | | | |
| Error | 27,704.9 | 60 | 461.8 | | | |
| Total | 41,425.8 | 79 | | | | |

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