

## SOME STUDIES ON TOBACCO DISEASES IN CEYLON—II

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### FIELD SPRAYING AGAINST FROG-EYE *CERCOSPORA NICOTIANAE* E. & E.

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**I**N the first part of this series of articles (1937), details were given of an experiment which demonstrated the efficacy of spraying tobacco seedlings in the nursery with colloidal copper in reducing the incidence of frog-eye infection on plants after transplanting into the field. Subsequent observations indicated that the protective effect of the fungicide was of limited duration in this instance. Leaves developing subsequent to transplanting were not sprayed and were therefore susceptible to the disease. Further, the sprayed leaves were removed at the first priming. The presence of unsprayed plants in the control blocks may have had a considerable bearing on the final extent of infection of the plants. It is not proposed to discuss here the ultimate effect of nursery spraying on the extent of frog-eye attack on tobacco in the field. It is obvious that the full value of such nursery spraying would be obtained only if large areas were planted up with sprayed nursery plants and, even then, the effect might well be cumulative and become more marked if the treatment were repeated in subsequent seasons. It is proposed to investigate this question further.

To revert, observations made about six weeks after transplanting indicated that frog-eye infection was present on every plant. The disease appeared to be of average severity, infection being considered to be neither exceptionally great nor

exceptionally light. No exact criteria of infection are available but it may be assumed that the extent of infection was fairly average.

In a high-class, flue-cured tobacco, the presence of numbers of frog-eye spots which appear before curing and of barn-spots which develop during curing and which have been shown to be due to infections by the same parasitic fungus, results in a considerable reduction in the value of the cured leaf. It was felt that, if the disease could be adequately controlled by one field spraying, such a spraying might be economically practicable and perhaps profitable.

The experiment described below was designed to determine the degree of control of frog-eye which could be obtained by a single spraying of plants in the field. The experiment was also framed to determine, within wide limits, the stage of growth of the tobacco at which such a spraying would give best results.

#### LAY-OUT OF THE EXPERIMENT

The lay-out took the form of five randomized blocks, each of four plots. Each plot consisted of four rows of 15 plants. Records were taken from the central 26 plants (2 rows of 13 plants) in each plot, the remaining 34 plants serving to overcome border effects. The experimental material consisted of a fairly uniform stand of plants of Harrison's Special tobacco grown at the Experiment Station, Wariyapola. These were derived from the nursery which was described in the first article of this series and were plants grown from seed surface-sterilized with silver nitrate solution, which had been sprayed in the nursery with colloidal copper. The area containing the plots was adjacent to the four-acre field on which were made the observations previously recorded.

The plants chosen for experiment were transplanted from the nursery during the period 14-16 November, 1936. The area was manured twice—once before and once after transplanting—with the following manure mixture:—

Superphosphate	..	224 lb.
Sulphate of potash	..	140 lb.
Nitrate of soda	..	184 lb.
		<u>548 lb. per acre</u>

The plants were primed for the first time on 28th December, 1936, and for the second time about a month later.

The plants were topped when the flowering shoot developed. At the time of the second spraying, on 26th January, 1937, about 30 per cent. of the plants had been topped, the remainder being topped soon after this date.

The following four treatments were randomized within each block :—

<i>Treatment No.</i>	<i>Treatment</i>
0	Unsprayed control
1	Sprayed with colloidal copper on 7-1-37
2	Sprayed with colloidal copper on 26-1-37
3	Sprayed with colloidal copper on 20-2-37

#### TECHNIQUE

*Spraying.*—Spraying was done with a pneumatic knapsack sprayer working at a pressure of 75 lb. per sq. in. The spray-lance was fitted with an angle-bend to facilitate the spraying of the lower surface of the leaves and the single nozzle was of the type which delivers a fine mist of spray.

The spray mixture used consisted of—

- 1 oz. ' Bouisol ' colloidal copper
- 1/16 oz. Agral 2
- 1 gallon water.

Bouisol is a proprietary fungicide consisting of a suspension of a basic copper derivative containing sulphite lye as the protective colloid. Agral 2 is a proprietary spreader having a composition allied to a sulphonate of an alkylated hydrocarbon. Six to eight gallons of the spray mixture were used at each spraying, which works out at about one gallon of spray to twenty plants. The plants were sprayed very thoroughly and it is probable that the amount of spray used was more than was necessary. It was certainly greater than the amount which would be used for the same number of plants in large-scale field spraying.

A noteworthy point is that no spray scorch was observed in the experimental plots at any time.

*Harvesting.*—The leaves from the experimental plots were harvested at the same time as those in the rest of the tobacco area. The usual practice adopted at Wariyapola is to harvest first all sand leaves. The leaves harvested in this first or preliminary harvest are not flue-cured but are air-cured and the resulting cured tobacco is of little value, being sold locally for chewing purposes. The main harvest which follows this includes all leaves which are mature and which are sufficiently clean to be worth flue-curing. Any leaves which are badly spotted are collected separately and air-cured. Leaves which are not sufficiently mature at the time of the main harvest are harvested later and flue-cured if sufficiently clean.

The time of the harvests may vary considerably from year to year with varying weather conditions. During the period of the experiment, the weather was relatively dry and, in consequence, the crop matured rather earlier than usual. The spraying programme, which had been laid down according to average conditions, was thereby not so well timed as it might have been. The first or preliminary harvest was made on 17th February, 1937, and the second or main harvest on 23rd February, 1937. It will be seen that the final spraying, which was done on 20th February, was carried out after the preliminary harvest and only three days before the main harvest. The intention was, when the experiment was planned, to carry out the final spraying ten days or so before the first harvest.

The early maturing of the tobacco was unfortunate in that, owing to a misunderstanding, the preliminary harvest was completed before a record of disease was made. It is considered, however, that this fact had no marked effect on the results obtained since all plants were of the same age and it will be shown later that the differences between the total numbers of leaves left after the preliminary harvest were not mathematically significant.

*Recording of Results.*—On the day before the second or main harvest, a count was made of the total number of leaves and of the number of “clean” leaves on each plant of the plots. The leaves examined at this time included many which were immature and which were not gathered with the main harvest. Leaves with 0 to 2 frog-eye lesions were classed as “clean” leaves.

On the following day, the leaves from each plot were harvested and flue-cured separately.

Following the usual practice, badly spotted leaves were not harvested for flue-curing, but were harvested separately and air-cured. The weights of the flue-cured leaf from the various plots were, in consequence, in themselves a measure of the relative freedom of the plants from frog-eye.

The harvested leaves from Block D were accidentally mixed with those from the remainder of the field and data of this block after curing were therefore unobtainable.

Rainfall records were kept throughout the experiment.

### RESULTS

It has been stated above that the plots on which the experiment is based consisted of 26 plants. In many of the plots vacancies occurred, the lowest number of plants in any one plot being 22 at the conclusion of the experiment. The data recorded below are therefore based on plots of 22 plants, the excess being eliminated at random in each instance.

The experimental area was selected for the uniformity of stand of tobacco and it may fairly be assumed that there were no significant differences in the numbers of leaves present on the plants in the various treatments before the first or preliminary harvest. In table I are recorded the total numbers of leaves and the numbers of "clean" leaves in the plots (of 22 plants) at the time of the first examination, five days after the preliminary harvest.

An analysis of variance of the total numbers of leaves yielded, for treatments, a value of  $z$  lower than the 5 per cent. point. From this it may be assumed that the population, as determined by numbers of leaves, was a homogeneous one after the first or preliminary harvest. It may therefore be concluded that this harvest did not take the form of a selective picking.

In the following analyses no correction has been made for leaf numbers, as such a correction was considered to be unnecessary. Differences shown to be significant by the crude analysis would merely have been accentuated if such a correction had been made.

TABLE I  
COUNT OF LEAVES IN PLOTS BEFORE MAIN HARVEST

	TREATMENTS						Totals		
	Unsprayed control (0)		Sprayed on				Total	" clean "	
	Total	" clean "	7-1-37 (1)	26-1-37 (2)	20-2-37 (3)	Total			
Block A	156	29	195	155	111	199	5	705	160
"	156	9	176	136	103	152	15	620	137
"	150	10	175	173	101	157	17	655	134
"	182	18	195	169	113	178	15	724	155
"	157	2	150	127	58	132	4	566	68
Totals	801	68	891	760	486	818	56	3270	654
Means	160.2	13.6	178.2	152.0	97.2	163.6	11.2	163.5	32.7

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*Results of Field Observations.*—In the observations recorded in table I, the term “clean leaves” was arbitrarily adopted to include those leaves which had up to two frog-eye spots on them. This standard was used since the presence of two spots on leaves was, for all practical purposes, negligible and the higher figures so obtained were more useful for comparison. An analysis of variance of the numbers of these “clean” leaves in the 22 plant plots is given in table II.

The value for  $z$  for treatments is very much higher than the 1 per cent. point, indicating a high level of significance. From this analysis, it may be concluded that the first and third sprayings had no effect on the incidence of frog-eye on the plants as measured immediately before harvesting. The mean numbers of clean leaves in these two treatments were actually less than in the controls although the differences were well within the limits of normal variation, and were therefore not significant. It is concluded that the first spraying was done too early and the final spraying, as was expected, was done too late to be effective. On the other hand, the effect of the second spraying, as judged by the number of clean leaves on the plants before harvesting, was most marked.

In comparing these figures with those of cured leaves, it should be remembered that these were derived from a count of all the leaves on the plants. Immature leaves and badly spotted leaves were not gathered for flue-curing at the main harvest.

*Results of Examination of Cured Leaf.*—As is stated above, the second or main harvest was flue-cured and the cured leaf was brought to Peradeniya for detailed examination in the laboratory. The cured leaf from each of the plots was first weighed and then counted. Subsequently, the cured leaves were classified according to the numbers of lesions, of both frog-eye and barn-spot, found on them. Here again, an arbitrary classification had to be adopted. Barn-spot lesions are usually small and a cured leaf with up to 50 lesions is, for all practical purposes, clean and of good appearance. The leaves were therefore classified in groups of leaves with 0–25, 26–50, 51–100 and more than 100 lesions.

The data obtained are recorded in table III.

TABLE II

ANALYSIS OF VARIANCE OF RECORD OF "CLEAN" LEAVES IN PLOTS BEFORE MAIN HARVEST

Due to	Degrees of Freedom	Sum of squares of Deviations	Variance	$\frac{1}{2} \log_e$	Z	One per cent. point
Blocks	4	1357.7	339.42	2.9136		
Treatments	3	27792.6	9264.2	4.5669		
Error	12	1315.9	109.66	2.3490	2.2179	0.8919
Total	19	30466.2				

Standard Deviation = 10.48

Co-efficient of variability = 32.1%

Mean Numbers of "Clean" Leaves per Plot	S.E. of Mean of 5 plots	Significant difference
(0)		
(1)		
(2)		
(3)		
General Mean		
13.6	4.68	15.25
8.8		
97.2		
11.2		
32.7		

TABLE III  
DATA FROM CURED LEAF

Treatment	Block	Wt. of cured leaf in Drachms	Total No. of cured leaves	Nos. of leaves with			
				0-25 lesions	26-50 lesions	51-100 lesions	> 100 lesions
(0) Unsprayed control	A	53	22	1	1	8	12
	B	32	15	0	1	1	13
	C	42	15	0	1	1	13
	E	42	24	0	1	1	22
(1) Sprayed on 7-1-37	A	38	17	0	1	1	15
	B	69	28	0	2	4	22
	C	42	16	0	1	0	13
	E	40	19	0	1	1	17
(2) Sprayed on 26-1-37	A	97	37	35	1	1	0
	B	77	35	31	3	1	0
	C	105	37	29	5	2	1
	E	56	31	25	3	1	2
(3) Sprayed on 20-2-37	A	60	37	4	12	14	7
	B	44	22	1	1	3	17
	C	47	18	0	1	1	16
	E	22	14	0	1	1	12

In table IV, the yields of cured leaf from the plots are subjected to an analysis of variance. The value of  $z$  for treatments is greater than the 1 per cent. point which indicates a high level of significance. This analysis again shows that the first and final spraying were without effect, the differences between the figures for these treatments and for the control being insignificant. The second spraying, however, resulted in a marked and significant increase in the yield of cured leaf. This increase was not due to a greater production of leaf tissue by the plants subjected to the second spraying, as can be seen from the pre-harvesting figures. The increase was due to the fact that heavily infected leaves were not harvested for flue-curing and that there was a relatively higher proportion of curable leaf than in the other three treatments.

The effect of the second spraying is further emphasized by a consideration of the numbers of lesions present on the cured leaf. In table V, cured leaves with up to 50 lesions (most of them were barn-spots) have been lumped together and considered to be "clean" and the figures subjected to an analysis of variance.

The value of  $z$  for treatments greatly exceeds the 1 per cent. point. It is shown that the results from the first and third sprayings are not significantly different from those of the control plants, whereas the second spraying effected a highly significant reduction in the amount of barn-spot on cured leaf.

*Effect of Spraying on Quality of Cured Leaf.*—Plants were examined after the field spraying. No deposit of fungicide was seen although the surface of leaves immediately after spraying looked rather more shiny than that of unsprayed ones. Examination of the cured leaf failed to reveal any differences in texture or appearance—other than in the amount of spotting—between the sprayed and unsprayed leaves. The cured leaf from the experimental plots was bulked with the rest of the tobacco and observations will be made later to determine, if possible, if the spray has any effect on the burning qualities of the leaf.

As has been noted above, no spray scorch was observed on any of the plants sprayed.

TABLE IV  
ANALYSIS OF VARIANCE OF WEIGHTS OF CURED LEAF FROM PLOTS AT MAIN HARVEST

Due to	Degrees of Freedom	Sum of squares of Deviations	Variance	$\frac{1}{2} \log_e$	Z	One per cent. point
Blocks	3	1148.75	382.92			
Treatments	3	4736.75	1578.92	3.6823	1.0061	0.9724
Error	9	1900.25	211.14	2.6762		
Total	15	7785.75				

Standard Deviation = 14.53  
Co-efficient of variability = 26.84%

Mean Weights of Cured Leaf per Plot in Drachms	S.E. of Mean of 4 plots	Significant difference
(0) 42.25		
(1) 47.25		
(2) 83.75		
(3) 43.25		
General Mean 54.125	7.26	25.15

TABLE V

ANALYSIS OF VARIANCE OF "CLEAN" LEAVES HARVESTED FROM PLOTS AT MAIN HARVEST

Due to	Degrees of Freedom	Sum of squares of Deviations	Variance	$\frac{1}{2} \log_e$	Z	One per cent. point
Blocks	3	78.75	26.25			
Treatments	3	2828.25	942.75	3.4244	2.1261	0.9724
Error	9	120.75	13.42	1.2983		
Total	15	3027.75				

Standard Deviation = 3.66

Co-efficient of Variability = 36.18%

Mean Numbers of "Clean" (0)	(1)	(2)	(3)	General Mean	S.E. of Mean of 4 plots	Significant difference
1.25	1.25	33	5	10.125	1.83	6.34

## METEOROLOGICAL DATA

The relevant rainfall records are given in table VI.

TABLE VI

DAILY RAINFALL RECORDS FROM 16th NOVEMBER, 1936 TO 25th FEBRUARY, 1937

(Rainfall measured at 9 a.m. each day for the previous 24 hours. Days on which no rainfall was recorded are omitted).

Date	Rainfall in.	Date	Rainfall in.
Nov. 16, 1936	.. .04	Jan. 1, 1937	.. .90
„ 17 „	.. .08	„ 3 „	.. .02
„ 18 „	.. .66	„ 6 „	.. .34
„ 20 „	.. .03	„ 7 „	.. .10
„ 22 „	.. .17	„ 10 „	.. .70
„ 24 „	.. .43	„ 13 „	.. .53
Dec. 8 „	.. .02	„ 14 „	.. .42
„ 9 „	.. .44	„ 15 „	.. 1.10
„ 11 „	.. .03	„ 19 „	.. .97
„ 12 „	.. .13	„ 30 „	.. .05
„ 13 „	.. .02	„ 31 „	.. .60
„ 14 „	.. 4.52	Feb. 2, 1937	.. .95
„ 15 „	.. 2.10		
„ 16 „	.. .36		
„ 17 „	.. .01		
„ 29 „	.. .90		
„ 30 „	.. .78		

During January, 1937, showers were relatively frequent and well distributed. The month included 10 wet days (more than 0.04 in.) and one rain day (0.01-0.04 in.). The total rainfall for January was 5.73 in. which was 1.9 in. below the average rainfall for this month during the five years 1931 to 1935.

The first spray was applied between 5 p.m. and 6 p.m. on the 7th January, there having been rain in the early morning. The leaves were dry at the time of spraying. Rain fell fairly regularly after this but it is not possible to say to what extent the fungicidal deposit was washed off.

The second spray was applied between 11 a.m. and noon on 26th January. No rain fell between the 19th January and the time of spraying. Between this date and the collection of the records of disease incidence there were only three wet days.

Rainfall during February was low. Apart from the heavy fall (0.95 in.) on 2nd February no rain fell during the whole month. The average rainfall for February for the period 1931–1935 was 2.33 in. This drought doubtless caused the early maturing of the tobacco and so upset the pre-arranged plan of experiment. It had been anticipated that the harvesting of the tobacco would not begin before the beginning of March. The drought may also have contributed to the efficacy of the second spraying in that the fungicidal deposit was not subjected to many showers which might have washed it off, at least in part.

The third spray was applied between 9 a.m. and 10 a.m. on 20th February. Weather conditions were dry and hot.

#### DISCUSSION

Field spraying of tobacco for the control of frog-eye and of barn-spot has not, apparently, been the subject of much experiment. Hill (1936) stated that field "spraying is uneconomic and unnecessary under Queensland conditions, since healthy seedlings can be obtained by applying control measures in the seed-bed." In a previous article on this subject, the writers (1937) concluded that nursery spraying is of great value in reducing the initial infection by frog-eye of tobacco after it is transplanted into the field. They also suggested that the soil is the main source of infection. Any new leaves which develop after the tobacco is transplanted, being unprotected by the fungicide applied in the nursery, are susceptible to attack by frog-eye. The plants used in the experiment described above were originally seedlings sprayed in the nursery and were, at the time of transplanting, free from frog-eye infection. The data recorded above indicate the extent of infection by frog-eye of the leaves of such plants at the time of harvest. These show clearly the need for the adoption in Ceylon of some control measure after the plants have left the nursery.

Meurs (1932) described the results of experiments on the control of frog-eye on tobacco in Sumatra. He recommended the field spraying of young plants with Bordeaux Mixture but pointed out that the spraying of plants more than twenty days after transplanting was inadvisable as a brownish-black or greenish-black deposit occurred on the fermented leaf from sprayed plants which completely spoiled the leaves for wrapper purposes. Hopkins (1934) reported that he had sprayed tobacco in the field for a number of years on an experimental scale. He mentioned the use of Bordeaux Mixture and also the less satisfactory use of fungicidal dusts. He concluded that field spraying was desirable but that further investigation was required to determine the number of sprayings required, the best times to spray and the kind of fungicide to be used and to solve practical questions, such as the discovery of the most satisfactory type of spray outfit.

From the above, it would appear that very little exact information on the field spraying of tobacco is available. The experiment described in the body of this article was therefore designed to determine if a single spraying would give satisfactory control of frog-eye and to determine, within wide limits, at what stage of the plant's growth the spray would be best applied. Bouisol was selected as the fungicide for use in the experiment partly because it is easy to use but chiefly because it leaves no obvious spray deposit on the leaves and so would be less likely to affect the appearance of the cured tobacco than Bordeaux Mixture, which had been adversely reported on by Meurs.

In the experiment under discussion the time from transplanting to the gathering of the harvest was 102 days. The different sets of plots were sprayed 55 days, 74 days and 99 days respectively after transplanting. The first and third sprayings were found to have no effect and it is concluded that the first was too early and the third too late to exercise any marked control of frog-eye infection. The second spraying, however, gave excellent results as judged by the number of frog-eye lesions present before harvesting, by the weight of flue-cured tobacco harvested and by the amount of barn-spot that occurred on the cured leaf. This second spraying was done at about the time of topping.

It is obvious that the determination of the optimum time of spraying should not be made purely by empirical methods and the experiment described above must be regarded as preliminary to more detailed experiments which are planned for next season. It is proposed to carry out then, side by side with a more elaborate experiment based on the results already obtained, a study of the course of infection by *Cercospora nicotianae* throughout the season and of the growth rate of the plants so that it may be determined on theoretical grounds at which stage the spray would be likely to be most effective.

It is obvious that, if a single field spraying has a marked effect on the amount of spotting, its practicability must receive careful consideration. No attempt has been made to consider the question of costs and to compare the costs with the increased value of the crop obtained in this experiment. The question will receive attention next season.

The effect of weather conditions on the incidence of frog-eye has not received special attention in this experiment. One point is, however, worthy of note. Weather conditions subsequent to the second spraying were unusually dry. The difference in the amount of spotting observed between the plots which were and which were not sprayed at this time was marked. This difference is due to infection which took place after the second spraying and it must therefore be concluded that frog-eye infection takes place in the absence of wet weather conditions.

### CONCLUSIONS

From the experiment described above the following conclusions are drawn :—

1. That a single field spraying of tobacco with colloidal copper, applied at about the time of topping, reduced markedly the amount of frog-eye infection, resulted in an increased crop of leaf suitable for flue-curing and reduced the amount of barn-spot on the cured leaf.
2. That single field sprayings applied about three weeks earlier and three weeks later than this had no effect.
3. That heavy frog-eye infection took place during dry weather.

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