

## SOME STUDIES ON TOBACCO DISEASES IN GEYLON—III

### THE EFFECT OF THE TIME OF SPRAYING AND OF THE NATURE OF THE FUNGI- CIDE ON THE CONTROL OF FROG-EYE (*CERCOSPORA NICOTIANAE* E. & E.)

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**I**N a previous number of this series (Park & Fernando, 1937 b) the writers described an experiment of a preliminary and exploratory nature, in which successful control of frog-eye (*Cercospora nicotianae* E. & E.) of cigarette tobacco was achieved by a single spraying in the field with a colloidal copper fungicide at about the time of topping. The single spray application resulted in a considerable reduction in the amount of frog-eye present on the leaves of the sprayed plants at the time of harvest and, in consequence, in an increased crop of leaf suitable for flue-curing. There was also a lower density of barn-spot on the cured leaf from the sprayed tobacco. In that experiment it was also shown that single spray applications made respectively three weeks earlier and three weeks later than the one described above produced no significant effect. From these results it was clear that, for successful frog-eye control by a single field spraying, the time of that spraying is a factor of major importance. The experiment described in this paper was therefore designed with a view to determine, *inter alia*, more accurately the optimum time of spraying.

In the previous experiment the only spray used was a proprietary colloidal copper fungicide at a concentration of 1 oz. per gallon. In the present experiment, the behaviour of lower concentrations of this fungicide and of cheap home-made substitutes was also investigated to determine if the cost of spraying could be reduced without sacrificing efficiency.

## DESIGN OF THE EXPERIMENT

The following are the formulae of the fungicides used :—

(1) Proprietary colloidal copper	..	..	4 oz.
Proprietary spreader	..	..	$\frac{1}{4}$ oz.
Water	..	..	4 gal.
(2) Proprietary colloidal copper	..	..	2 oz.
Proprietary spreader	..	..	$\frac{1}{4}$ oz.
Water	..	..	4 gal.
(3) Proprietary colloidal copper	..	..	1 oz.
Proprietary spreader	..	..	$\frac{1}{4}$ oz.
Water	..	..	4 gal.
(4) Copper emulsion :—			
Copper sulphate	..	..	$2\frac{1}{2}$ oz.
Soft soap	..	..	16 oz.
Water	..	..	4 gal.
(5) Ammoniacal copper emulsion :—			
Copper sulphate	..	..	$2\frac{1}{2}$ oz.
Soft soap	..	..	13 oz.
Ammonia (66%)	..	..	1 oz.
Water	..	..	4 gal.
(6) Unsprayed control.			

The above fungicidal treatments were combined with 4 times of spray application. The spray applications were made at weekly intervals after some, at least, of the plants had reached the stage of topping. The following were the dates of spray application :—

- A — January 13, 1938.
- B — January 20.
- C — January 27.
- D — February 3.

The previous year's experience of marked contrasts between the effects of spraying at different times suggested that even with a finer subdivision of the interval between the first and the final spray applications, the differences would be sufficiently striking to become evident in the face of a relatively large experimental error. No information was, however, available regarding the fungicidal differences, and a closer comparison by the apportioning of a greater number of degrees of freedom for the estimate of experimental error for fungicidal effects was considered desirable. These considerations determined the selection of the complex latin square design illustrated in Fig. 1. The 4 times of spraying (A, B, C, and D) were distributed over the main plots of the  $4 \times 4$  latin square, and the 6 fungicidal treatments were randomized within each main plot. The statistical advantages apart, the complex latin square lay-out was conducive to convenient handling of the experimental

2	1	4	6	1	5	2	3				
6	A	4	2	B	5	2	D	3	6	C	5
3	5	3	1	6	4	1	4				
6	5	3	5	1	2	3	1				
3	B	4	6	C	2	4	A	5	6	D	5
1	2	1	4	6	3	4	2				
4	5	3	1	4	2	5	3				
1	C	6	5	D	2	6	B	3	2	A	6
2	3	4	6	5	1	4	1				
1	6	5	6	4	5	1	6				
2	D	5	4	A	2	6	C	3	5	B	3
3	4	3	1	2	1	2	4				

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FIG. 1.—DESIGN OF EXPERIMENT.

material and rendered the experiment relatively fool-proof—factors of some importance when much of the experimentation has to be done with unskilled labour.

### EXPERIMENTAL METHODS

*Experimental Material.*—The sub-plots were square and consisted of 36 plants spaced 3 feet  $\times$  3 feet, *i.e.*, the size of each sub-plot was about 1/135 of an acre. Adjacent sub-plots within main plots were separated by 2 guard rows. Adjacent main plots were separated in the direction of their long axes (see Fig. 1.) by 8 border rows, and in the direction of their shorter axes by wide, shallow drains.

The experimental material consisted of a comparatively uniform area of Harrison's Special tobacco grown at the Experiment Station, Ganewatta, on a light, sandy, non-lateritic soil. Tobacco is one of the routine rotation crops at this station and the present experiment was carried out in the usual rotation area. The rotation consists of tobacco or a cereal crop, such as maize, during the *maha* season (September-October to March) and a green manure crop like sunnhemp, grown and ploughed in during *yala* (March-April to June-July). Tobacco is therefore grown on the same area during the *maha* season of alternate years.

The nursery from which the plants were obtained was prepared with the aseptic and antiseptic precautions described previously (Park & Fernando, 1937 a), except that the seed was not disinfected with silver nitrate.

The nursery consisted of 12 beds, 8 of which were sown during the period September 11–19, 1937, and the rest during the period October 1–7, 1937. Most of the experimental plants were contributed by the beds which had been sown early. Accidental damage to the spraying machine disorganized the routine spray programme, and a certain amount of frog-eye appeared in the nursery before transplanting. The amount of infection was not great and it was possible to use apparently healthy seedlings only for transplanting.

Seedlings were transplanted from the nursery on October 31, 1937, and during the period November 2–4, 1937. The supplying of vacancies was begun on November 11. The total quantity of manure applied per acre was as follows :—

			lb.
Superphosphate	..	..	224
Sulphate of potash	..	..	140
Nitrate of soda	..	..	84
			<hr/>
			448
			<hr/>

This quantity was divided into 3 dressings, the first of which was applied before transplanting. The manure was applied in spoonfuls to the planting holes and forked in. The second and third applications were made 3 weeks and 6 weeks after transplanting, respectively.

The supplying of vacancies, which was made necessary by the damage caused by heavy rains shortly after transplanting, and the replacement of young plants badly attacked by stem-borer (*Phthorimaea heliopa*) introduced some degree of heterogeneity in the experimental material. In addition, the presence of termite nests in the experimental area was a disturbing factor. Plants growing on the sites of existing and old nests grew much more vigorously than the remainder. To avoid undue coarseness in the leaves, they were left untopped but, in spite of this, many leaves from these plants were too coarse at the time of harvesting for flue-curing. The number of plants so affected was not great but proved to be a factor increasing the heterogeneity of the experimental material and, in consequence, raising the experimental error obtained.

The plants were primed two weeks before the first spraying. Subsequently, no priming was done in the experimental area. Some plants were ready for topping by January 10, but the first topping was not done until January 20, the day on which the second spray application was made. Subsequently, rounds of topping were done at intervals of about one week.

*Preparation of Fungicidal Mixtures.*—The formulae of the fungicides used in this experiment are given above. For brevity, the numbering employed there will be used throughout the paper.

Formulae (1), (2), and (3): The colloidal copper and the spreader used in these formulae are identical with those used in the 1937 experiments (Park & Fernando, 1937 b). The colloidal copper is a proprietary fungicide containing 22 per cent. copper oxychloride, 50 per cent. water and 28 per cent. of an organic complex (Jacquemain & Gravier, 1932). The spreader is a proprietary product having a composition allied to a sulphonate of an alkylated hydrocarbon.

Formulae (4) and (5): The addition of copper sulphate solution to an excess of soap results in the formation of a finely divided precipitate of "copper stearate" in a condition well suited for spraying. If the soap is not in excess, an unpleasant sticky putty is formed which tends to block the nozzle of the sprayer. Formula (4) is based on a recipe by Wormald and Wormald (1919). The concentration of soap in formula (4) is higher than in the original formula. This increase in concentration was adopted because the amount of soap recommended

in the latter formula was found to give unsatisfactory results with hard water. In preparing this fungicide, the copper sulphate and the soft soap were dissolved separately each in two gallons of water. The copper sulphate solution was then poured into the soap solution with continuous stirring.

The soap in this formula can be replaced to some extent by ammonium hydroxide. The partial replacement of soap by ammonia is desirable as it reduces costs appreciably. In formula (5), 1 oz. ammonia (66 per cent.) is substituted for 3 oz. of the soap. The substitution of part of the soap by ammonia affects the texture of the spray mixture, the precipitate becoming coarser as the proportion of ammonia is increased. In the preparation of fungicidal mixture (5), the copper sulphate and the soap were dissolved separately in 2 gallon quantities of water as in formula (4). The ammonia was added to the soap solution and the copper sulphate was then poured in with continuous stirring.

*Spray Application.*—Field notes on the times of spraying and the weather conditions at the times of spraying are given below :—

- January 13, 1938 .. Spraying A. Treatments (1), (2), and (3) done between 11 A.M. and 4.30 P.M. Nearly  $2\frac{1}{2}$  gallons of spray per sub-plot of 36 plants. Strong N. E. wind during spraying : jute hessian screen, 5 feet long by 45 inches high used to check drifting of spray. Hot and sunny.
- January 14, 1938 .. Spraying A concluded. Mixtures (5) and (4) applied. Weather conditions and amount of spray used as above.
- January 20, 1938 ... Spraying B. 7.30 A.M. to 5 P.M. Spraying done in the order (1), (2), (3), (4), (5).  $2\frac{1}{2}$  gallons of spray used per sub-plot. Weather hot and sunny with a strong N.E. wind. Screen used.
- January 27, 1938 .. Topping in progress during spray application. Spraying C. 9.15 A.M. to 1.40 P.M. treatments (1), (2), (3), and (4). 3 P.M. to 4.15 P.M. treatment (5). Fairly heavy showers 7.30 to 8.30 A.M. Leaves not completely dry when treatment (1) was commenced ; plants oversprayed in this treatment to compensate for dilution ( $3\frac{1}{4}$  gallons per sub-plot). Leaves dry when treatment (2) was begun. Weather cloudy during the whole day. No wind. Spraying conditions ideal.  $2\frac{1}{2}$  to  $2\frac{3}{4}$  gallons of spray per sub-plot used in treatments (2), (3), (4), and 5. Screen used.
- February 3, 1938 .. Spraying D. 8 A.M. to 2 P.M. Dew not completely dried when spraying was begun. Leaves dried quickly. Spraying done in the order (1), (2), (3), (4), (5).  $3\frac{1}{4}$  gallons spray per sub-plot for treatment (1),  $2\frac{3}{4}$  gallons per sub-plot for treatment (2), (3), (4), and (5). Weather bright with some cloud, wind moderate. Screen used.

It will be seen from the above that, in at least one spraying, each of the treatments was applied during the hottest part of the day, so that it is possible to ascertain the relative importance of time of spraying in the occurrence of spray injury as observed in the field and on the cured leaves from the different treatments. The amount of spray used in the experiment, varying from  $2\frac{1}{4}$  to  $3\frac{1}{4}$  gallons per sub-plot of 36 plants, was considerably more than would be used in large-scale field spraying, but it was essential that both sides of every leaf should be sprayed thoroughly and over-spraying could not be avoided. No attempt was made to study the economic aspect of the problem in this experiment.

*Developmental Records.*—At the time of spraying, a plan was made of each sub-plot, a cypher being allotted to indicate the stage of maturity of each plant. As is explained below, at the time of harvest, it was found necessary to reduce the number of plants harvested in each sub-plot to 30. In table I are given

TABLE I

## Record of Stages of Development of Plants at Different Times of Spraying

Time of Spraying	Fungicidal Treatment	No. of plants				
		No appreciable stem Development	Stem present but not fully elongated	Stem fully elongated or nearly so	Flower buds visible	Topped or at topping stage
A (13.1.38)	(1)	18	40	44	14	4
	(2)	18	41	41	18	2
	(3)	15	25	68	12	0
	(4)	15	39	45	11	10
	(5)	21	65	21	9	4
	Total	87	210	219	64	20
B (20.1.38)	(1)	4	16	43	36	21
	(2)	6	17	31	20	46
	(3)	6	26	30	10	48
	(4)	0	7	36	4	73
	(5)	6	56	33	3	22
	Total	22	122	173	73	210
C (27.1.38)	(1)	3	18	13	11	75
	(2)	0	11	28	22	59
	(3)	0	8	10	29	73
	(4)	0	13	25	15	67
	(5)	0	12	16	2	90
	Total	3	62	92	79	364
D (3.2.38)	(1)	1	5	5	1	108
	(2)	0	6	5	0	109
	(3)	1	1	11	5	102
	(4)	0	1	4	0	115
	(5)	1	3	3	0	113
	Total	3	16	28	6	547

the records showing the stage of maturity of the 120 plants, subsequently harvested, in the four replications of each treatment, at the time of spraying. In the last column of the table are included the number both of plants actually topped and of those which had reached the stage of topping, but which had not been topped. The figures for the unsprayed control sub-plots are not included as they are irrelevant; the plants were, however, at the same stage of development as those in the neighbouring sub-plots at the time of each record. The figures given permit of the ready assessment of the size of the plants at the times of spraying. At the time of the first spraying (A) the majority of the plants were at the stage when the stem was elongating and only 20 plants out of the 600 had reached the topping stage. At the time of the second spraying (B), about one-third of the plants (210 out of 600) had reached the topping stage. At the time of the third spraying (C), over one-half of the plants (364 out of 600) had reached the stage of topping and a further 181 were nearly full grown. At the time of the last spraying (D), almost all the plants (547 out of 600) had reached the stage of topping.

*Harvesting.*—The time of the harvesting of cigarette tobacco is determined to a large extent by weather conditions. During the present experiment, dry weather which set in about a month after transplanting hastened the development of the plants so that harvesting would have been unusually early had it not been for the rains which fell shortly after the last spraying and delayed the maturing of the leaves. The first harvest of leaves for flue-curing was gathered on February 15 and the second and final harvest on February 22, fourteen and fifteen weeks after transplanting respectively. Following the usual practice, leaves badly blemished by frog-eye were not harvested for flue-curing so that the weights of total cured leaf from plots subjected to the various treatments provide a measure of the efficiency of these treatments in frog-eye control.

#### PHYTOCIDAL EFFECTS

In the field the sprayed leaf was periodically examined for phytocidal damage. A mild scorch was observed on a few of the leaves; but as the same type of scorch occurred to some extent on the control plants, it was argued that the injury was not caused by the fungicidal chemicals; accumulations of water on the leaf surface appeared to be responsible for the injury. The damage was negligible.

A rather more serious type of spray injury however occurred on some of the cured leaf which had been sprayed with the copper emulsions; the injury was more severe with the ammoniacal copper emulsion. The damage took the form of minute,

suberized, warty eruptions on the lower surface of the leaf. The eruptions often coalesced into irregular masses. The percentage of leaves affected was however comparatively low, and the damage did not appear to affect markedly the appearance of the cured leaf. The cured leaf which had been sprayed with various concentrations of colloidal copper was free of this blemish.

#### METEOROLOGICAL DATA

In an equable tropical climate of the type prevailing in Ceylon, seasonal fluctuations in temperature and sunshine are not sufficiently marked to be of much consequence. In Ceylon, the volume and distribution of rainfall are the major factors conditioning plant growth. In the present paper certain conclusions have been drawn concerning the relation between the stage of maturity of the tobacco crop and the optimum time for spraying. The maturing of the crop is determined largely by the prevailing rainfall. A protracted drought would result in rapid ripening of the leaf and would necessitate early spraying. Heavy and continued precipitation would delay the maturing process. Generalizations regarding the optimum spraying time would be possible only if the same type of rainfall curve recurs regularly in successive tobacco seasons. Fig. 2 illustrates the distribution of the daily precipitation at Ganewatta during the present season and the four preceding seasons. The periods of transplanting and of harvesting have also been recorded. The total precipitation for the 5 month period, November 1937–March, 1938, which was roughly the duration of the experimental season, was 36.24 inches. This did not differ appreciably from the normal rainfall for the corresponding period in previous years; the average calculated from the preceding four years' records was 33.5 inches. There were however certain peculiar features in the rainfall distribution during the 1937–38 season. December is normally a wet month. In 1937, the precipitation for this month was 1.17 inches, *i.e.*, only 16.6 per cent. of the average for the 4-year period. Again, February, the month during which the leaf usually undergoes rapid maturing, is as a rule comparatively dry. During the experimental season, the precipitation for this month was 7.70 inches, as against an average of 2.67 inches for the preceding four years. The maturing of the tobacco crop was hastened by the dry spell in December, but was subsequently delayed by the February rains; this delay resulted in a severe epiphytotic of frog-eye and in a consequent reduction in the yield of cured leaf; the unsprayed area yielded as little as 22 lb. per acre.

Despite the occurrence of abnormal weather conditions during December and February, the growing period in the field of the experimental crop—as estimated by the interval between the

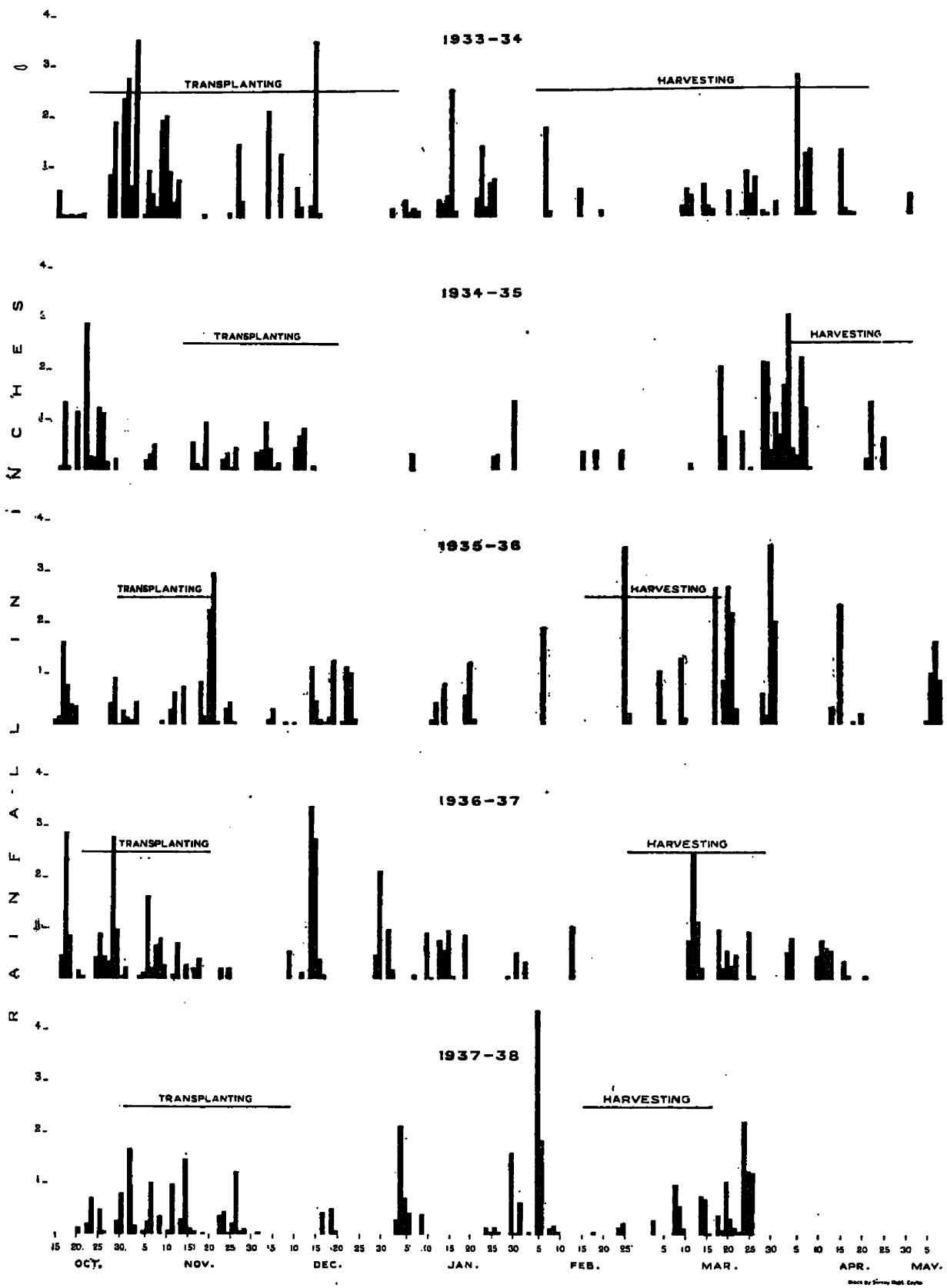


FIG. 2.—RAINFALL RECORDS.

commencement of transplanting and the commencement of harvesting—did not differ considerably from that of previous years; the growing period for the experimental season was 107 days and the four-year average was 125 days.

Although topping was not begun till January 20, some plants were ready for topping by January 10, *viz.*, 10 weeks after the commencement of transplanting. In a normal year with a wet December, the interval between topping time and the commencement of transplanting would probably have been rather longer. The most efficient spraying was done on January 27, *viz.*, 12 to 13 weeks after the commencement of transplanting; at this time 60 per cent. of the plants had been topped. It is not possible at this stage, to generalize on the applicability of these results to years of normal precipitation.

It is generally accepted that frog-eye damage is most severe in wet weather. This is at least in part due to the fact that the crop stays longer in field under wet weather conditions. The direct effect of rainfall on the intensity and spread of the disease is however rather obscure. The present experiment provides little relevant information in this connection.

### COURSE OF INFECTION

The times chosen for spraying the plants in this experiment were based on the results of the experiment carried out by the writers in the corresponding season in 1936–37 (Park and Fernando, 1937 b), but they were nevertheless empirical and not based on records of the progress of the disease on plants in the field. It was therefore felt that the experiment would not be complete unless it was accompanied by records which would indicate why spraying at any one time should be more effective than at other times.

During the course of this experiment, observations were made on plants throughout the season, from the time they were transplanted until they were harvested. The first series of observations was made on plants immediately after transplanting. It is stated above that, owing to an accident, the nursery beds from which the plants used in this experiment were obtained were not subjected to the proper spraying programme and that, in consequence, some frog-eye appeared in the nursery. As far as could be ascertained by visual observation, no plants were used in the experimental area which had been attacked by frog-eye, but it is possible that some incipient infections did exist. The early records of the course of infection by frog-eye were therefore carried out at Wariyapola Experiment Station, which is less than ten miles from Ganewatta. At Wariyapola, the nurseries were planted with the same seed as

at Ganewatta, regular spraying was done and no frog-eye infection occurred in the nurseries up to the time of transplanting. It is not proposed here to discuss in full the results of those observations, which will be the subject of another paper, but it may be stated that, although the plants were free from frog-eye at the time of transplanting, within six to eight weeks of that time infection was present on practically every plant, frog-eye infection in this case being judged by the presence of one or more frog-eye lesions on the plants. Similarly, casual observations of the experimental area at Ganewatta indicated that all plants were infected by the end of December, 1937, and it may be assumed that this state of affairs is normal for tobacco grown in that area.

While the plants are young and still in the rosette stage, it is not uncommon to find frog-eye lesions on all leaves. The situation, however, changes once the stem begins to develop; upper leaves of plants with definite stem development do not commonly become infected by frog-eye. At this stage of the development of the plants infection is confined to the lower leaves.

It was not practicable to keep detailed records of the number of frog-eye lesions present on each leaf of a large number of plants and detailed records were therefore made of sixteen plants only. Plants were selected which were of the same growth-type, with approximately the same number of leaves and in the same stage of development. One plant was selected from each of two of the sub-plots of the treatments A (1), B (1), C (1), D (1), *i.e.*, plants sprayed with spray (1)—the proprietary colloidal copper spray at 1 oz. in 1 gallon—at the four different times of spraying. At the same time, single plants were selected from each of the corresponding unsprayed control plants, giving eight unsprayed plants. Records were made of the number of frog-eye lesions on each leaf, starting from the top of the plants and working downwards. The results are best seen in graph form. Fig. 3 shows the progress of the disease on each leaf of the plants in the different treatments. Each little curve represents the number of lesions present on a leaf on three occasions—February 4, 10, and 14 respectively—and each is the average, with the sprayed plants, of the corresponding leaves on two plants and, with the unsprayed plants, of the corresponding leaves on eight plants. The number of leaves on most plants examined was 15 or 16, but one plant had only 12 and another 18. The lowest leaves were all severely spotted throughout the record period and the averages are therefore confined to those of the top 12 leaves. The parts of the curves which are dotted indicate that the curves should have been continued until they met the time ordinate: the

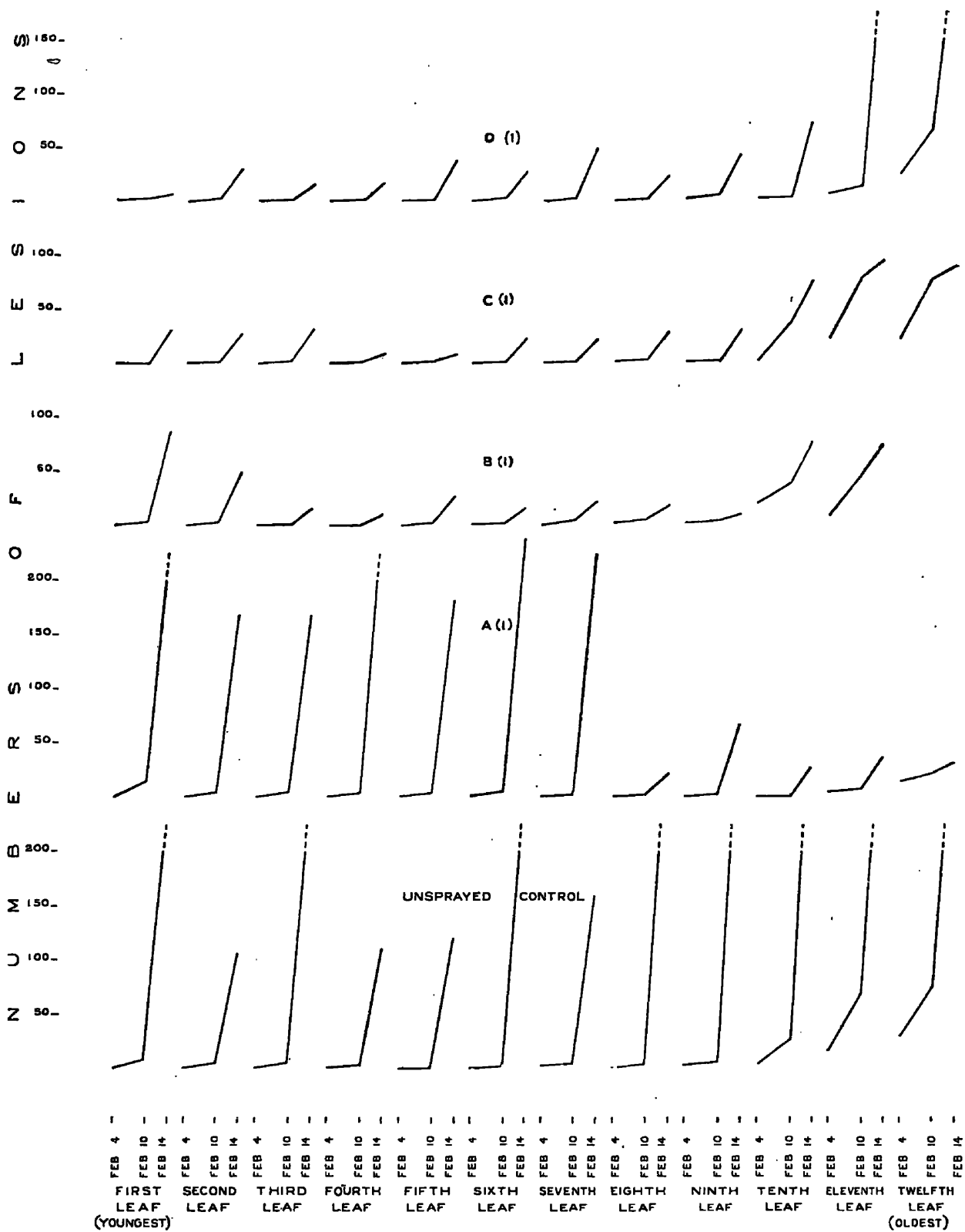


FIG. 3.—COURSE OF FROG-EYE INFECTION.

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completion of these curves would have necessitated the use of an inconvenient scale and it was felt that their slope would convey all the information required.

It will be seen that, even with the unsprayed plants, infection, as judged by frog-eye spotting, did not occur to any great extent on most of the leaves of the plants until after February 10. The last spraying was done on February 3. Later records were not possible as the plants were harvested, in part at least, on February 15, and earlier records would not have given much information.

The suddenness of the appearance of large numbers of frog-eye lesions on the leaves of unsprayed plants is remarkable and is possibly connected with changes which take place in the leaves when they mature. On February 10, the numbers of frog-eye spots on most of the leaves were insignificant whereas on February 14—only four days later—the minimum number of spots on a leaf was over 100.

Inferences can be drawn from these curves, which are worthy of note. It will be seen by comparison with the control curves that some of the D (1) leaves, *i.e.*, leaves sprayed on February 3 were markedly protected by the spray. On February 14, the numbers of frog-eye lesions on these leaves were considerably lower than on the corresponding leaves on unsprayed plants. It may therefore be assumed that most of the infections of the unsprayed leaves which had developed as frog-eye lesions on February 14 took place *after* the time of spraying. That gives an upper limit of 11 days to the incubation period of frog-eye. It may also be argued that, as there was no marked difference in the number of lesions on the corresponding leaves on February 10, the lower limit of this incubation period is 7 days, but here the total number of new lesions occurring on February 10 was not sufficiently great to warrant so definite a conclusion on this point.

The curves or symptom-pictures also show clearly the relative position on the plants of the leaves protected by the sprays. In the spraying at time A, the first seven leaves developed the same number of lesions as those of the control plants. The obvious explanation is that, at the time of spraying, these leaves were insufficiently expanded to receive adequate protection. In the spraying at time B, the protection range has moved, and the first two leaves were partially protected, the third to the ninth leaves were completely protected and the tenth and eleventh were again partially protected. In the spraying at time C, the first nine leaves were completely protected and the remainder only slightly protected. In the spray at time D, there is evidence of protection up to the tenth leaf but there is

also an indication that some infection had taken place at the time of spraying. The last two leaves were obviously already infected heavily at the time of spraying.

It will be seen that a combination of the protection provided by sprayings at the times A and C would have given complete protection.

## RESULTS

Although in the original design the sub-plots consisted of 36 plants, the occurrence of vacancies—in some cases as many as six—necessitated the restriction of the data to the produce of 30 plants from each sub-plot, the excess in each instance being eliminated at random.

Table II records the weights of total cured leaves in the various treatments; the figures are the sums of the yields from the four replications of each treatment. These data have been subjected to an analysis of variance in table III. In tests of significance, Snedecor's  $F$  is used in preference to Fisher's  $Z$ ; the use of  $F$  eliminates the need for calculating natural logarithms of the variances and simplifies the arithmetic considerably. It may be mentioned in this connection, that  $X$  of Mahalanobis (1932) is synonymous with Snedecor's  $F$ . The analysis of variance of weights of total cured leaf indicates that the effects of times of spraying and of fungicides and the time-fungicide interaction are all highly significant,  $F$  in all cases being well above the one per cent. point. It is probable that the inclusion of the figures for the untreated control plots in the data from which the calculations were made, was responsible for the significance of the time-fungicide interaction: this is therefore of little interest in the present connection. The ratio of the two error variances provides a measure of the relative accuracy of the main-plot and sub-plot comparisons.

The significant difference between two values is the product of the standard error of the difference between the two values and the appropriate value  $t$ . A 5 per cent. level of significance is usually assumed. Hoblyn (1931) states that the number of degrees of freedom for  $t$  is the sum of the numbers of degrees of freedom contributed by the two values. Most statistical authorities however use the error number of degrees of freedom in estimating  $t$ . In the previous papers in this series (Park & Fernando, 1937 a & b), Hoblyn's method was employed. It was recently pointed out to the writers by Dr. T. Eden \* that the second method provided a more valid estimate of  $t$ ; this method has accordingly been adopted in the present paper. It may be mentioned, in this connection, that the adoption of Hoblyn's

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\* Personal communication to the writers

method did not vitiate the conclusions made in the previous papers. The significant differences were merely slightly inflated and the tests of significance were consequently made unnecessarily severe.

**TABLE II**  
**Weights of Total Cured Leaves in Grammes**

Treatment	Time of spraying				Total
	A	B	C	D	
(1)	2,061	3,635	4,430	3,476	13,602
(2)	1,385	2,717	3,358	2,398	9,858
(3)	1,062	2,016	2,906	2,519	8,503
(4)	2,203	3,158	3,380	2,712	11,453
(5)	2,030	3,093	4,312	2,950	12,385
(6)	381	314	251	238	1,184
Total	9,122	14,933	18,637	14,293	56,985

**TABLE III**  
**Weights of Total Cured Leaf**

ANALYSIS OF VARIANCE

	D.F.	Sum of Squares	Variance	F	1 per cent. point	5 per cent. point
Rows	3	686,654·11	228,884·70	17·21	9·78	4·76
Columns	3	51,869·11	17,289·70	1·30		
Times	3	1,917,101·45	639,033·82	48·04		
Error (a)	6	79,799·31	13,299·89			
Fungicides	5	6,202,614·35	1,240,522·87	105·6	3·34	—
Interaction : Times × Fungicides	15	661,822·61	44,121·51	3·755	2·40	—
Error (b)	60	705,202·22	11,753·37			

Standard error of the 24 sub-plot totals for times A, B, C, and D: 565·0 or 3·97 per cent.

Standard error of the 16 sub-plot totals for fungicides (1), (2), (3), (4), (5), and (6): 433·7 or 4·57 per cent.

**Effect of Time of Spraying on Yield of Total Cured Leaf**

	A	B	C	D	Mean	Significant Difference
Yield per 24 sub-plots in gm.	9,122	14,933	18,637	14,293	14,246	1,955
Yield in lb. per acre (4,840 plants.)	113·2	185·2	231·2	177·2	176·8	24·25

**Effect of Quality and Quantity of Fungicide on Yield of Total Cured Leaf**

	(1)	(2)	(3)	(4)	(5)	(6)	Mean	Significant Difference
Yield per 16 sub-plots in gm.	13,602	9,858	8,503	11,453	12,385	1,184	9,497·5	1,226·54
Yield in lb. per acre	252·9	183·3	158·1	212·9	230·3	22·0	176·6	22·8

The following conclusions may be drawn from the statistical analysis :—

*Time of Spraying.*—The yield from the plants sprayed at the time C is significantly greater than the yields from the plants sprayed at times B, D, and A ; the yields from the plants sprayed at times B and D are both significantly greater than the yield from the plants sprayed at time A ; the difference between the yields from plants sprayed at time B and D is not significant. In brief, spraying at time C gave the best results, with B and D, bracketed second and A the least effective.

*Fungicides.*—The results obtained with the fungicidal treatments are singularly happy. The difference between the weights of cured leaves obtained from any two treatments exceeds the significant difference so that it is possible to place them in a definite order of efficiency: the order is as follows:—(1) > (5) > (4) > (2) > (3) and all are better than the unsprayed control.

In examining the cured leaf from the experimental plots, it was seen that there was a marked variation in the amount of spotting, due to frog-eye and barn-spot, on the cured leaves. It was therefore decided to grade the cured leaves from the sub-plots according to the degree of spotting. For this, the adoption of an arbitrary standard was necessary. It was found that leaves with up to 200 lesions (frog-eye and barn-spot) were sufficiently clean for the spotting not to affect their market value. The leaves were therefore graded into two classes, *viz.*, those with 0–200 lesions and those with more than 200 lesions. To assist in grading, leaves which just came on the border-line between the two classes were kept as standards. By reference to these, it was found possible to sort with ease the majority of the leaves: doubtful cases were verified by an actual count of the lesions. In table IV. are given the weight of the “clean” leaves, *i.e.*, those with 0–200 lesions, so obtained. The figures were subjected to an analysis of variance, the results of which are given in table V. In this analysis, the figures from the unsprayed control sub-plots were omitted, since it is obvious that all the fungicidal sprays had a mathematically significant effect and since, by omitting these figures, it was possible to determine whether there was a significant time-fungicide interaction. It was found that the effect of time of spray application, the effect of fungicides and the time-fungicide interaction were all significant, *F* exceeding the one per cent. point in every instance.

TABLE IV  
Weights of Clean Cured Leaves in Grammes

Treatment	Time of Spraying				Total
	A	B	C	D	
(1) ..	1,023 ..	2,408 ..	3,242 ..	2,417 ..	9,090
(2) ..	279 ..	1,252 ..	1,843 ..	1,336 ..	4,710
(3) ..	121 ..	353 ..	631 ..	1,017 ..	2,122
(4) ..	1,225 ..	2,281 ..	2,431 ..	1,737 ..	7,674
(5) ..	1,182 ..	1,873 ..	3,736 ..	2,018 ..	8,809
(6) ..	2* ..	27* ..	23* ..	45*	
Total (5 treatments)	3,830	8,167	11,883	8,525	32,405

\* Weights of clean cured leaves from unsprayed controls were not used in the analysis of variance

TABLE V

## Weights of Clean Cured Leaf

## ANALYSIS OF VARIANCE

	D.F.	Sum of Squares	Variance	F	1 per cent. point
Rows .. .. .	3	340,179·8	113,393·3		
Columns .. .. .	3	109,882·7	36,627·6		
Times .. .. .	3	1,630,454·9	545,485·0	18·89	9·78
Error (a) .. .. .	6	173,248·1	28,874·7		
Fungicides .. .. .	4	2,236,689·8	559,172·5	49·93	13·93
Interaction: Times × Fungicides	12	541,261·3	45,105·1	4·03	3·78
Error (b) .. .. .	48	537,754·1	11,203·2		

Standard error of 20 sub-plot totals for times A, B, C, and D: 759·9 or 9·38 per cent.

Standard error of 16 sub-plot totals for fungicides (1), (2), (3), (4), and (5): 423·3 or 6·53 per cent.

## Effect of Time of Spraying on Yield of Clean Cured Leaf

	A	B	C	D	Mean	Significant Difference
Yield per 20 sub-plots in gm.	3,830	8,167	11,883	8,525	8,101·25	2,630
Yield in lb. per acre	57·0	121·6	176·8	126·8	120·6	39·1

## Effect of Quality and Quantity of Fungicide on Yield of Clean Cured Leaf

	(1)	(2)	(3)	(4)	(5)	Mean	Significant Difference
Yield per 16 sub-plots in gm.	9,090	4,710	2,122	7,674	8,809	6,481	1,197
Yield in lb. per acre	169·1	87·6	39·5	142·7	163·8	120·6	22·26

The conclusions to be drawn from the statistical analysis are only slightly different from those obtained with the weights of total cured leaf. The order of efficiency in the times of spraying is exactly the same as before. Here again, the spraying at time C gave the best results, with B and D bracketed second, and A the least effective.

The order of efficiency of the fungicides is the same as with the weights of total cured leaves. The only difference is that, with the weights of clean cured leaves, fungicide (1) is not significantly superior to fungicide (5) nor is fungicide (5) significantly superior to fungicide (4), although the difference between (5) and (4) approaches significance. Fungicide (1) is, however, significantly superior to fungicide (4) and fungicides (1), (4), and (5) are all significantly better than fungicides (2) and (3); fungicide (2) is better than fungicide (3). The order of efficiency is tabulated below:—

$$\left. \begin{array}{l} (1) \not> (5) \not> (4) \\ (1) > (4) \end{array} \right\} > (2) > (3)$$

where  $>$  represents a difference mathematically significant, and  $\not>$  a difference not mathematically significant.

Finally, the significance of the time-fungicide interaction indicates that there were some differences between the relative efficiencies of fungicides at the different times at which they were applied. It is proposed to discuss the point more fully below.

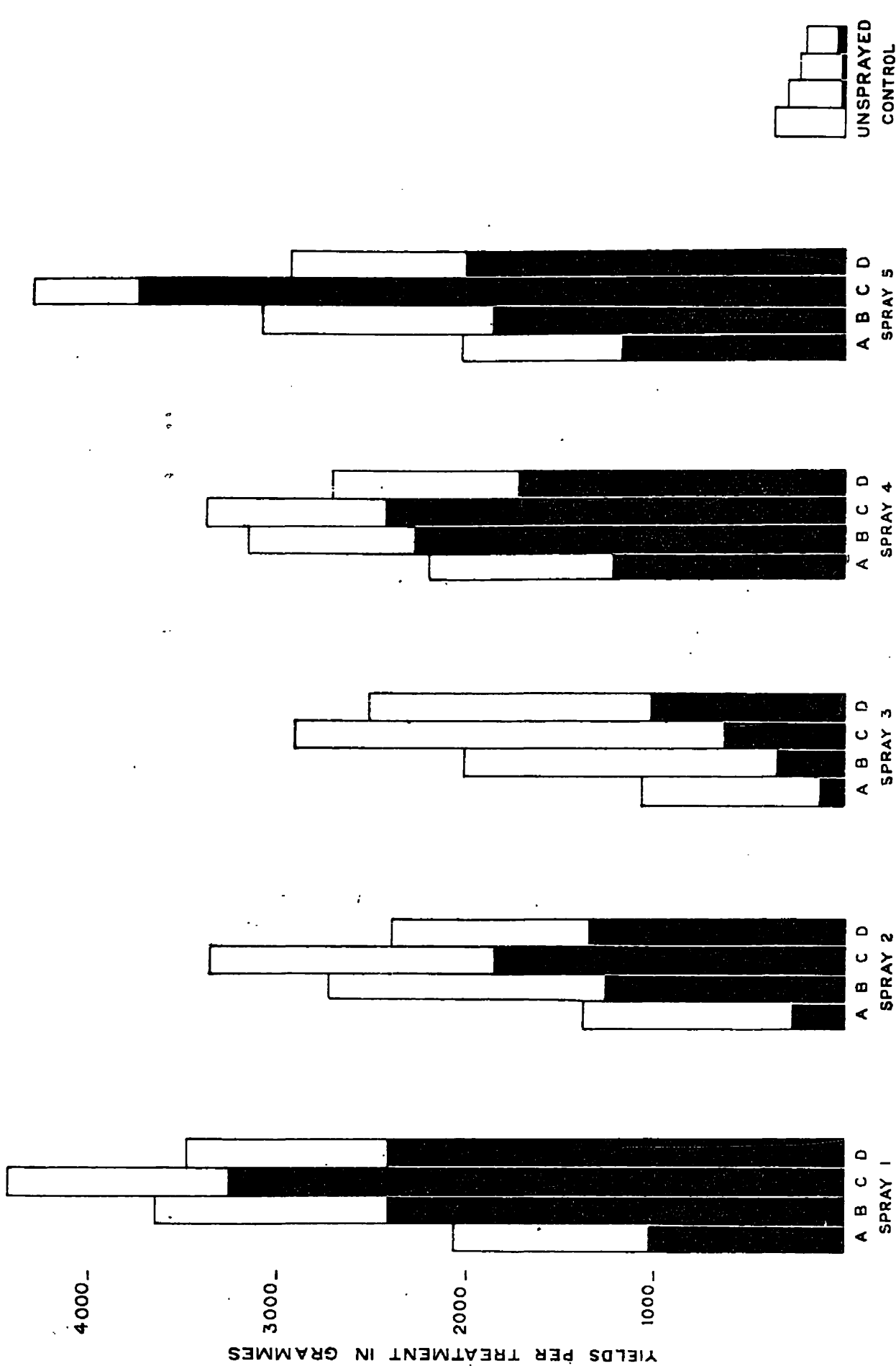
The relation between the weights of clean cured leaves and the weights of total cured leaves is clearly shown in Fig. 3 in which the solid columns represent the weights of clean cured leaf and the whole columns the weights of total cured leaf.

### DISCUSSION.

*Fungicides.*—The fungicides (1), (2), (3), (4), and (5) produced increases in yield of 230, 161, 136, 190, and 208 lb. cured leaf per acre respectively over the control. In addition, the average quality of the cured tobacco was higher with the sprayed leaf. The economics of spraying will not be discussed in the present connection. The results of a subsidiary spraying trial in which spraying costs were worked out in detail and of which an account is published in this journal (Park and Fernando, 1938), demonstrate that spraying is economic and profitable under Ceylon conditions.

The two copper emulsions contain 0·10 per cent. copper and the fungicide (1) contains 0·09 per cent. copper. The latter, however, provides a significantly greater measure of control. There is a significant difference in efficacy between the two copper emulsions, although their copper content is the same. The explanation of the differences in behaviour between these three fungicides must therefore lie in some property other than their copper content, *e. g.*, their wetting and adhesive powers or the degree of subdivision of the fungicidal particles. With the proprietary fungicide, dilution leads to a significant reduction in efficiency at each level.

*Interactions.*—The significance of the time-fungicide interaction in the analysis of variance of total cured leaf is of little interest as the presence of the control evidently contributed largely to this significance. In the analysis of variance of the clean leaf, a significant time-fungicide interaction exists, despite the exclusion of the control figures. The explanation of this significant interaction lies in the aberrant behaviour of the fungicidal mixture (3) which is considerably more effective at the time D than at C. This difference is shown clearly in Fig. 4. This fungicidal mixture, on account of its higher dilution and its consequent low copper content, is probably affected by expansion of leaf surface and by leaching due to rains, more severely than the more concentrated fungicides.



Block by Survey Dep.: Caylon. 7-6-38.

FIG. 4.—YIELDS OF TOTAL AND CLEAN LEAF FROM VARIOUS TREATMENTS.

*Time of Spraying.*—Two factors are of obvious importance in determining the suitability of any particular time for spraying, *viz.*, the rate of expansion of leaf surface and the drift of frog-eye infection. Spraying during a period of rapid extension of leaf surface is of little value, as new unprotected tissue is being continually exposed to infection. The curve representing the increase in surface area of the leaves of a tobacco plant with time would be sigmoid, and spraying should, on theoretical grounds, be delayed till the curve begins to flatten out. The limiting factor to this delay would be the increase in the volume of infection. Protective spraying would of course, be comparatively valueless if considerable penetration by the pathogen has already occurred. The infection curve would also be sigmoid if the leaves were left unharvested on the plant. In actual practice, the leaves are harvested before the flattening out of the curve becomes evident.

In the field, the appearance of flower buds provides an index of the stage at which the rate of leaf expansion begins to decline rapidly. The plants are topped soon after they flower. Subsequently, there is no increase in leaf number, while the extension of surface area of existing leaves ceases soon after topping. The percentage of topped plants in the field is accordingly an estimate of the proportion of plants at the stage of maximum extension of leaf surface. The percentages of topped plants and of plants which had reached the topping stage in the experimental plots at the time of spraying were 3·3, 35·0, 60·7 and 91·2 for the dates A, B, C and D, respectively. Date C was found to be the optimum time of spraying although the percentage of topped plants was only 60·7 as against 91·2 for D. Fig. 3, which illustrates the appearance of the symptom picture in the experimental plots, provides an explanation of this result. It has been shown above that, under Ganewatta conditions, the incubation period of a frog-eye lesion appears to be under eleven days. The curves in Fig. 3 imply the existence of parallel infection curves, the symptom picture curves having a lag of not more than eleven days behind the infection curves. The lesion numbers were still very low in the control plots on February 10. The spraying date C, *viz.*, January 27, is at least three days ahead of the infection date corresponding to the symptom picture of February 10; the infection level on spraying date C must therefore have been very low. The curve of infection at this stage appears to have been logarithmic, and the volume of infection one week later, *viz.*, on spraying date D, had consequently increased considerably. It is suggested that this rise in infection level or volume of infection was the cause of the reduced efficacy of the spraying at time D in spite of the fact that the leaves of the plants at this stage had expanded more nearly to their maximum. On dates A and B the leaves

were evidently still undergoing rapid expansion, the percentage of topped plants being 3·3 and 35·0 respectively, and this explains the imperfect protection afforded by the sprays at these times.

Spraying date C appears to have been the optimum because it included the maximum proportion of topped plants, *i.e.*, the maximum leaf area, compatible with a minimum infection level.

A consideration of the symptom picture curves indicates that two sprayings, at intervals of about a fortnight, would give better control of frog-eye than any one single spraying. More than two sprayings would be unlikely to give significant additional control.

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