STUDIES ON THE BACTERIAL LEAF SPOT DISEASE OF BETEL

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INTRODUCTION

THE bacterial leaf spot disease of betel (Piper betle), caused by *Pseudomonas betle* (Ragunathan) Burkholder (10, 2), has long been known as a devastating disease transforming luxurious cultivations into masses of rotten vines within a very short time. The disease which was first recorded about the turn of the century (10) is particularly severe in the low country wet zone of Ceylon. A detailed description of the disease has been given by Park and Fernando (8).

Early investigations on the disease were carried out by Ragunathan (10) who isolated and characterised the causal organism. However, all efforts to bring the disease under control with spray materials proved futile.

In recent years, the role of antibiotics in crop protection has received increasing attention and their value in controlling bacterial diseases which have hitherto defied control by conventional fungicides recognised. Antibiotics have the great advantage in that they can act systemically; they can be absorbed into plant tissues and translocated within them so as to combat pathogenic bacteria which have already gained entrance into plants. Streptomycin, for example, has been shown to be very effective in controlling fire blight of pear (*Erwinia amylovora* (Burrill) Winslow et al.) (1, 3), halo blight of beans (*Pseudomonas phaseolicola* (Burkholder) Dowson) (11), bacterial canker of tomato (*Corynebacterium michiganense* (Smith) Jensen) (5), and other diseases. Vancomycin has been found to be effective against a wide spectrum of pathogenic bacteria (7). The potential use of antibiotics even against fungus diseases is being explored, for example, Antimycin Blastmycin and Blastcydin S in the control of rice blast (*Piricularia oryzae* Briosi *et* Cavara) (6). In bioassay investigations, *Pseudomonas betle* too was found to be sensitive to antibiotics (9).

This paper presents the work carried out in the Division of Plant Pathology, Peradeniya, on the casual organism of bacterial leaf spot and its control by antibiotics.

ISOLATION OF PSEUDOMONAS BETLE

Isolations are best made from the minute oily lesions of tender infected leaves. In more advanced lesions secondary fungi are invariably encountered. The bacterium grows satisfactorily in potato dextrose agar and more rapidly in potato dextrose peptone agar.

INOCULATION EXPERIMENTS

These experiments were carried out to determine whether the disease could be artificially induced under experimental conditions. The materials used for inoculation included fresh detached leaves maintained in moist chambers, leaf segments placed on moist filter paper in petri dishes, stem cuttings maintained in nutrient solutions and plants established in pots. Fresh isolates of the bacterium were used for inoculation and several methods were explored, viz. smearing the leaf surface with the bacterium, spraying a bacterial suspension with an atomiser, spraying a suspension under high pressure, immersing leaves in a bacterial suspension and applying the bacterium at points on the leaf surface where abrasions were caused by fine needles. In all cases the leaves were kept under moist conditions before and after inoculation. Spraying the lower surface of leaves with a bacterial suspension gave the best results, the time taken for the appearance of symptoms being generally 15-24 days. The bacterium was reisolated from the lesions induced. However, certainty of positive infection even by this method was not assured. In one instance, a three day old culture which had been subcultured thrice at approximately weekly intervals since its initial isolation was successful in inducing the disease. This indicates that the pathogen does not loose virulence shortly after transfer to artificial media.

BIOASSAY STUDIES

Bioassay studies were carried out with the antibiotic preparations Phytomycin and Agri-mycin 100. The former is a Japanese streptomycin preparation, the latter a U. S. formulation containing both streptomycin (15%) and terramycin (the trade name of Chas. Pfizer & Co. Inc. for oxytetracycline) (1.5%). Potato dextrose agar was used and the bacterium was dispersed in it by two methods. In the first method the organism was introduced into the medium while still liquid and plated. In the second method which proved more satisfactory, the bacterial suspension was sprayed on the surface of PDA plates with a sterile atomiser. Filter paper discs soaked in the test antibiotics were placed on the surface of the inoculated medium. Clear zones of inhibition of the bacterium were produced by both Phytomycin and Agri-mycin 100. The effect of various concentrations of Agri-mycin 100 on the bacterium was also investigated. The diameters of the zones of inhibition produced are given in Table 1.

TABLE 1

Inhibition zones produced by three concentrations of Agri-mycin 100

Concentration in p.p.m.			*Diameter of zone of inhibition in mm.**
Streptomycin 200 Terramycin 20	..	..	30·99
Streptomycin 400 Terramycin 40	..	..	34·36
Streptomycin 600 Terramycin 60	..	..	36·37

* Mean value from 28 measurements.

FIELD TRIALS

The field trials were conducted in cultivators' fields in Beligala. In these cultivations the vines were grown in long raised beds 45 ft. × 4 ft. with 44 rows of vines, each row with 4 stakes and generally 2 vines per stake. These beds were divided into plots for the various treatments with a buffer row between adjacent plots. The cultivations, once luxuriant, were heavily infected when taken over for the trials. The infected vines were pruned and defoliated at the commencement of the experiment. Sprays were applied at 10 day intervals. Prior to the application of each spray all infected leaves were plucked off.

In assessing infection, counts were made of the number of leaves per plot as well as the number of infected leaves. A 0-5 rating scale was used and infected leaves were classified into five groups depending on the serevity of infection, each group being allotted a numerical score corresponding to the scale:

0 — No infection
1 — Very slightly infected
2 — Slightly infected
3 — Moderately infected
4 — Severely infected
5 — Very severely infected

The intensity of infection was then expressed by the aggregate of the number of leaves in each infection group multiplied by the corresponding numerical infection score. From this a disease index was calculated as follows:

$$\text{Disease index} = \frac{\text{Score of infection attained}}{\text{Maximum score of infection attainable}} \times 100$$

The formulations screened in these trials are given in Table 2.

TABLE 2

Formulations screened and their active materials

Formulation		*Active material*
Merculine	..	6% Phenyl mercuric salycilate
Fixtan	..	10% Phenyl mercuric dinaphthyl methane disulphonate
Phytomycin	..	50,000 I.U. Streptomycin-HCL per cc.
Agri-mycin 100	..	15% Streptomycin and 1·5% Oxytetracycline (Terramycin)

Glycerol was added to the antibiotic sprays to achieve better penetration (4).

Two trials were carried out.

Trial 1.

The following treatments were evaluated:

(1) Merculine at 0.00003 gm Hg/cc.
(2) Fixtan at 0.00003 gm Hg/cc.
(3) Phytomycin at 1cc./500 cc. water.
(4) Agri-mycin at 400 ppm. streptomycin and 40 ppm. terramycin plus 1% glycerol.
(5) Control.

The treatments were replicated twice with 30 vines per plot. The variety used was 'Ratadalu'. Five sprays were applied in treatments 3 and 4. In treatments 1 and 2, spraying was discontinued after the third spray due to heavy phytotoxicity. Table 3 shows the course of infection over the period of observation and this is illustrated graphically in Fig. 1. (see page 75). Increased infection was found to coincide with periods of very wet weather.

TABLE 3

Disease indices based on infection in periods between successive sprays

Treatment	Number of sprays applied				
	1	2	3	4	5
1. Merculine	24·9	20·2	23·0	*	—
2. Fixtan	15·7	14·4	19·1	*	—
3. Phytomycin	7·6	7·7	15·8	22·0	10·9
4. Agri-mycin 100	20·2	5·2	4·1	7·6	4·9
5. Control	28·0	27·3	28·3	49·1	†

* Spraying discontinued due to phytotoxicity.

† Vines practically dead.

The antibiotics achieved a significant measure of control of the disease, Table 4. The percentage of leaves infected and the severity of infection were comparatively low while leaf production was maintained at a fair rate in marked contrast to the untreated controls and the organomercurial treatments which showed severe phytotoxic symptoms.

TABLE 4

Disease indices based on infection during entire period of treatment

Treatment	Disease Index
1. Merculine	22·38
2. Fixtan	16·73
3. Phytomycin	10·89
4. Agri-mycin 100	7·70
5. Control	27·79
Significant difference (5%)	3·35

Trial 2.

This was also carried out on the same lines as the previous trial with the following treatments:

(1) Agri-mycin 100 at 200 ppm. streptomycin and 20 ppm. terramycin plus Merculine at 0.0001 gm Hg/cc. plus 1% glycerol.

(2) Agri-mycin 100 at 400 ppm. streptomycin and 40 ppm. terramycin plus 1% glycerol.

(3) Agri-mycin 100 at 600 ppm. streptomycin and 60 ppm. terramycin plus 1% glycerol.

(4) Control.

The treatments were replicated twice with 30 vines per plot. The variety used was 'Ratadalu'. Five sprays were applied. Table 5 shows the course of infection over the period of observation and this is illustrated graphically in Fig. 2 (see page 76).

TABLE 5

Disease indices based on infection in periods between successive sprays.

Treatment	*Number of sprays applied*				
	1	*2*	*3*	*4*	*5*
1. Agri-mycin at 200–20 plus Merculine	11·5	15·6	9·3	5·5	1·4
2. Agri-mycin at 400–40	8·4	15·4	6·3	5·3	0·6
3. Agri-mycin at 600 –60	8·8	12·7	3·5	5·5	0·6
4. Control	19·3	20·7	20·6	*	—

* Vines practically dead.

Agri-mycin 100 was again found to be very effective in controlling the disease even at the lowest dosage. The phytotoxic effects which Merculine produces were observed to a lesser extent at the reduced rate of application in combination with Agri-mycin 100. The results are shown in Table 6.

TABLE 6

Disease indices based on infection during entire period of treatment

Treatment	*Disease Index*
1. Agri-mycin at 200–20 plus Merculine	10·95
2. Agri-mycin at 400–40	9·43
3. Agri-mycin at 600–60	7·53
4. Control	20·03
Significant difference (5%)	2·44

DISCUSSION

These preliminary trials give a clear indication of the potentialities of agricultural antibiotics in combating the bacterial leaf spot disease of betel which has hitherto resisted all attempts at control by other chemical formulations. It is evident that the success of these antibiotics is largely due to their systemic action. Another factor which has enhanced their efficacy has been the use of glycerol to secure better absorption of the antibiotics into the plant tissues.

Antibiotics are a relatively recent development in biological science. With the successful development of penicillin, they have found extensive application for medicinal purposes. It seems probable that in crop protection, too, antibiotics will play an increasingly important role in the near future. At the present time, the relatively high cost of agricultural antibiotic formulations is an important factor to be considered. Nevertheless, a reduction in prices should be expected with increasing production as antibiotics find wide usage. This will enable a greater measure of control of those plant diseases, especially due to bacteria, which have long defied attempts at control.

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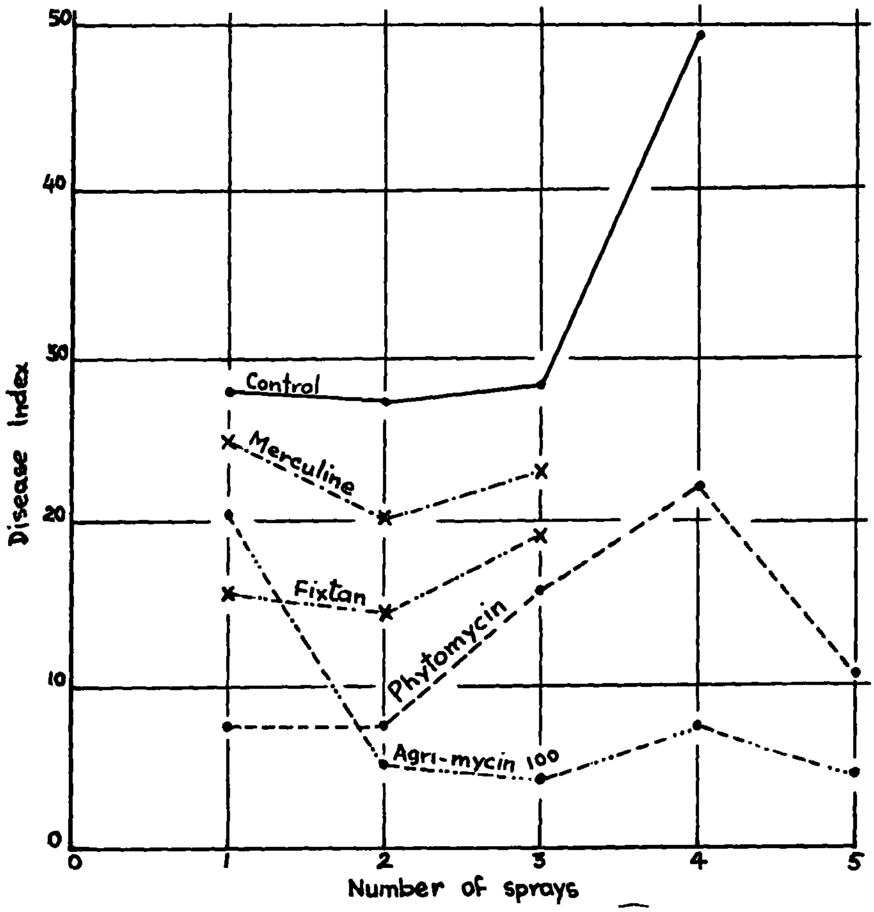

Fig. 1. Disease indices based on infection in periods between successive sprays.

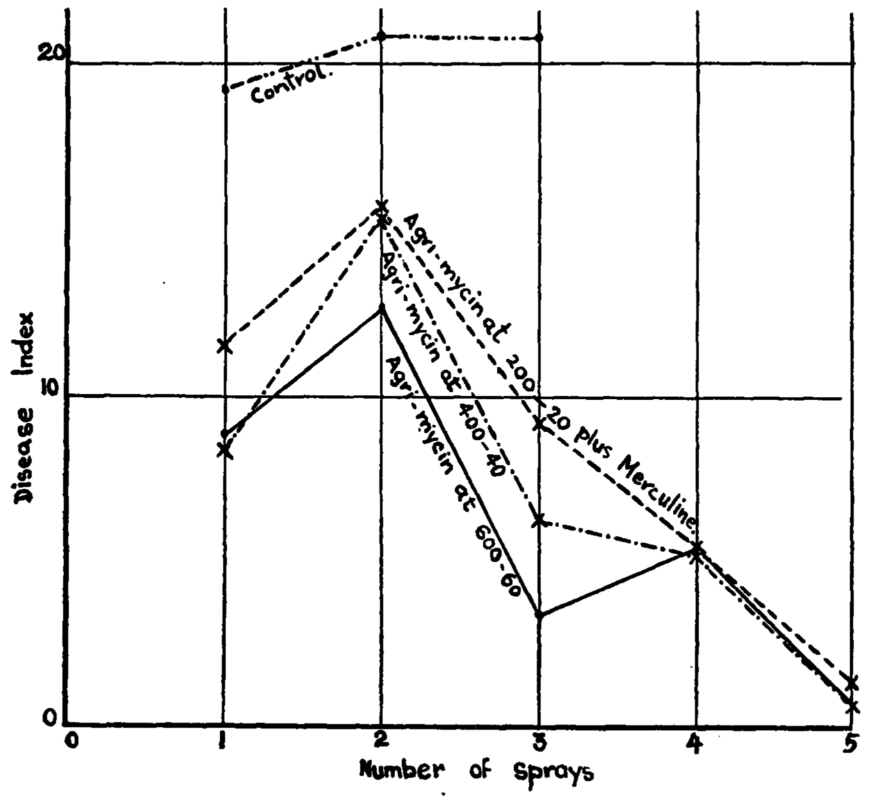

Fig. 2. Disease indices based on infection in periods between successive sprays