Effect of Seed Protectants on the Control of Bacterial Blight of Cotton

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INTRODUCTION

BACTERIAL BLIGHT is a collective name for the different phases of a destructive rain spread disease of cotton caused by the bacterium *Xanthomonas malvacearum* (E. F. Sm.) Dowson. It has been recognized as a major disease of cotton in several countries particularly in Sudan and the U. S. A. where climatic conditions for severe epiphytotics are favourable (3, 5). It is a disease of considerable complexity and the manifestation of its symptoms show striking variation according to the part of the plant attacked. The disease on the leaf is descriptively named ' Angular Leaf Spot ' and ' Vein Blight ', on the fruiting structure as ' Bract Spot ' and ' Boll Blight ' and on the stem as ' Black Arm '.

The causal organism perennates on the surface of the seed and on the fibres attached to it, but rarely inside the seed (6, 12). Contaminated seed often provides the initial source of infection in a crop while infected crop residues, wind, warm humid conditions, dew and strong wind-driven rains are conducive to the rapid dissemination of the pathogen (1, 2, 7).

The ultimate solution for the control of bacterial blight of cotton devolves on breeding varieties resistant to attack by the bacterium. Control measures in the nature of seed treatment, destruction of infective crop residues, crop rotation and other cultural operations are interim palliative measures which will help to minimize the severity of the disease.

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Seed disinfection to control the primary infection has been the subject of great deal of field experimentation in the last 3 to 4 decades. In the U. S. A. delinting of seed as a ' wet process ' with sulphuric acid or as a " dry process " with fumes of sulphuric acid and subsequent dusting of the acid delinted seeds with an organo-mercurial fungicide has been successfully employed (4, 9). This method, although expensive and machine operated, has the advantage of effective control of the disease and improved seedling emergence (10). In the U. S. S. R. treatment of seed with formalin has been proved to be effective. In African countries a mixture of mercuric chloride and mercuric iodide has been used for many years as a dry seed dressing. Recently ' short wet process ' of seed treatment with organo-mercurial formulations has given promising results in reducing seed borne infection (8, 11). In Ceylon, with the expanded cultivation of cotton on a commercial scale this disease has gained prominence as a major threat. The study reported in this paper summarises the results of field experiments conducted at the Cotton Research Station, Hambantota, where organo-mercurial, antibiotic and other organic fungicide formulations were evaluated for their relative efficacy in controlling seed borne infection of bacterial blight.

MATERIALS AND METHODS

The design of the field experiment consisted of 4 blocks within each of which 10 treatments were randomised. The plot size was 63′ × 12′ or 1/60th of an acre in area. The seeds were hand sown into holes spaced 2′ × 3′. Seed for the trial was selected from a heavily diseased crop of the previous season. Seed of a highly susceptible variety originating from a heavily diseased crop was therefore adequate to provide the inoculum sufficient for field evaluation of the seed protectants.

The treatments included 5 organo-mercurial formulations " Mema " A, B, C, and D containing 2-methoxy ethyl mercury acetate as active ingredient in different proportions, Ceresan wet (ethyl mercury acetate), an antibiotic formulation of Streptomycin sulphate and three organic fungicides ' Orthocide (Captan 50 per cent.), Orthophaltan (50 per cent. N-trichloromethylthiophthalimide), and Difolatan (N (1, 1, 2, 2,-tetrachloroethylthio) 4 cyclohexene-1, 2-dicarboximide 80 per cent). Machine delinted infected seeds were soaked in the treat-

ments for 2 hours, sun dried and sown into regularly spaced holes at the rate of 6 to 7 seeds per hole. Vacancies were filled approximately 12 days after sowing.

In obtaining disease estimates it was essential to differentiate between the amount of residual seedborne infection that survived the 2 hours exposure to the different treatments and the amount of infection incited by the subsequent secondary spread of the disease. The former was determined at fortnightly intervals by estimation of diseased lesions on cotyledonary leaves and the new flush of leaves. With the advance in the age of the crop the observations on seedborne primary infection became increasingly obscured by the secondary spread of the disease within and between plots. After the secondary spread of the disease set in, the severity of the different phases of the disease was estimated on the following numerical scale from 0 to 4 :—

0 = No infection
1 = Mild infection
2 = Moderate infection
3 = Severe infection
4 = Very severe infection

The colour, shape, size and the manner of development of individual lesions formed the basis of determination, of the severity of infection. Small sized restricted lesion without an initial water soaked appearance was regarded as mild infection while large sized water soaked spreading type of lesion was rated as very severe infection.

RESULTS

Seedling emergence

Resowing to fill vacancies was not extensive in most treatments, and ranged from 9.6 per cent vacancies in Streptomycin treated to 19.4 per cent in ' Orthophaltan ' treated seed. The maximum reduction in emergence was in seed treated with Mema C where in addition inhibition of germination phytotoxic symptoms of stunting, distortion of cotyledonary leaves and drying of leaf margins persisted for two to three weeks. The plots treated with Mema C continued to have vacancies even after resowing due to the marked reduction of over 50 per cent in seedling emergence. Significantly higher seedling emergence was

recorded with Mema A in comparison to that of untreated seed. Table 1 compares the per cent germination of seed treated with Mema A and the untreated control over a period of three successive cropping seasons.

TABLE 1.

Treatments	*Percentage Seedling Emergence in*		
	Maha 1963–64	*Maha 1964–65**	*Maha 1965–66*
Mema A ..	88	73	89·5
Untreated Seed ..	81·5	68	82·5

* The crop was affected by severe drought Significant at P = 0·05.

Primary Infection of Seedlings

The first two ratings chiefly constituted the disease arising from the residual seed-borne infection while the accuracy of the third disease rating was somewhat reduced on account of the secondary spread of the disease. A summary of the estimation of residual seed-borne infection and the severity of the disease at the end of the 7th week together with per cent emergence of seedlings, yield of cotton and the relevant statistical data appear in Table 2. The arc sinangular transformation was used in the analysis of the data.

Table 2.—Primary Infection of Bacterial Blight at 3 Stages of the Crop

Seed Protectant	*Dosage per bushel*	*Per cent Emergence*	*Infected Seedlings in arc sin transformation*			*Disease Severity Rating in 7 weeks*	*Yield in cwt. per acre*
			2 weeks	*4 weeks*	*7 weeks*		
Mema A	1½ fluid oz.	88·0	0·62	2·17	5·68	2·35	4·4
Mema B	1½ fluid oz.	84·0	0·62	3·22	10·01	2·68	3·14
Mema C	1½ fluid oz.	47·6	2·36	6·00	8·60	2·56	3·12
Mema D	3 fluid oz.	89·2	2·25	2·55	16·19	5·77	3·05
Ceresan Wet	1½ fluid oz.	84·8	7·63	8·82	19·69	7·75	3·52
Streptomycin	500 parts per million	90·4	8·30	9·85	28·46	8·01	3·43
Orthocide	1½ oz.	82·7	7·28	9·54	33·77	8·27	3·47
Orthophaltan	1½ oz.	80·6	8·05	9·11	36·57	8·69	3·99
Difolatan	1½ oz.	83·5	8·47	10·81	37·16	9·17	4·06
Control		81·5	7·60	10·44	36·31	9·52	3·43
L. S. D.			1·98	3·19	4·56	1·99	not significant
C. V.			25·6	30·48*	13·57	21·49	do.
P = 0·05							do.

Vacancies were not completely filled due to phytotoxicity of the chemical

* A high C. V. value in the 4th week may have been due to filling of vacancies.

As illustrated in Table II in the first two disease estimations the Mema formulations effectively reduced seed-borne infection and were outstanding as seed protectants for the control of bacterial blight. None of the other chemical formulations tested in this trial gave statistically significant reduction of seed-borne infection when compared to the untreated control. Much emphasis cannot be placed on the statistically significant differences among the treatments noted in the assessment at the end of the 7th week due to the error introduced by the secondary spread of the disease. However, both in respect of reduction in the number of diseased plants and severity of infection the Mema formulations proved to be significantly superior to all other treatments even at the end of 7 weeks though to a lesser extent.

The efficacy of control of seed-borne infection by Mema C was not correctly assessed due to the very high percentage of vacancies that needed resowing and partly due to the vacancies that existed even after resowing. The phytotoxic symptoms observed initially were also reflected in reduction of plant height measurements.

Both Mema A and B reduced seed-borne infection more effectively than Mema D, giving no infection in 2 weeks and 2.17 and 3.22 infected seedlings respectively at the end of 4 weeks compared to 2.25 and 2.55 infected seedlings at 2 and 4 weeks respectively for Mema D. These initial differences in primary infection persisted and enlarged thereafter with the advance in age of the crop. Thus in 7 weeks, 5.68 and 10.01 infected seedlings were recorded in the plots treated with Mema A and B respectively and in contrast 16.19 infection was recorded in plots treated with Mema D. Although these differences in the efficacy of control of primary infection among the Mema series of chemicals were not statistically significant, on close examination of the data Mema A appears to be superior to the other formulations.

Streptomycin as a treatment, although not effective on the control of seed-borne infection, promoted emergence and a full stand of vigorous seedlings in all plots.

The explosive epiphytotic of the disease after the 4th week may be ascribed to the heavy monsoonal rainfall of 28″ experienced at the early stages of the crop. The distribution of rainfall during this period was as follows :—October 9 rainy days with an aggregate of 10.55″ from date of sowing, November 19 rainy days with an aggregate total of 13.91″ and December 7 rainy days with 3.92″ of rainfall. This

heavy rainfall during the seedling phase of the crop provided sufficient moisture for the rapid dissemination of the pathogen as can be seen from data on disease intensity illustrated in Table II and III.

An examination of the data in Table II reveals two important and major effects of seed treatment. They are, firstly, an initial reduction of seed-borne infection resulting in a delayed outbreak of the disease thereby enabling the crop to escape disease during the most susceptible stage, and secondly the reduction in the severity of the disease which prevents an initial set back that could be caused to the crop.

Relative severity of the disease in different treatments

The severity of the different phases of the disease, viz. angular leaf spot, vein blight, black arm, and boll blight was estimated when the crop was 2½, 3 and 4 months old. In the analysis of the results the average severity ratings were transformed into arc sine square. The results are summarised in Table III.

TABLE 3. Severity of the Different Phases of the Disease as affected by different seed protectants

Treatments	*%Diseased plants at 2½ months*	*Mean Disease in Arc Sine*			
		Angular Leaf Spot	*Vein Blight*	*Black Arm*	*Boll Blight*
Mema A	19·4	11·9	0·96	0·0	0·0
Mema B	35·5	14·46	7·86	0·0	0·0
Mema C	23·9	18·94	13·23	0·0	0·0
Mema D	59·3	28·45	13·83	8·22	0·0
Ceresan Wet	76·9	33·67	27·75	18·52	1·35
Streptomycin	87·6	35·33	23·51	14·76	7·10
Orthocide	86·1	38·67	28·83	28·92	12·89
Orthophaltan	72·4	44·80	26·71	28·47	15·51
Difolatan	79·2	37·53	23·43	18·29	5·29
Control	78·4	47·75	30·58	29·58	15·18
Significant at P = 0·05					
L. S. D.		13·31	10·65	14·11	11·95

As illustrated in Table III there is a close similarity in the primary disease resulting from seed-borne infection and the severity ratings of the different phases of the disease appearing towards the latter part of the crop. The Mema treated plots were free from attack of the more severe phases of the disease, and highly significant differences in the severity ratings were noticed between the Mema treated plots

and the untreated control, especially in regard to angular leaf spot and vein blight phases of the disease. The other seed protectants tested failed to show statistical differences between them and the untreated control. Among the Mema formulations the reduction of disease in Mema A treated plots was statistically significant when compared to Mema D, while the differences in the infection ratings in Mema B, C and D were however not statistically significant.

The heavy rainfall in November and December accounted for the rapid dissemination of the pathogen and the explosive ephiphytotic of the disease. The increase in severity of the disease was observed in January and February during which months precipitation in the form of dew provided ample moisture for a progressive spread of the disease. The severity ratings showed highly significant increases from 2½ months to 3 months and from 3 months to 4 months.

The reduction in the disease ratings in Mema treated plots may be ascribed to two major causes ; firstly, the highly effective reduction in seed-borne infection resulting in a reduction in the potential inoculum for disease development ; and secondly the possible persistent and systemic effect of the chemical reducing the incidence of secondary spread and thereby reducing the severity of the disease.

DISCUSSION

The rainfed cotton crop in Ceylon experiences climatic conditions favourable for severe epiphytotics of the bacterial blight disease. The monsoonal rain during the months of October, November and December favour the rapid dissemination of the pathogen, while dew in subsequent months in conducive to a slow spread and increase in severity of the disease. In the absence of varieties that are immune to the disease palliative measures designed to minimise the initial reservoirs of the pathogen are essential. Sowing seed naturally infected with the pathogen, and volunteer seedlings originating from seed dispersed from unpicked infected bolls of the previous season constitute the major sources of initial infection in a new crop, while infected crop residues left on the field to a great extent favour the secondary spread of the disease.

The application of seed protectants in this trial has varied benificial effects although the yield differences were not statistically significant when related to the untreated control. The Mema formulation in particular Mema A effectively eliminated the pathogenic organism on the seed and this resulted in a delayed outbreak of the disease in the Mema treated plots. The infected seedlings originating from seed

treated with Mema A in the first seven weeks were in the order of no infection in 2 weeks, 2.17 in 4 weeks and 5.68 in 7 weeks which is a negligible trend compared to the untreated control where 7.60 in 2 weeks, 10.14 in 4 weeks and 36.31 in 7 weeks were recorded.

The maximum spread of the disease was in the first two and half months when the rainfall was also high. The relatively slow spread of the disease in the Mema treated plots may be attributed to the reduction in the amount of inoculum arising from residual seed-borne infection that survived treatment and also to a possible systemic property of the chemical which prevented recondary invation by the bacterium. These initial differences in reduction of seed-borne infection persisted to a lesser extent in the mature crop due mainly to the secondary spread of the disease. Despite the susceptibility of the crop, the Mema treated plots were less infected with the more serious phases of the disease. It is unlikely that the Mema formulations exercised a persistent effect sufficient to last 3 to 4 month in the test area. On the contrary it is probable that the initial reduction in residual seed-borne infection considerably depressed the inoculum for secondary spread.

In addition to the great efficacy of control of seed-borne infection the Mema formulation promoted emergence of a full stand of vigorous seedlings which may be ascribed to prevention of attack of seedlings by both soil and seed-borne pathogenic organisms. Treatment of seed with an organo-mercurial fungicide such as Mema may therefore result in effectively reducing the seed rates currently used and in getting greater seedling emergence, which will obviate the necessity to fill vacancies.

SUMMARY

"Short Wet" process of seed treatment of heavily infected cotton seed with Mema formulations containing 2-methoxy ethyl mercury acetate as active ingredient considerably reduced seed-borne infection. The formulation Mema A was superior to the other Mema chemicals and promoted better stands and improved emergence. Mema C was phytotoxic and inhibited germination by over 50 per cent.

The disease was observed in a mild form in the Mema treated plots due to its delayed development possibly caused by the systemic effect of the chemical in the seedlings. The other seed protectants tested including an antibiotic and a common organo-mercury seed dressing were ineffective and could not bring about a substantial reduction in the primary infection of cotton seedlings. On the basis of these results Mema A could be profitably used in the control of seed-borne infection of Bacterial Blight of cotton in Ceylon.

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