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Technique to create epiphytotics of angular leaf spot (*Xanthomonas malvacearum* (Smith) Dowson) of cotton. (ii) Field Trials *

by

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Synopsis: Studies conducted by the author to evolve a method by which epiphytotics of angular leaf spot of cotton could be created under field conditions are described in this paper.

Introduction: Angular leaf spot otherwise known as blackarm of cotton is a major disease of cotton and occurs in many cotton growing countries. In India, this disease occupies the primary importance in view of the loss it causes to the yield of cotton especially in certain States like Andhra Pradesh, Madras, Mysore and Kerala. This disease is a limiting factor in the cultivation of Sea Island cotton along the west coast areas and in places of heavy rainfall. Attempts are being made to exploit the source of resistance to this disease from a number of species and varieties of cotton.

In evaluating the genetic stocks for their resistance to this disease, a suitable technique to create epiphytotics of this disease is essential as in the absence of such a technique the grading of a particular variety varies from year to year or from place to place according to seasonal variations. In the past, various methods have been described by different authors which have been briefly enumerated by Sundaram (1961). However, these techniques were found to be inadequate to create epiphytotics of the disease under field conditions. Sundaram (1961) described a technique to create the disease in an epiphytotic form under laboratory conditions based on which further studies were conducted to evolve a method to create the disease under field conditions and the results are briefly reported in this paper.

Materials and Methods: The variety MCU-3 [*G. hirsutum*] selfed seeds were used throughout the experiment. The seeds were delinted with sulphuric acid and treated with Agrosan GN (1:350) before sowing. The seeds were dibbled in ridges with spacing of 2½' between rows and 1' along the rows. Two seeds were dibbled per hole but thinned out to one 40 days after germination. Basal dose of N:P:K at 40:30:30 in the form of urea, superphosphate and muriate of potash was applied. A second dose of 20 lb. N. was applied 45 days after germination.

Inoculum was prepared by collecting cotton leaves showing blackarm infection grades of 5 and above, which were air dried under shade for 3 to 4 days, powdered and kept free from dampness. The leaf infusion for spray inoculation was prepared by soaking 1 oz. in 1 gallon of water for 2 hours with squeezing at frequent intervals and strained through thin muslin cloth. The spraying was done with a pressure retaining high volume knapsack sprayer at 60 lb. pressure. The

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culture was prepared by freshly isolating the bacteria on nutrient agar medium and 18-24 hours old culture kept under the room temperature was mixed in water and spray inoculated.

Covering the plants under field conditions was done with transparent polythene sheet (300 gauge) supported by bamboo stakes (Fig. I). The spray inoculations were done for two consecutive days at any one spray schedule. In all, the plants were spray inoculated for 4 periods.

The cotyledon infection was graded as light, medium and heavy with corresponding numericals 1, 2 and 3 (Fig. II) and the true leaf infection with 7 grades viz., 0. Totally free, 1. Few minute scattered spots, veins free, 2. Greater number of spots scattered, veins free, 3. Spots larger, more in number, veins free, 4. Lesions large coalesce, typically angular spreading along the small veins also, 5. Lesions large, coalescing, more in number and the bigger veins also are affected. The spots become brownish-black and dry up in patches, 6. Veins and midrib infection covering to pulvinus spot forming prominent necrotic area, extending to the petiole, black, drying up of larger portion of the leaf, 7. Leaf crumples, drying, petiole with sooty black bands extending to stem; shoot engirdled resulting in death. (Fig. III.)

Experimental results: The experiment was laid in split plot design during the year 1961-'62 and replicated four times with the combination of presoaking the seeds with blackarm culture, addition of infected leaf debris in planting holes and spray inoculation with or without covering. The details of the treatments are furnished below.

Main treatments: Two

1. Covered with alkathene cage 3 days before and after spray inoculation.
2. Not covered.

Sub-treatments: Eight

1.	Seeds soaked in bacterial suspension	Sown in debris	Spray inoculated with leaf infusion.
2.	,,	,,	Not spray inoculated with leaf infusion.
3.	Seeds soaked in bacterial suspension	Sown without debris	Spray inoculated with leaf infusion.
4.	,,	,,	Not spray inoculated with leaf infusion.
5.	Seeds not soaked	,,	Spray inoculated with leaf infusion.
6.	,,	,,	Not spray inoculated with leaf infusion.
7.	,,	Sown in debris	Spray inoculated with leaf infusion.
8.	,,	,,	Not spray inoculated with leaf infusion.

The infected leaf debris was added to the planting hole at 2 oz. per row of 20 feet long (2·5 g per hole). The spray inoculation was uniformly given between 11 a. m. and 2 p. m. repeating the same the following day also. The preliminary observation on the cotyledon infection was recorded on the 15th day after sowing when the water soaked spots were clearly visible. The results of observation made during 1961-'62 are recorded in table 1.

FIG. I. Plots covered with polythene sheets before and after spray inoculation.

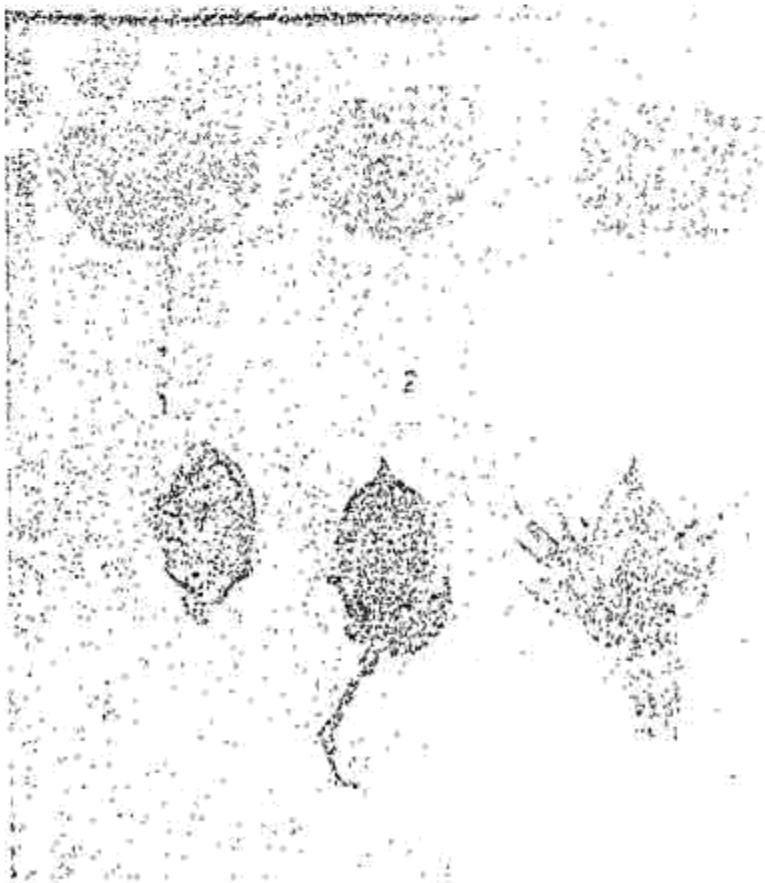

FIG. II. Upper row: 1, 2, 3: Cotyledon infection under artificial creation.
Lower row: Boll infection (a) Infection on lint and seed, (b) severely infected boll, (c) Initial symptom of infection on boll and bracts (under artificial creation).

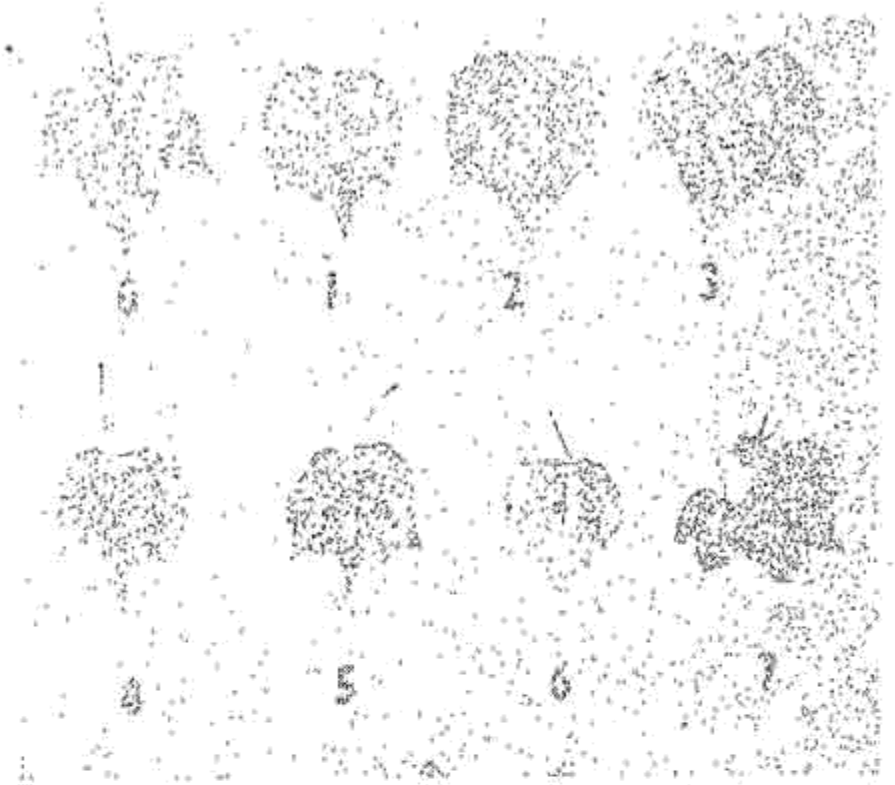

FIG. III. Grades of leaf infection 0—7.

FIG. IV. Seedling blight and branch infection on a very susceptible variety (under artificial creation).

TABLE I.

Mean intensity of cotyledon infection (1961–'62)

S. No.	Details of treatments	Mean intensity of infection
1.	Seeds presoaked and sown in infected leaf debris	1.43
2.	Seeds presoaked and sown without debris	1.52
3.	Seeds not presoaked and sown in infected leaf debris	1.21
4.	Seeds not presoaked and sown without debris	0.41

0: Free 1: Light 2: Medium 3: Heavy.

A high degree of cotyledon infection was created by presoaking the seeds with culture solution and sowing in leaf debris added to the planting hole. However, there was no difference between treating the fuzzy seeds with freshly isolated bacterial culture before sowing and dibbling the seeds in holes applied with infected leaf debris of cotton.

The average grades of leaf infection representing the mean of 4 observations made 10 days after each set of spraying are recorded in table II.

TABLE II.

Mean grades of leaf infection (1961–'62)

S. No.	Details of treatments	No spray inoculated		Spray inoculated	
		Not covered	Covered	Not covered	Covered
1.	Seeds presoaked and sown in infected leaf debris	2.0	1.8	5.2	5.6
2.	Seeds presoaked and sown without debris	1.5	1.5	4.8	6.0
3.	Seeds not presoaked and sown in infected leaf debris	1.9	2.3	4.6	5.6
4.	Seeds not presoaked and sown without infected leaf debris	1.5	1.4	4.3	5.4

	'F' test	S. E.	C. D. (at 5% probability)
Spraying *vs.* No spraying	Significant	= 0.10	0.30
Covering *vs.* No covering	,,	= 0.14	0.42

It was found that the treatments spraying *versus* no spraying and covering *versus* no covering alone were significant.

The spray inoculation has given significantly increased infection over unsprayed plots irrespective of other treatments. Covering the plants in combination with spray inoculation was found significantly superior to "no covering"

plots. The other treatments *viz.*, soaking the seeds with culture solution and applying infected leaf debris to soil before sowing did not create secondary infection to any significant level.

In view of the difficulties experienced in providing a cover for the plants on the field before and after spray inoculation and due to the narrow difference in the intensity of infection between the treatments, the trial was laid out during 1962–'63 and repeated during 1963–'64 with slight modifications as detailed below :-

Details of treatments :

1.	Seeds sown without debris	No spray inoculation.
2.	,,	Spray inoculated with culture solution.
3.	,,	Spray inoculated with leaf infusion.
4.	Seeds sown with debris	No spray inoculation.
5.	,,	Spray inoculated with culture solution.
6.	,,	Spray inoculated with leaf infusion.

Method of raising the plants, spray time, etc., were kept as in the previous year.

The cotyledon infection as well as leaf, branch and boll infections (Fig. II and IV) recorded during 1962–'63 and 1963–'64 are furnished in table III.

The addition of infected leaf debris to the planting hole did not result in significant increase in secondary infection as compared to other methods except treatment No. 1. Spray inoculating the plants between 11 a. m. and 2 p. m. either with leaf infusion or bacterial culture gave the highest degree of infection. Significant increase in the per cent branch and boll infection was also observed in spray inoculated plots. There was no perceptible difference between spraying either culture or leaf infusion in the per cent boll or branch infection.

Another trial was laid during 1962–'63 and 1963–'64 with the following treatments in order to stimulate conditions prevailing under natural condition and to study their effect in creating disease in an epiphytotic condition. The details of the treatments are furnished below :

1. Seeds sown without debris — No spraying (Control).
2. Seeds sown in debris — Debris dusted in the previous day and water sprayed next day between 11 a. m. and 2 p. m.
3. Seeds sown in debris — Freshly isolated bacterial culture sprayed between 11 a. m. and 2 p. m.
4. Seeds sown in debris — Leaf infusion sprayed between 11 a. m. and 2 p. m.

The method of cultivation, manurial schedule, spacing, etc., were adopted as in the previous experiment. Observations were made at the end of 15 days after sowing for the incidence of primary infection on the cotyledons and at monthly intervals on the true leaves. Four sets of spray inoculations in all, on the true leaves were given and the mean grades of infection of the cotyledons, true leaves, branches and bolls are furnished in table IV.

Seeds sown without treating either with culture solution or dibbled merely in soil were the least affected with primary infection. Treating the seeds either with freshly isolated culture or sowing in debris gave significantly higher primary infection on the cotyledons. In the case of leaf, branch and boll infections, dusting the leaves with powdered infected leaf debris of cotton in the previous evening and following by water spraying the next day between 11 a. m. and 2 p. m. gave higher leaf, branch and boll infection which is found to be on par with spray inoculating the plants with bacterial culture or leaf infusion.

Discussion: Different methods were adopted in the past to create artificial infection of angular leaf spot disease *(Xanthomonas malvacearum)* in the laboratory or field to test cotton varieties for their resistance (Knight and Cloustan, 1939; Logan, 1958; Last and Dransfield, 1959; Innes, 1961). However, the methods described are either inconvenient to be adopted in testing large number of cultures or are only partially effective. A simpler laboratory method by spray inoculating the plants with bacterial leaf infusion between 11 a. m. and 2 p. m. was described by Sundaram (1961). With suitable modifications of the above laboratory methods, a technique was evolved to create epiphytotics of the disease under field conditions.

The optimum temperature for the growth and multiplication of the cotton blackarm bacterium was found to be 31° - 32° C (Patel and Kulkarni, 1950) and in nature it is known to occur in a virulent form during warmer months combined with high humidity and wet weather. The disease appeared under field conditions during the study in a virulent form under artificial infection when the day temperature varied between 31° - and 35° C.

Addition of infected leaf debris in planting holes or soaking the seeds in bacterial culture created higher intensity of primary (cotyledon) infection under field conditions (Table 1). However, these treatments had little influence on the intensity of secondary infection in comparison to spray inoculating the plants with bacterial culture or leaf infusion (Table II), thus indicating that even high cotyledon infection may not lead to secondary infection in an epiphytotic condition under unfavourable weather conditions. It was observed that the disease created on the true leaves in a severe form though causing the shedding of the leaves, did not spread to other healthy leaves under Coimbatore condition. For further spread of the disease, continued wet weather combined with bright sun during the noon appear to be quite essential. The plants therefore, should be tested in places like Coimbatore where the weather is mostly dry during the major

TABLE III.

Mean grades of primary and secondary infection.

No.	Particulars of treatments	1962—'63				1963—'64			
		Mean intensity of infection on							
		Coty ledons	True leaves	Branches per cent	Bolls per cent	Coty-ledons	True leaves	Branches per cent	Bolls per cent
1.	Sown without debris-No spray inoculation	0.27	0.60	1.70	2.10	0.06	0.96	1.50	0.90
2.	do. culture sprayed	0.26	6.30	24.50	44.20	0.08	1.74	37.60	12.30
3.	do. Leaf infusion sprayed	0.32	6.00	30.10	53.90	0.06	3.56	41.70	17.40
4.	Sown in derbis-No spray inoculation	1.58	1.20	0.30	0.70	0.71	1.25	0.60	1.20
5.	do. culture sprayed	1.59	6.20	27.60	49.60	0.70	2.82	39.20	19.30
6.	do. Leaf infusion sprayed	1.50	6.20	27.90	49.80	0.65	3.68	44.10	14.50
	'F' test	S	S	S	S	S	S	S	S
	S. E. (±)	0.37	0.12	3.18	4.37	0.04	0.20	6.6	1.13
	C. D. (at 5% probability)	0.90	0.37	9.45	12.98	0.12	0.54	19.14	3.27

S : Significant

TABLE IV.

Mean grades of primary and secondary infection.

No.	Particulars of treatments	1962—'63				1963—'64			
		Mean intensity of infection on							
		Coty-ledons	True leaves	Branches per cent	Bolls per cent	Coty-ledons	True leaves	Branches per cent	Bolls per cent
1.	Sown without debris - No spray inoculation	0.15	0.62	2.1	3.05	0.20	0.97	1.82	2.1
2.	Sown in debris - Debris dusted and water sprayed	1.52	5.80	27.5	51.52	0.63	4.12	23.20	31.2
3.	do. Culture sprayed	1.60	5.70	29.5	52.78	0.65	3.78	27.52	37.5
4.	do. Leaf infusion sprayed	1.52	5.30	34.2	52.68	0.68	4.75	32.30	41.7
	'F' Test	S	S	S	S	S	S	S	S
	S. E. (±)	0.349	0.33	4.50	7.96	0.147	0.165	4.8	5.9
	C. D. (at 5% probability)	0.74	0.70	9.68	16.84	0.311	1.46	13.4	16.5

S : Significant

part of the growth phases of the crop creating high humid condition. It was, however, observed that the variety MCU—3, a moderately susceptible one to angular leaf spot disease, showed no difference in the intensity of infection during different periods of its growth.

Covering the plants with transparent alkathene cages proved to be highly efficient in creating the disease both under field and laboratory condition. But for purposes of testing resistance of the breeding materials under field conditions, the intensity of infection created on plants without covering was also high enough (up to grade 6) to test their resistance provided high humidity is maintained around the crop by adequate irrigation.

The spray inoculations resulted in satisfactory development of boll and branch infection and it was observed that there was positive correlation between leaf infection and branch and boll infection by adopting the present method of spray inoculation.

Dusting infected leaf debris on the leaves and spraying water at noon created angular leaf spot up to grade 6 and was found to be on par with spraying the leaf infusion. This may prove to be efficient especially in places like west coast areas where continued wet weather prevails.

The factors involved in the successful creation of the disease when the plants are spray inoculated at noon when the day temperature reaches its maximum are being worked out.

Summary: 1. Soaking the fuzzy seeds of cotton in bacterial culture suspension [*Xanthomonas malvacearum*] or applying infected leaf debris in the planting holes created high intensity of primary infection on the cotyledons.

2. Covering the plants with transparent alkathene sheets 3 days before and after spray inoculation helped in creating higher intensity of infection when compared to "no cover" treatment.

3. The differences in the intensity of infection was negligible between spray inoculation with freshly isolated bacterial culture solution and infected leaf debris infusion.

4. The primary cotyledon infection did not create as high an intensity of secondary infection compared to spray inoculation with culture solution or leaf infusion.

5. There was close correlation between the intensity of leaf infection and the per cent boll or branch infection in the variety MCU—3.

6. Dusting infected leaf debris of cotton in the evening followed by water spraying during the following day between 11 a. m. and 2 p. m. resulted in satisfactory creation of angular leaf spot disease.

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