Reaction of Cotton Species and Cultivars to Four Isolates of Ramularia areola

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ABSTRACT

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Twenty-four cultivars of cotton were tested in the glasshouse for resistance to isolates of *Ramularia areola* from Madagascar, Tchad, Ivory Coast, and India. Infection symptoms on cotyledons differed from leaf symptoms and were described. The Madagascar isolate was the most aggressive followed by the isolate from Tchad. Ivory Coast and Indian isolates were weakly aggressive. Cultivars BJA 592, Réba BTK 12 of *Gossypium hirsutum*, and cultivars Tadla 16, Pima 67 of *G. barbadense* were hypersensitive against the four isolates.

Additional key words: nightly wetting and day drying regime.

Gray mildew (*Ramularia areola* Atk.) occasionally becomes a problem to cotton cultivation (2). Differences in cultivar susceptibility to a single local isolate have been reported (2, 5, 10). No information, however, is available on pathogenic variation. The objectives of the present study were to screen cotton cultivars for resistance to *R. areola* and to study the variability among isolates of *R. areola*.

MATERIALS AND METHODS

Seedlings of cotton cultivars were grown in 12-cm diameter plastic pots (three per pot) in the glasshouse (25-30 C) with supplemental lighting (2,000 lux) for 14 hours daily. Monoconidial isolates from Madagascar (R1), Tchad (R2), Ivory Coast (R3), and India (R4) were used to inoculate 3- to 4-week-old seedlings, except the *Gossypium arboreum* and *G. herbaceum*, which were inoculated at the age of 5-6 weeks. The inoculum, consisting of conidia (1×10^5/ml), was grown on V8 juice agar (7) and sprayed with a paint gun on both the upper and lower surfaces of leaves until run-off. The four isolates were tested simultaneously for homogeneity. At 1930 hours, plants were inoculated, covered with moist plastic bags, and individual pots were placed in cement trays with water. The plants were uncovered the next morning (0730 hours), left there throughout the day (temperature 25-32 C, relative humidity 50-75%), and covered again at 1930 hours after wetting them with a spray of sterile, deionized water. This process was continued during a 9-day period (total wetness duration, 108 hours). Night temperature fluctuated between 20 and 24 C.

Disease severity was rated as percent leaf area diseased on the most severely diseased leaf (6) (generally the second youngest leaf of each plant at the time of inoculation). A dead fallen leaf was rated as 100% leaf area infected. Although the experiment was repeated several times over a period of three years, only the representative results of one trial are given here. Cultivars were graded for the degree of resistance according to the scale given below.

Percent infected leaf area	Reaction class
0	Highly resistant
0.1-1.0	Resistant
1.1-10.0	Moderately resistant
10.1-60.0	Susceptible
>60.0	Highly susceptible

RESULTS

Cultural characteristics of the isolates.—Visual differences in growth rate and colony morphology were observed when single spore cultures of the four isolates were grown on V8 juice agar. The R1 isolate, which was the fastest growing, produced a mound of whitish growth. The growth rates of isolate R2 and R3 were similar but were slower growing than that of R1; single spore colonies were raised and typically ash-gray in color. Isolate R4 had the slowest growth rate; growth of the latter was exclusively stromatic. Difficulty was encountered in initiating sporulation from a mycelial suspension, prepared by blending the stromatic growth. The diameters of 24-day-old single spore colonies of R1, R2, R3, and R4 isolates were 7, 4, 4, and 2 mm, respectively. Similarly, in an earlier study (8) R1 isolate produced the most growth.

In various inoculation tests symptoms appeared within 9-11 days. Plants from 3-8 weeks old were equally susceptible to *R. areola*.

Cotyledonary symptoms.—Although the occurrence of cotyledonary infection of cotton plants in nature has been reported (4), no symptomatic description was available. During this study considerable cotyledonary infection was noticed, and it differed from those on true leaves. This may facilitate detecting primary infection in the field. The incubation period for cotyledonary infection was longer (16 days) than that for true leaves (9-11 days). The earliest symptoms on cotyledons were circular, water-soaked, dark green patches when viewed with backlight. Later, infected cotyldeons became chlorotic and spots turned reddish brown (Fig. 1). Severely infected cotyledons withered. No sporulation occurred on the cotyledonary lesions. Thus the causal agent could be determined only after incubating cotyledons in moist petri plates to induce sporulation (Fig. 2).

Cultivar susceptibility.—The responses of all the cultivars to pathogen isolates are given in Table 1. Cultivars BJA 592, Réba BTK of *G. hirsutum* and Tadla 16, Pima 67 of *G. barbadense* gave a hypersensitive-fleck reaction to the four isolates. Flecks were minute, reddish

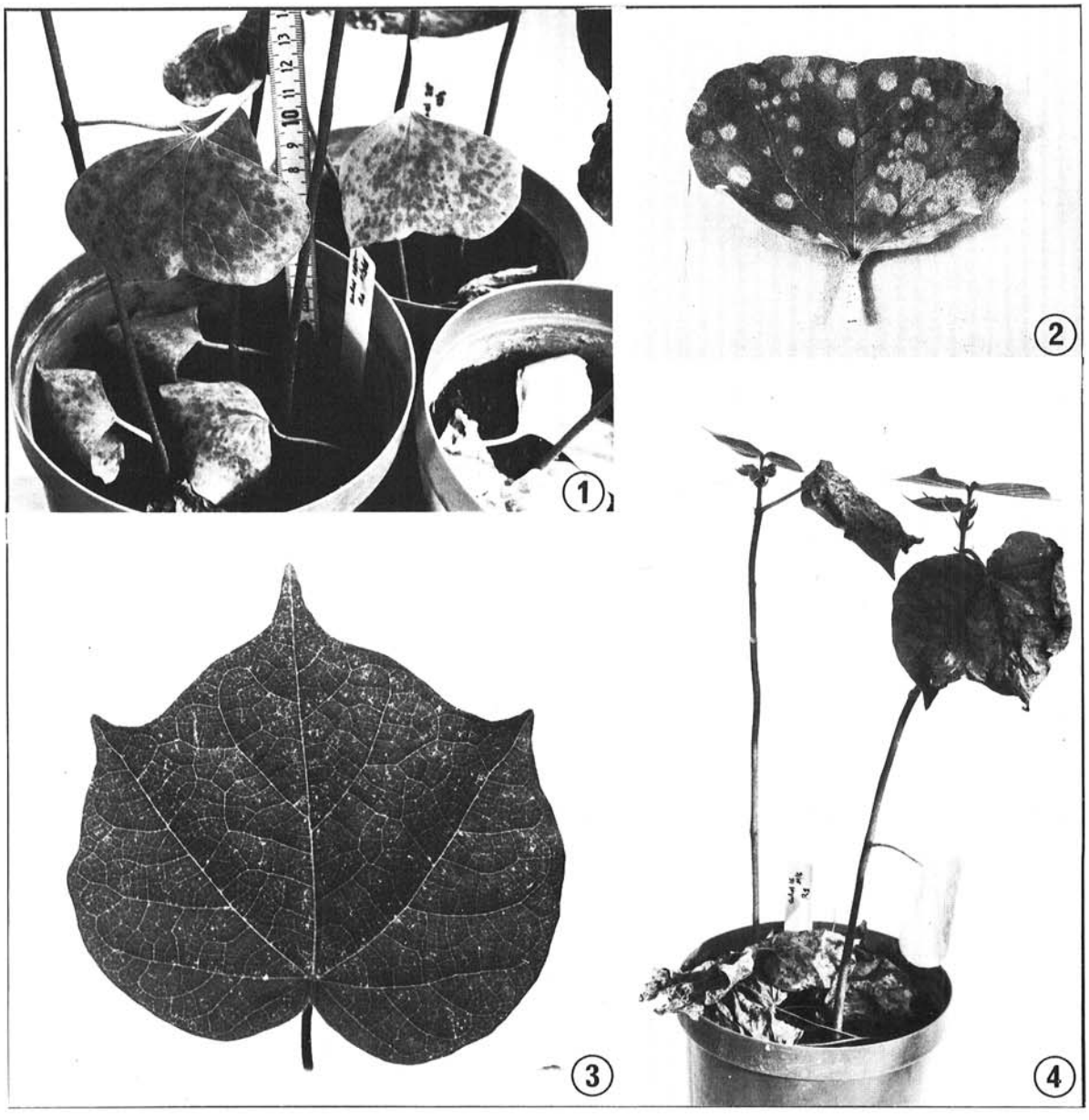

Fig. 1-4. *Ramularia areola* on cotton leaf. **1)** Cotyledonary symptoms on cultivar Sahel 17 days after inoculation; note withering of the severely infected cotyledons. **2)** An infected cotyledon when kept in moist petri plates for 48 hours showing sporiferous growth. **3)** Flecks of hypersensitive response on cultivar Réba BTK 12, of *G. hirsutum*. **4)** Highly susceptible reaction of the Sahel 16 to R1 isolate; 20 days after inoculation, showing defoliation.

brown, and encircled by a chlorotic zone when examined with transmitted light (Fig. 3). They were more pronounced on *G. barbadense* than on *G. hirsutum*.

The four isolates differed in their aggressiveness: R1 was the most aggressive isolate. Isolate R2 was moderately aggressive, and R3 and R4 were least aggressive. However, R4 isolate induced a highly susceptible reaction on the *G. herbaceum* cultivars from which it was originally isolated, and a susceptible reaction on the two *G. arboreum* cultivars. In general, the Indian isolate was least pathogenic to the cultivars of *G. hirsutum* except for Stoneville 7A, Glandless 1372, Sahel 19, Sahel 29, and Sahel 39, on which it induced a susceptible reaction. The triple hybrid, HAR M 327-4 was susceptible to R1, R2, and R4, but moderately resistant to R3 isolate. All seven lines of Sahel were highly susceptible and susceptible to R1 (Fig. 4) and R2 isolates of the pathogen, respectively. Cultivars Acala 1517 BR-2, which showed good tolerance to natural infection in Madagascar (2) gave a susceptible reaction to R1, R2, and R3, and a moderately resistant reaction to the R4; Stoneville 7A, Glandless LY 1472, Glandless LY 1372, Coker 417, Acala 1517 V, and DP 45 were susceptible to R1 and R2, but moderately resistant to R4.

DISCUSSION

Lower levels of infection on the older leaves were probably associated with their low wettability. Certain correlation between the growth rate and aggressiveness appeared to exist: the most aggressive isolate, R1, grew the most rapidly and the least aggressive isolate, R4, grew the least rapidly in a culture medium. The highly resistant reaction of *G. barbadense* was in contrast with Ehrlich and Wolf's report (3). Perhaps the U.S. isolate was different from our isolates. Identical reactions of BJA 592 and Réba BTK 12 cultivars could be explained by the fact that both were derived from *G. hirsutum* var. *punctatum* (1). Gray mildew resistance genes are probably present in the latter. It is noteworthy that the most resistant cottons in this study also are resistant to bacterial blight (caused by *Xanthomonas malvacearum*) and to boll rot (1).

Susceptibility of Stoneville 7A to the Madagascar isolate confirmed a similar report from Madagascar (2). The susceptible reaction of Acala 1517 BR-2 to R1 was in contrast with the results obtained in Madagascar (2). Perhaps this may be due to the nightly wetting and day-drying regime in this study, which is rigorous for resistance. Resistant to moderately resistant reactions of the *G. hirsutum* cultivars to the Indian isolate substantiate the report that *hirsutum* cottons are not generally attacked in India (5). Susceptibility of *G. herbaceum* and *G. arboreum* cultivars to the Indian isolate corroborate the report that these cottons are heavily infected in India (9).

In conclusion, cultivars BJA 592 and Réba BTK 12 of *G. hirsutum,* and cultivars Tadla 16 and Pima 67 of *G. barbadense* can be used as sources of resistance for breeding resistant cultivars.

TABLE 1. Reaction of cultivars of *Gossypium* species to four isolates (R1, R2, R3, and R4) of *Ramularia areola*

Gossypium spp. and cultivars[a]	Reaction to isolates[b]			
	R1	R2	R3	R4
G. hirsutum				
BJA 592	HR	HR	HR	HR
Réba BTK 12	HR	HR	HR	HR
Réba B 50	S	S	MR	R
SB 70 bulk 3	S	S	MR	R
Stoneville 7A	S	S	MR	S
Glandless LY 1472	S	S	MR	MR
Glandless LY 1372	S	S	MR	S
Coker 417	S	S	MR	MR
Acala 1517 V	S	S	MR	R
Deltapine 45	HS	HS	MR	MR
Acala 1517 BR-2	S	S	S	MR
Sahel 7	HS	S	MR	MR
Sahel 8	HS	S	S	MR
Sahel 14	HS	S	MR	R
Sahel 16	HS	S	MR	MR
Sahel 19	S	S	MR	S
Sahel 29	HS	S	MR	S
Sahel 39	HS	S	MR	S
G. arboreum 'Nanking'	HS	HS	S	S
G. arboreum A2	S	S	MR	S
G. herbaceum var. *acerifolium*	HS	MR	S	HS
G. herbaceum A1	S	HS	S	HS
G. barbadense				
'Tadla 16' and 'Pima 67'	HR	HR	HR	HR
G. herbaceum × *G. arboreum* × *G. reimondii* 'HAR M 327-4'	S	S	MR	S

[a]Seeds of all the above cultivars were kindly supplied by R. Lagière, Institut of Research for Cotton and Overseas Textiles, Paris, France.

[b]Rating scale: HR = highly resistant, MR = moderately resistant, R = resistant, S = susceptible, HS = highly susceptible.

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