Charcoal Rot of Guayule in Arizona

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ABSTRACT

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Fifteen experimental lines of guayule were tested for resistance to the charcoal rot pathogen, *Macrophomina phaseolina*, in greenhouse and field tests. Although no immunity to the pathogen was shown in greenhouse or field tests, charcoal rot incidence in the field was quite low, indicating a high level of field tolerance. We could not confirm previously reported differences among lines. Greenhouse tests of the interaction between saline irrigation water and charcoal rot demonstrated enhanced mortality when tap water was amended with NaCl or KCl at −0.05 to −0.5 MPa. In a field experiment with nonsaline and saline irrigation water, however, no difference was observed in rates of mortality caused by *M. phaseolina*. The wide distribution of symptomless infection in this field plot suggests a potential for widespread disease should the appropriate environmental triggers occur.

Guayule (*Parthenium argentatum* A. Gray; Compositae) is a native of the Chihuahuan Desert of northeastern Mexico and southern Texas (8). Since the turn of the century, periodic efforts have been made to commercially cultivate this plant as a domestic source of natural rubber (5). Recent interest has focused on commercialization of guayule production in the arid southwest region of the United States (5). In recent work dealing with factors that constrain guayule seed germination, seedling establishment, and survival of transplanted seedlings, Miyamoto et al have shown that guayule seedlings are particularly sensitive to saline irrigation water (12–15). To enhance seedling survival, they recommend modification of cultural practices to reduce the accumulation of salts on the soil surface (12,15).

Charcoal rot of guayule, caused by the soilborne fungus *Macrophomina phaseolina* (Tassi) Goidanich, was first reported during July and August of 1944 in a 2-yr-old, nonirrigated experimental field in south Texas (17). The disease was characterized by dark brown, sunken lesions on the stem at or near the soil line. Preliminary evidence suggested that disease incidence was higher where plant density was lowest (17). That the charcoal rot disease affected both seedlings and mature plants was shown by reports from Texas in 1949 (2-yr-old plants) (20) and in 1951–1952 (8-wk-old transplants and 18-mo-old plants) (16). In the latter investigation, the authors suggested that USDA line 4265 was more tolerant to charcoal rot than lines 109, 130, and 593 (16). All reports suggested that disease development was favored by high temperatures and severe drought stress. However, no evidence was presented confirming the pathogenicity of this fungus through the completion of Koch's postulates.

The effects of water quality on disease development is a subject of concern in areas where water quality is declining because of increased salinity. In studies of the microflora of sugar beet (*Beta vulgaris* L.), the mycelial growth of several fungi, including *Rhizoctonia solani* Kühn, *Sclerotium rolfsii* Sacc., and *Fusarium* spp., was stimulated by increasing the salinity of culture media (3). Further work indicated that *S. rolfsii* caused more disease on sugar beets as the salinity of irrigation water increased (2). Salinity stress has also been found to increase the susceptibility of Penncross creeping bentgrass (*Agrostis stolonifera* L. var. *stolonifera* 'Penncross') to infection by *Pythium aphanidermatum* (Edson) Fitzp. (18). In a field study with charcoal rot of sunflower (*Helianthus annuus* L.), disease increased with increasingly saline irrigation water (4).

The first objective of our study was to confirm the pathogenicity of *M. phaseolina* to guayule and evaluate available germ plasm for resistance to the pathogen. The second objective was to examine the influence of saline irrigation water on the incidence of charcoal rot of young guayule plants.

MATERIALS AND METHODS

Inoculations and isolations. All inoculations were with cornmeal-sand inoculum (CMIn), containing microsclerotia of *M. phaseolina* prepared after the method of Jiménez-Díaz et al (6), modified with a volumetric ratio of sand, cornmeal, and distilled water (1.1:0.4:0.4, v/v). Sterile cornmeal-sand medium (CMSt) served as a control. All reisolations of *M. phaseolina* from plant roots

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used the MPA semiselective medium previously described (9).

Evaluations for resistance. Fourteen guayule lines from the USDA National Seed Storage Laboratory were used in all tests: N565, N396, N576, 11605, 11591, 11619, 12229, 4265-XF, A48118, N565-II, N596, 11646, 12231, and 593. The tetraploid interspecific hybrid AZ-101 was also used. Greenhouse evaluations were made using 4- to 8-wk-old seedlings or 12- to 28-wk-old plants.

To evaluate the susceptibility of seedlings, we placed an area of silica sand (15 × 20 cm, 3–4 mm deep) on each of several horizontal sterile glass plates (20 × 20 × 0.2 cm). Five guayule seedlings were placed on the sand such that their hypocotyls rested at the point where sand and glass met. Seedling roots were covered with CMIn or CMSt (3–4 ml per seedling). All seedlings were then covered with another 3- to 4-mm layer of silica sand, after which each glass plate was enveloped with aluminum foil to hold the area covered by sand firmly in place. Three replicate plates of inoculated seedlings (15 total) and three plates of controls were used for each line. All plates were carefully positioned on a greenhouse bench so that the seedlings were vertical and the sand, medium, and inoculum were not disturbed. Plates were incubated at day and night temperatures (at least 35 and 30 C, respectively). Seedlings were watered at the top of the foil envelope with distilled water or with half-strength, modified Hoagland's solution (1) on alternate days. At the end of each test, the entire root system of each seedling was cultured on MPA to confirm the presence of *M. phaseolina*. Seedling pathogenicity tests were repeated two or three times for each of the 15 lines.

Greenhouse tests. Resistance to *M. phaseolina* was evaluated in 12- to 28-wk-old plants of the 15 guayule lines in five greenhouse tests. A soil mixture (SMIX) of sand and potting soil (2.5:1.0, v/v) was combined with CMIn or CMSt (3.5:0.05, v/v) and used in all experiments. Two plants of each line were transplanted into each of 20 plastic pots (10.2 cm in diameter) containing SMIX and CMIn. Each experiment was arranged in a randomized complete-block design of 20 blocks to minimize the effects of environmental variability in the greenhouse. Six plants of each line, planted in SMIX and CMSt, were used as controls. The number of wilted plants was monitored for 7–9 wk. Throughout each experiment, we made isolations on MPA from the roots of all wilted plants to confirm the presence of the pathogen. At the end of each experiment, the lines were ranked on a 1–15 scale according to incidence of charcoal rot. Plants in rank 1 had the lowest incidence and those in rank 15 had the highest. Rank data from five tests were analyzed using the nonparametric Friedman test for aligned ranks (7).

Field tests. The 15 guayule lines were evaluated for charcoal rot resistance in a field plot at the University of Arizona experimental farm in Marana, Arizona. The soil was a Gila loam. Pathogen population was determined by taking a soil sample from each transplant site and assaying it using procedures previously described (10). A randomized complete-block design with three blocks was used. Each block consisted of 15 contiguous beds (15.24 m long, 1.02-m centers). On 1 April 1985, 150 3.5- to 5-mo-old plants of each line were hand-transplanted to the plot at intervals of 0.3 m. Within a block, each line was represented by a single bed of 50 plants. Because of poor establishment of seedlings in the greenhouse, plants as young as 2 mo old were used for lines N565-II and 12229, and line 11646 was represented in only two blocks. A block of *Euphorbia lathyris* L. was established immediately to the north of the guayule on 31 March 1985. *E. lathyris* is a hydrocarbon-producing plant highly susceptible to infection by *M. phaseolina* (19). Seeds of *E. lathyris* were hand-sown at intervals of 0.15 m on five contiguous beds (6.1 m long, 1.02-m centers). The soil population of the pathogen was determined for each planting site as previously described (10). The field plot was monitored during four growing seasons, as charcoal rot of guayule can affect both young and mature plants. All guayule and *E. lathyris* plants showing severe wilt symptoms were excavated, and root isolations were made on MPA to determine the association of *M. phaseolina* with observed symptoms. Monitoring of the field was discontinued on 3 November 1988.

Evaluation of salinity effects. *In vitro tests.* The effect of salinity on the mycelial growth of *M. phaseolina* was tested in vitro using culture media amended with NaCl. Two tests were run, each using the fungal isolates MP68 (ATCC 62589) from guayule, MP97 from corn (*Zea mays* L.), and MP120 from *Salicornia* sp. Isolates were cultured on cornmeal agar (CMA), containing 8.5 g/L Difco cornmeal agar and 4.5 g/L Difco Bacto agar, and on potato-dextrose agar (PDA), containing 19.5 g/L Difco potato-dextrose agar and 4.5 g/L Difco Bacto agar. Each medium was amended with NaCl (w/w) to achieve electrical conductivity levels of 0, 7.8, 15.6, 39.1, 78.1, and 156.3 decisiemens (dS) per meter (1 dS/m = 640 ppm). For each fungal isolate, a small inoculum block (5 × 5 mm) was placed in the center of a petri dish (100 × 15 mm) containing one of the test media. Three replicates were prepared for each combination of fungal isolate, culture medium, and salt amendment. All petri dishes were incubated in the dark at 34 C. Colony diameters were measured after 24, 48, and 72 hr.

Greenhouse tests. The effect of saline irrigation water on the incidence of charcoal rot of guayule was examined in several greenhouse tests using guayule line 593 and *M. phaseolina* isolate MP68 from guayule. For all experiments, 5- to 9-mo-old plants were transplanted into pots (10.2 cm in diameter, two plants per pot) containing SMIX and CMIn (inoculated) or SMIX and CMSt (control). In a given experiment, the inoculated and control plants were apportioned equally among seven different salinity treatments that included NaCl and KCl solutions (three concentrations each) and a control solution of unamended tap water. For both salts, the three concentrations were selected from the following: −0.01, −0.05, −0.1, −0.3, −0.5, and −1.0 MPa. For NaCl, these levels correspond to 0.2, 0.9, 1.8, 5.4, 9.0, and 17.9 dS/m, respectively. For KCl, they correspond to 0.2, 1.1, 2.3, 6.8, 11.4, and 22.8 dS/m, respectively. Each experiment was arranged in a randomized complete-block design of three blocks in a greenhouse at day and night temperatures of at least 35 and 30 C, respectively. As mortality occurred, isolations on MPA were made from the roots of all dead inoculated plants to confirm pathogen presence. With the exception of the two most-concentrated solutions (−0.5 and −1.0 MPa), each salt solution was included in at least three experiments.

Field test. A field test of the interaction between saline irrigation water and charcoal rot of young guayule transplants was conducted in a test plot (13.4 × 121.9 m) at the Maricopa Agricultural Center of the University of Arizona. Fifty soil samples were taken in a 5 × 10 point grid in October 1986. The population of microsclerotia of *M. phaseolina* was determined by assaying (9) one 20-g subsample from each of the 50 samples. Because the inoculum level was low (1.1 microsclerotia per gram), we planted *E. lathyris* in an attempt to increase the pathogen population. On 14 October, *E. lathyris* seeds were planted on beds (115.8 m long, 1.02-m centers) at a rate of 80 g of seed per bed. By 8 July 1987, more than 90% of the plants were dead and microsclerotia of *M. phaseolina* were visible in their lower stems. All plants were incorporated into the plot by disking.

In October 1987, the plot was divided in half lengthwise, with a levee separating the halves. Six beds on 1.02-m centers were prepared in each half. On 6 October, we hand-transplanted 4- to 6-mo-old guayule plants of lines N596, N396, 11591, and A48118 into the center four beds of each half of the plot. Each half was arranged in a randomized complete-block design with three blocks. Within each block, each line was planted on two

adjacent 15.3-m bed segments, with 0.31 m between plants. One hundred plants from lines N596, 11591, and A48118 were used per block, while 70 plants per block were used for line N396. Border rows were planted with guayule plants of a similar age. On 6 and 9 October, both halves of the plot were irrigated with nonsaline (SWEET) water (0.9–1.2 dS/m total soluble salts). Subsequently, the north half of the plot was irrigated with saline (SALT) water (2.2–3.3 dS/m total soluble salts) and the south half was irrigated with SWEET water. Irrigation dates were 16 October, 23 October, and 7 December 1987 and 16 February, 17 March, 14 April, 20 May, 13 June, and 26 August 1988. On 6 October 1987, we took two soil samples for each guayule line in each replicate of the SALT and SWEET halves of the plot, for a total of 48 samples. The population of microsclerotia of *M. phaseolina* was then determined by assaying one 5-g subsample from each soil sample (9). As plants died, they were removed and isolations were made from roots on MPA to confirm the association of *M. phaseolina* with mortality. In October 1988, 10 adjacent, apparently healthy plants of each line were removed from each replicate in both the SWEET and SALT halves of the plot (240 plants total). Tops were clipped, air-dried, and then weighed. Isolations were made on MPA from the roots of all plants to determine the level of symptomless infection by *M. phaseolina*. As part of the isolation procedure, roots were washed for 3–5 min in running tap water, then soaked for 4–7 min in a 10% solution (v/v) of sodium hypochlorite to minimize the possibility of contamination by *M. phaseolina* propagules in the rhizosphere or rhizoplane.

RESULTS AND DISCUSSION

Charcoal rot of guayule has been observed at least 25 times in experimental plantings since 1980 in Maricopa, Pima, Pinal, and Yuma counties, Arizona. Symptoms have included a change in leaf color from blue-green to gray-green, partial defoliation, dark stem lesions just above the soil line, cortical rotting of lower stems and roots, wilting, and eventual death. Microsclerotia of *M. phaseolina* have been seen in the cortical tissues of roots of severely affected plants. In one case, pycnidia of the fungus were also observed. As reported previously (11), *P. aphanidermatum* and *M. phaseolina* have been isolated from the same diseased plant on six different occasions. Although charcoal rot of guayule has been observed in Arizona throughout the year, it is most prevalent from July through October. Among the guayule lines that have been affected by charcoal rot in Arizona are 11701, 12231, 591, 593, N576, 395, N565, and N396. The Arizona tetraploid interspecific hybrid AZ-101 was also affected by charcoal rot in fields near Sacaton. Because these observations were the result of occasional field visits rather than systematic survey, it was impossible to determine whether some guayule genotypes are consistently unaffected by charcoal rot. Plants ranging in age from 4 wk to 4 yr have been found to be affected by charcoal rot in the observations since 1980, thus confirming earlier observations that the disease can affect plants of any age.

Pathogenicity and germ plasm evaluation. Inoculated seedlings wilted and died within 3–4 days after inoculation. Often, microsclerotia of the fungus were seen in small roots that became nearly transparent. In all tests involving the 15 lines, at least 30% of inoculated seedlings showed symptoms. The pathogen was recovered from at least 73% of inoculated seedlings. For all lines, there was at least one test in which a minimum of 80% of inoculated seedlings showed symptoms. Generally, less than 25% of the control seedlings wilted because of severe temperature and water stress conditions in the test system; *M. phaseolina* was never recovered from these noninoculated plants. In greenhouse evaluations of 12- to 28-wk-old plants, we found no statistical difference among the 15 lines for proportion of plants expressing charcoal rot symptoms (Table 1). For several lines, there was a great deal of variability in disease development among the five tests; this may be partially attributable to the genetic variability within lines.

Tests of seedlings and older plants confirmed the pathogenicity of *M. phaseolina* to the 15 guayule lines through the completion of Koch's postulates. The pathogen was reisolated from 81–100% of symptomatic plants. These tests further showed that none of the tested guayule lines were immune to infection by the pathogen. We could not confirm earlier suggestions that line 4265 (from which 4265-XF was selected) is tolerant to charcoal rot (16).

During field evaluation of the 15 guayule lines, both overall mortality and mortality associated with *M. phaseolina* were low (Table 1). Based on these observations, it was not possible to identify differences among lines in field tolerance to charcoal rot, although the low disease incidence did indicate a high level of field tolerance for all 15 lines. The population of *M. phaseolina* microsclerotia found in the three guayule blocks before planting ranged from one

Table 1. Resistance of 15 guayule lines to *Macrophomina phaseolina* infection in greenhouse and field experiments

	Greenhouse test[a]										Field test[b]			
	A		B		C		D		E		Mortality		Charcoal rot	
Line	PS	Rank	PS	Rank	PS	Rank	PS	Rank	PS	Rank	Prm	Rank	Prc	Rank
N565	0.13	3	0.03	2	0.45	8	0.83	12.5	0.65	14.5	0.020	6.5	0.007	8.5
N396	0.58	15	0.35	13.5	0.58	11.5	0.70	6.5	0.38	4	0.013	3	0.000	3
N576	0.40	13	0.05	3.5	0.60	13	0.73	9	0.30	1	0.020	6.5	0.007	8.5
11605	0.05	1	0.35	13.5	0.53	10	0.90	14	0.40	6	0.080	13	0.020	12.5
11591	0.28	10	0.18	9.5	0.63	14.5	0.83	12.5	0.40	6	0.013	3	0.000	3
11619	0.18	6	0.23	11	0.28	3	0.70	6.5	0.40	6	0.040	9	0.007	8.5
12229	0.25	8	0.28	12	0.38	6	0.80	11	0.48	9	0.047	10	0.000	3
4265-XF	0.28	10	0.38	15	0.25	2	0.75	10	0.53	11	0.173	15	0.053	15
A48118	0.18	6	0.08	5.5	0.48	9	0.58	2	0.33	2.5	0.020	6.5	0.007	8.5
N596	0.13	3	0.00	1	0.23	1	0.43	1	0.63	13	0.113	14	0.007	8.5
N565-II	0.13	3	0.15	8	0.63	14.5	0.65	4	0.43	8	0.007	1	0.007	8.5
11646	0.18	6	0.08	5.5	0.37	5	0.70	6.5	0.53	11	0.070	12	0.020	12.5
12231	0.33	12	0.18	9.5	0.40	7	0.70	6.5	0.33	2.5	0.060	11	0.040	14
593	0.43	14	0.05	3.5	0.58	11.5	0.63	3	0.65	14.5	0.020	6.5	0.000	3
AZ-101	0.28	10	0.10	7	0.35	4	0.95	15	0.53	11	0.013	3	0.000	3

[a] PS = Proportion of 40 plants expressing charcoal rot symptoms. For each test, ranks of 1 and 15 are assigned to lines with the lowest and highest charcoal rot incidence, respectively. Average ranks are assigned to ties.

[b] Prm = Proportion dead from all causes. Prc = Proportion of dead plants from which *M. phaseolina* was recovered. Each line was represented by 50 plants in each of three replicates. Line 11646 was represented in only two replicates. The proportions Prm and Prc were calculated for all plants of a given line over the four growing seasons of the field experiment. Ranks were assigned as for greenhouse experiments.

to 62 microsclerotia per gram of soil, with an average population of 6.5 per gram (10). In the *E. lathyris* block, the preplant population ranged from one to 25 microsclerotia per gram, with an average of 9.8 per gram. Additional details on the spatial pattern of inoculum in this field have been published elsewhere (10). Soil samples associated with the 25 dead guayule plants from which *M. phaseolina* was recovered had preplant soil populations of one to 27 microsclerotia per gram. In contrast, 83.5% of 200 *E. lathyris* plants developed severe charcoal rot symptoms during the first growing season (1985); the pathogen was recovered from 82% of the symptomatic plants. The preplanting population of *M. phaseolina* associated with symptomatic *E. lathyris* had the same range and mean as the population associated with the entire *E. lathyris* block. Clearly, pathogen population was not a limitation to infection of guayule or to disease development. While it is possible that the propagules of *M. phaseolina* in the test field were of a strain able to infect *E. lathyris* but unable to infect guayule, there has not been enough research on the identification of strains of the fungus to properly evaluate this possibility.

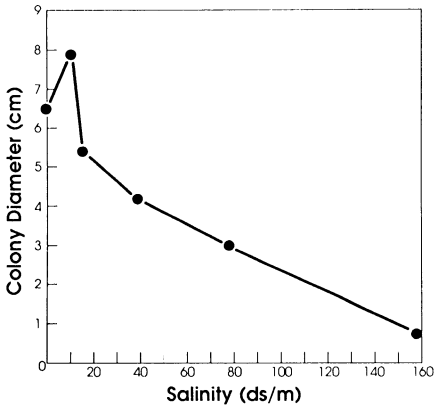

Fig. 1. Growth of *Macrophomina phaseolina* on medium amended with NaCl. Salinity expressed as decisiemens per meter. Colony diameter (measured at 48 hr) is the average of three replicates.

Because charcoal rot of guayule has been associated with water stress in the past (16,17,20), we varied irrigation practices over the four growing seasons studied in an attempt to trigger widespread symptom development. In 1985, the first irrigation was applied immediately after transplanting on 1 April, and light irrigations were continued weekly through mid-June to facilitate plant establishment. Subsequently, the plot was irrigated once in both July and September to supplement the 9.6 cm of rain recorded from April through October. In 1986, only two irrigations in April supplemented the 12.7 cm of precipitation that fell between April and October. During this second growing season, all plants showed symptoms of severe water stress (including leaf curl and death of older leaves). However, vigorous growth and flowering resumed for nearly all plants almost immediately after a rainfall of at least 0.75 cm. In 1987, one irrigation was applied monthly from April through October to supplement the 11.84-cm rainfall, which created a condition of water stress lower than that prevailing during 1986. In 1988, only three irrigations were applied (in June, July, and October) to supplement rainfall from April thru October (20.9 cm). The data in Table 1 suggest that water stress alone does not trigger widespread charcoal rot development. At least one additional coincident stress factor, not present in this field study, may be necessary.

Saline irrigation water and charcoal rot. Mycelial growth of *M. phaseolina* was detectable even when culture media were amended with 156.3 dS/m of NaCl (Fig. 1). The growth response shown in Figure 1 was typical of all isolates, regardless of the culture medium. For all isolates, mycelial growth at 48 hr was significantly enhanced by a NaCl amendment of 7.8 dS/m as compared to the nonamended control or higher NaCl amendments.

All plants irrigated in the greenhouse with water of at least −0.05 MPa had greater mortality than those irrigated

Table 2. Mortality of guayule plants challenged with *Macrophomina phaseolina* and saline irrigation water

					NaCl (MPa)											
			No salt		−0.01		−0.05		−0.1		−0.3		−0.5		−1.0	
Test	N[a]	Day	C[b]	I[c]	C	I	C	I	C	I	C	I	C	I	C	I
A	20	12	0.0[d]	1.0 zv[e]	...	...	...	...	...	...	6.3	10.3*[f]	7.0	17.7**[g]	18.7	20.0 zv
B	20	14	0.0	0.3 zv	0.0	0.7 zv	...	...	2.0	7.0*	7.3	20.0 zv	...	...	...	...
C	20	15	0.0	0.3 zv	1.0	0.3 zv	...	...	2.3	10.7*	19.0	19.3	...	...	...	...
D	10	15	0.3	1.0 zv	0.7	3.3	2.0	4.3*	4.0	8.0	...	...	...	...	...	...
	10	20	0.7	1.7	2.3	4.3	3.3	7.3*	6.0	9.3	...	...	...	...	...	...
E	20	15	0.0	0.0 zv	0.3	1.7*	2.0	1.7	5.0	7.0 zv	...	...	...	...	...	...
	20	20	0.0	0.0 zv	0.7	2.0 zv	3.3	2.3	9.0	13.7	...	...	...	...	...	...
F	18	11	0.0	0.7 zv	0.0	0.0 zv	1.9	2.5	3.8	9.3*	...	...	...	...	...	...
	18	18	0.4	1.0	0.7	1.1	5.8	14.1*	14.7	17.6	...	...	...	...	...	...

					KCl (MPa)											
			No salt		−0.01		−0.05		−0.1		−0.3		−0.5		−1.0	
Test	N[a]	Day	C[b]	I[c]	C	I	C	I	C	I	C	I	C	I	C	I
A	20	12	0.0[d]	1.0 zv[e]	...	...	...	...	...	...	6.7	17.3**	15.0	20.0 zv	20.0	20.0 zv
B	20	14	0.0	0.3 zv	1.0	0.3	...	...	3.0	12.7*	13.0	20.0 zv	...	...	...	...
C	20	15	0.0	0.3 zv	1.3	1.7	...	...	7.4	14.0	19.7	20.0 zv	...	...	...	...
D	10	15	0.3	1.0 zv	1.0	1.7 zv	3.3	9.7**	2.0	6.7**	...	...	...	...	...	...
	10	20	0.7	1.7	2.3	3.7	5.7	10.0 zv	5.3	7.0	...	...	...	...	...	...
E	20	15	0.0	0.0 zv	0.3	1.0	2.3	5.7	7.7	16.3*	...	...	...	...	...	...
	20	20	0.0	0.0 zv	0.7	1.3	5.0	9.3	13.3	19.3	...	...	...	...	...	...
F	18	11	0.0	0.7 zv	0.0	0.7 zv	1.1	3.3	1.2	12.0*	...	...	...	...	...	...
	18	18	0.4	1.0	6.6	2.1	7.3	13.1	12.7	17.7	...	...	...	...	...	...

[a] N = Number of plants in each of three replicates.
[b] C = Noninoculated controls.
[c] I = Plants inoculated with microsclerotia of *M. phaseolina*.
[d] Values are the average number of dead plants over three replicates for each treatment. The C and I means for each test were compared for each salt treatment using Student's *t* test.
[e] zv = Zero variance (0.0) for at least one mean of the pair, such that the assumption of homogeneity of variance was not met and a *t* test was inappropriate.
[f] * = Pair of means was significantly different at $P \leq 0.05$.
[g] ** = Pair of means was significantly different at $P \leq 0.01$.

with nonamended tap water (Table 2). At 10–20 days after the start of the experiment, plants in treatments subjected to both challenges often had significantly greater mortality associated with *M. phaseolina* than controls receiving only saline irrigation water (Table 2). By the end of each experiment (30–35 days), however, the surface accumulation of salts was sufficient to kill plants and mask the interaction between saline water and charcoal rot (Fig. 2). The data summarized in Table 2 represent observations made up to 20 days after experiment initiation.

Initially, a separate least-squares regression line was fit to the mortality data for inoculated and noninoculated plants from each of the six experiments. With one exception (test D, day 15, KCl, noninoculated), the slope of the regression line was significantly different from zero, indicating a rise in mortality as the salinity of irrigation water increased. For each test, the slopes of the regression lines for inoculated and noninoculated plants were compared. The lines were statistically different in only one case (test B, NaCl), implying that the dose-response relationship was the same regardless of pathogen presence. For tests A, B, and C, the most-saline treatment caused similarly high mortality among both inoculated and noninoculated plants. Regression lines for these tests had similar slopes even though mortality in treatments of intermediate salinity was often greater among inoculated than among noninoculated plants (Table 2). For tests D, E, and F, variance among the replicates was often quite low or zero (indicated by zv in Table 2). On the other hand, variance could also be so high that the assumption of homogeneity of variance (implicit in regression analysis) was violated. Owing to the difficulties encountered in the regression analysis, and because the overriding concern in these experiments was to identify the salinity treatments that enhanced disease incidence, the data were then analyzed using Student's *t* test. In a number of tests—particularly those in which the salinity treatment was between −0.05 and −0.3 MPa—the challenge of both pathogen and salinity caused significantly greater mortality than the challenge of salinity alone (Table 2). However, when there was a variance of 0.0 (Table 2), Student's *t* test was inappropriate because of the violation of the assumption of equality of variances. In several cases (for example, test B, NaCl, −0.3 MPa and test D, KCl, −0.05 MPa, day 20), mortality was clearly greater among inoculated plants than among noninoculated plants, despite the absence of a confirming statistical test (Table 2). These data demonstrated a clear negative response of guayule seedlings to increasingly saline irrigation water. Although the interaction between *M. phaseolina* and saline irrigation water was observed less consistently, the phenomenon was sufficiently common to warrant a field investigation.

The soil population of *M. phaseolina* in the field experiment increased from 1.1 microsclerotia per gram in October 1987 to 11.3 per gram 1 yr later, after *E. lathyris* infected by *M. phaseolina* was incorporated into the soil. Average mortality during the first year after transplanting was 10–18% for plants receiving SWEET water and 15–32% for plants receiving SALT water. There were no significant differences in mortality attributable to line or irrigation water. At least 75% of first-year mortality occurred within 1 mo of transplanting and was attributable to the stress associated with transplantation. Mortality associated with *M. phaseolina* was quite low (1–3%) and did not appear to vary appreciably between guayule lines or irrigation treatments. *M. phaseolina* was recovered from the roots of dying plants within 13 days after transplanting. These observations suggest that irrigation water of the salinity tested here (2.2–3.3 dS/m total soluble salts) does not increase the risk of transplant mortality caused by charcoal rot. These levels of salinity correspond approximately to the −0.1 MPa level used in the greenhouse experiments (Table 2). The salt mixture in the water used for field irrigation may have been less detrimental than the single-salt solutions tested in the greenhouse.

During the course of the field study, plants receiving saline irrigation water appeared somewhat larger and more robust than those receiving nonsaline water. When dry weights of shoots were compared for 30 plants of each line in the two irrigation treatments, no statistical differences were found in dry weight within each treatment. In the case of line A48118, average shoot dry weight was significantly greater for plants in the SALT treatment (205 g) than for plants of the same line in the SWEET treatment (139 g) ($P = 0.013$, *t* test). When isolations were made from roots of these 240 apparently healthy plants, *M. phaseolina* was recovered from 239 of them. This is evidence for widespread symptomless infections that could become a problem when environmental conditions favor active disease development. It also shows the high level of field tolerance in the four lines included in this study.

Beginning on 9 April 1988, soil temperatures were monitored hourly at the 5- and 20-cm depths. At 5 cm, the maximum temperature recorded was 41.6 C, and a daily maximum in excess of 40 C was recorded on 18 days during the growing season. At 20 cm, the maximum temperature recorded was 38.7 C, and a daily maximum in excess of 35 C was recorded on 60 days. It would appear that high soil temperature alone is not sufficient to trigger active charcoal rot of guayule.

In both of the field studies reported here, conditions of severe drought or heat stress were created, yet development of charcoal rot was minimal. Further work is needed to identify the combination of critical factors governing disease development.

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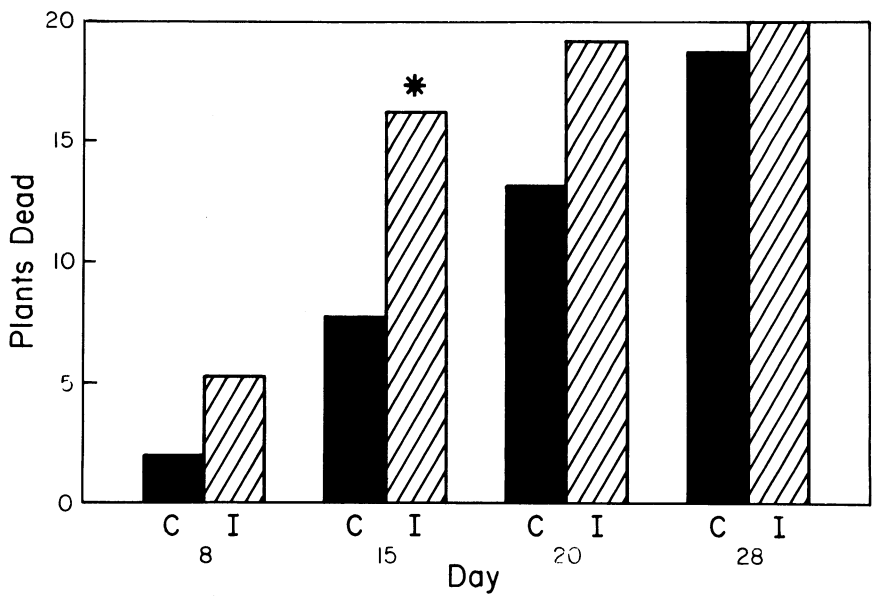

Fig. 2. Mortality of guayule irrigated with a KCl solution of −0.1 MPa. Each bar is the average of three replicates. I = Inoculated, potting mixture amended with microsclerotia of *M. phaseolina*. C = Noninoculated control. * = C and I treatments are statistically different at $P \leq 0.05$ using Student's *t* test.

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