Screening Mimosa (*Albizia julibrissin*) Seedlings for Resistance to Nematodes and Fusarium Wilt

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ABSTRACT

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About 7,600 seedlings of *Albizia julibrissin* were used in screening procedures designed to select individuals resistant to the combined effects of the wilt fungus *Fusarium oxysporum* f. sp. *perniciosum* and the root-knot nematodes *Meloidogyne incognita* and *M. javanica*. Mortality increased significantly when *Fusarium*-inoculated seedlings were grown in media infested with the nematodes. Survival of seedlings derived from intercrossing among trees originally selected for wilt resistance was significantly higher than that of seedlings from unselected parents. Of 6,000 seedlings from wilt-resistant parents, 2,818 were alive after growing in a nematode-infested medium for 70 days after *Fusarium* inoculation. Six hundred of the most vigorous survivors were reinoculated with *Fusarium*, and although 312 of these were still alive after 7 mo, only 78 were symptomless the following spring. The surviving seedlings will be field-planted and evaluated during the next 5 yr to select wilt-resistant cultivars with superior horticultural characteristics.

The mimosa, or silk tree (*Albizia julibrissin* Durazz.), has been planted extensively and has frequently become naturalized in the southeastern United States and in other areas with mild climatic conditions. Mimosa, which is native to a region extending from Iran to central China (10), was introduced first to England in 1745 and later to the United States in about 1814 (1). It is valued for its rapid growth, delicate bipinnately compound leaves, and whitish pink to red flowers blooming during midsummer to late summer. The foliage and feathery flowers create a tropical effect unique among landscape trees of the temperate zone.

The value of mimosa as a landscape tree has been greatly reduced by a destructive and usually fatal disease. Since Hepting (8) first reported a vascular wilt of mimosa in the Carolinas in 1936, the disease now occurs almost everywhere the tree is planted or naturalized. The causal agent of mimosa wilt is *Fusarium oxysporum* f. sp. *perniciosum* (Hepting) Toole, a fungus with many strains of variable virulence and growth types in culture (13).

Artificial inoculation experiments conducted by the USDA Forest Service (15) resulted in the release of two wilt-resistant cultivars, Charlotte and Tryon, in 1949 (14). Wilting and death of planted trees of both resistant cultivars were reported in 1964 (4), and pathogenic strains of the fungus were isolated from apparently healthy trees (5). Another wilt-resistant cultivar, Union, was released in 1979 (7), but it has not been widely tested.

The effects of root-knot nematodes (*Meloidogyne* sp.) on the expression of wilt symptoms was reported in 1958 (2). A larger percentage of seedlings wilted when grown in soil infested with both *Fusarium* and either *M. incognita* (Kofoid & White) Chitwood or *M. javanica* (Treub) Chitwood than when grown in soil infested with the fungus alone. Therefore, the nematodes increased the incidence of mimosa wilt but were not necessary for wilt to occur.

The wilt fungus is generally considered to be disseminated in infested soil, but Stipes and Phipps (12) found that sporodochia in the lenticels of infected trees could be local sources of inoculum, and Gill (6) noted the possibility of seed transmission, even from surface-sterilized seed. Chemical control of the wilt is largely ineffective (9).

Stipes (11) showed that several tropical species of *Albizia* were highly resistant to the mimosa wilt fungus and that of the eight species tested, *A. julibrissin* was the most susceptible. However, because none of the tropical species would be cold-hardy in most areas where *A. julibrissin* is planted, screening for disease resistance should concentrate on that relatively hardy species.

The loss of wilt resistance by Charlotte and Tryon could have been caused by the pathological effects of root-knot nematodes in combination with the wilt fungus. There is therefore a need to develop selections resistant to the fungus-nematode complex in order to eventually produce superior cultivars with desirable horticultural attributes.

MATERIALS AND METHODS

Mimosa seed collection. In 1970, the U.S. National Arboretum, Washington, DC, received from D. L. Gill (USDA, ARS, Tifton, GA) rooted cuttings of six clones (18 plants, two to six per clone), including Union, that had been selected for wilt resistance. These trees were planted randomly as a group on a site where mimosas had previously died of wilt. As of 1983, many trees were declining, perhaps because of mimosa wilt, although only one tree showed obvious signs of the wilt fungus (sporodochia within lenticels). These trees, however, had the unique potential for producing open-pollinated progeny with significant wilt resistance, because there were no other mimosas within 1 km of this planting. We decided to rely on open-pollinated seed because seed set to natural crossing was only 1.9% in one study (3) and the difficulties of controlled pollination in this insect-pollinated species would probably further reduce seed yield.

Seed pods from 11 of the trees in our planting were collected when the pods had matured in mid-November 1983. Although the identification labels on many trees had been lost, all six original clones, including Union, were represented. In addition, seed was collected from two unselected trees growing along nearby highways to total 13 seed lots. Seeds were shelled and stored in glass jars.

Propagation of root-knot nematodes. About 175 seeds of Rutgers tomato were pregerminated and sown in flats. The seedlings were transplanted singly 10 days later to 20-cm-diameter containers in a soil mix of composted soil, peat, and perlite with a wetting agent and micronutrients added.

Inocula of two species of root-knot nematode (*M. incognita* and *M. javanica*) were obtained from A. Morgan Golden (USDA, ARS, Beltsville, MD) as infected tomato roots and soil. The tomato roots were chopped finely and mixed thoroughly with the soil to constitute the inoculum. About 30 cm^3 of this inoculum was added to each pot of 4-wk-old tomato by digging two holes on opposite sides of the plant and placing 15 cm^3 of inoculum in each hole; the holes were then filled with uninfested soil. The

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amount of available inoculum allowed inoculation of about two-thirds of the tomatoes with *M. javanica* and of the remaining one-third with *M. incognita*. At 15 wk, the roots of these tomato plants and the soil in which they were growing were used as inoculum for the mimosa seedlings.

Propagation of *Fusarium oxysporum* f. sp. *perniciosum*. A composite-isolate inoculum of the mimosa wilt fungus was obtained from R. J. Stipes (Virginia Polytechnic Institute and State University, Blacksburg) on milled oats and was stored at −18 C. A small amount of inoculum was placed on potato-dextrose agar and allowed to grow for about 2 wk. At this time, the mycelial mass completely covered the plate (100-mm petri dish) and conidia were abundant.

Mimosa seedling inoculation and disease screening. Mimosa seeds were scarified in concentrated sulfuric acid for 45 min, rinsed, dried, and stored at 4 C overnight. About 1,000 seeds of each of the 13 seed lots were sown on 14 February 1984 in flats of sterilized soil of the same mix as described previously.

Seed lots O and I were used in an experiment to test pathogenicity of the two root-knot nematode species and the mimosa wilt fungus, alone and in all possible combinations. Seed lot O was collected from the single tree with obvious signs of mimosa wilt, and seed lot I, from a tree showing no wilt symptoms. Three-week-old seedlings of seed lots O and I were removed from the flats and subjected to various combinations of fungal and nematode inoculation (Table 1). Those treated with the fungus were dipped for 1 min in an inoculum prepared by blending 2-wk-old cultures with distilled water in a ratio of four plates to 100 ml. The seedlings were transplanted into a medium consisting of equal parts sterilized soil and soil-tomato root mixture.

From 400 to 1,000 3-wk-old seedlings of each seed lot (except O and I) were transplanted and inoculated with the two species of root-knot nematode and the mimosa wilt fungus in a second experiment. A root dip consisting of one culture plate per 100 ml of distilled water was used. The nematode inoculum (soil and roots) was combined with soil from the seed flats in a 1:1 ratio. The nematode inoculum was combined in a 2:1 ratio of *M. javanica* to *M. incognita*. The mimosa seedlings were pulled and their roots dipped in the fungal suspension and transplanted to flats containing the nematode-infested soil. Controls were transplanted without inoculation (100 seedlings of each seed lot). Seventy days after transplanting and inoculation, the mortality and condition of all seedlings in both experiments were recorded by seed lot. The numbers of dead and wilted or chlorotic seedlings were noted.

After the 70-day evaluation, the most vigorous seedlings of the progenies from resistant parents, up to a maximum of 10% of the number of seed sown, were transplanted individually to 10-cm-diameter pots and all were reinoculated with the wilt fungus at the same time. In addition, *all* of the symptomless seedlings from parents of unknown susceptibility and 10 each of the control seedlings of these progenies were similarly handled. Two plates of mimosa wilt fungus were blended with 100 ml of distilled water, and the seedling roots were dipped into this mixture. Soil from the flats in which the treated seedlings had been growing was used to fill the containers and thus continue the root-knot nematode association. These seedlings were fertilized monthly with Peters 20-20-20 at one-half recommended strength.

Beginning 4 June 1984, the plants were observed periodically, and those that were obviously dead or showing severe wilt were discarded at 2-wk intervals until 15 August. On 28 September, symptomless plants were transplanted to 20-cm-diameter containers and placed in a cool greenhouse. A final, preplanting evaluation for wilt and chlorotic symptoms was made on the potted plants after growth had resumed in 1985.

Table 1. Mortality and condition of two seed lots of *Albizia julibrissin* seedlings 70 days after various treatments (100 seedlings per treatment)

	Seed lot I			Seed lot O		
Treatment[a]	Dead (no.)	Wilt/ chlorosis (no.)	Total (no.)	Dead (no.)	Wilt/ chlorosis (no.)	Total (no.)
C	0	0	0	0	0	0
J	0	4	4	7	10	17
N	1	3	4	2	19	21
JN	1	5	6	0	3	3
F	16	14	30	42	11	53
FJ	32	37	69	66	2	68
FN	25	31	56	39	27	66
FJN	34	30	64	52	8	60

[a] C = Control, J = *Meloidogyne javanica*, N = *M. incognita*, and F = *Fusarium*.

Table 2. Mortality and condition of *Albizia julibrissin* seedlings 70 days after treatment with Fusarium wilt and two nematode (*Meloidogyne*) species[a]

Seed lot[b]	GA no.[c]	Seedlings (no.)	Dead (%)	Wilt/ chlorosis (%)	Total (%)
C[d]	2	800	17	27	44
F	···	400	19	23	42
J	65-15	800	21	19	40
D	···	400	28	24	52
L	16	800	28	26	54
P	32	1,000	33	26	59
I	48	100	34	30	64
Q	65-88	800	35	24	59
M	···	400	36	30	66
S	65-88	400	47	16	63
O	···	100	52	8	60
CT[e]	···	400	74	22	96
TU	···	400	76	21	97

[a] Controls of all seed lots had no more than 2% dead or chlorotic seedlings.
[b] Seed lots listed in order of increasing mortality.
[c] Selection number of parent assigned by D. L. Gill, USDA, ARS, Tifton, GA.
[d] Seed lots identified by single letters derived from natural intercrossing among trees selected for resistance to Fusarium wilt.
[e] Seed lots identified by two letters derived from landscape trees of unknown origin and wilt resistance.

RESULTS AND DISCUSSION

Data from our experiments are presented in Tables 1–3. Our testing procedures precluded all but the most rudimentary statistical treatment, using *t* tests with groups of equal or unequal sizes with each flat of 50 or 100 seedlings as an entry. Even so, the results were adequate for our purpose.

In the first experiment, mortality was significantly lower in treatments with nematodes alone than in those with *Fusarium* or *Fusarium*-nematode combinations (Table 1). The major influence of the nematodes was to induce leaf chlorosis, but this chlorosis, coupled with incipient wilt, placed many seedlings in the wilt/chlorosis category. Differences between seed lots in the treatments with nematodes alone may have been caused by some seed transmission of the wilt fungus (6), because seed lot O had been taken from a wilt-infected tree. Short-term mortality was significantly higher in

treatments where the root-knot nematodes, especially *M. javanica*, were added to the growing medium of *Fusarium*-inoculated seedlings. Because of the apparent superiority of *M. javanica* in inducing wilt symptoms, the proportion of this species was doubled in the growing medium for our longer-term tests.

The data in Table 2 show significant differences in mortality and percentage of symptomatic seedlings among progenies derived from supposedly wilt-resistant parents. However, some of these differences disappeared in longer-term tests (Table 3). The major differences among seed lots were between those derived from resistant parents and those from parents of unknown origin. Of 6,000 seedlings from resistant parents, mortality after 70 days was 29% (1,746 seedlings) and the number of symptomatic seedlings was 3,192 (53%). This compares with a mortality of 75% and a symptomatic rate of 96% for the seedlings of untested parents.

Not all of the 2,808 short-term survivors of the original 6,000 seedlings were vigorous plants, however, and the selection of seedlings for longer-term testing (Table 3) eliminated most of the weaker-growing plants and also served to conserve space. Many of the differences in treatment responses among seed lots noted earlier no longer existed, and the survival averages clustered closely around the mean of 52%. This average survival rate (from selected parents) is significantly different from the mortality observed in progenies from unselected parents, where only three of the original 800 plants (0.38%) survived. None of the 20 plants of similar progenies that had not been subjected to the earlier screening survived the long-term tests.

After our various tests, we were left with 312 living seedlings of the 6,000 seedlings from wilt-resistant parents originally grown for the screening process. Evaluation of these seedlings in the spring of 1985 showed that 22 plants did not resume growth and that only 78 plants were symptomless. A spot check showed nematodes on both symptomless and symptomatic plants, and *Fusarium* was isolated from the roots of plants showing wilt.

We anticipate that more mortality will occur after outplanting and during the subsequent years before the plants reach sexual maturity. Apparently, resistance to the combined effects of root-knot nematodes and Fusarium wilt is rare in *A. julibrissin*. Whether we will be able to select trees with such resistance, and with superior growth and flowering characteristics, remains a challenge.

Table 3. Mortality of seedlings of *Albizia julibrissin* 7 mo after initial treatment with Fusarium wilt and two nematode (*Meloidogyne*) species and 130 days after *Fusarium* reinoculation at time of potting

Seed lot[a]	GA no.[b]	Seedlings tested (no.)	Seedlings alive 28 Sept. 1984 (no.)	Mortality (%)
C[c]	2	80	36	55.0
F	···	40	18	55.0
J	65-15	80	33	58.8
D	···	40	22	45.0
L	16	80	54	32.5
P	32	100	52	48.0
I	48	10	6	40.0
Q	65-88	80	43	46.3
M	···	40	23	42.5
S	65-88	40	20	50.0
O	···	10	5	50.0
CT[d]	···	17	1	94.1
CT-C[e]	···	10	0	100.0
TU	···	15	2	86.7
TU-C[e]	···	10	0	100.0

[a] Seed lots listed in order of increasing mortality.
[b] Selection number of parent tree assigned by D. L. Gill, USDA, ARS, Tifton, GA.
[c] Seed lots identified by single letters derived from natural crossing among trees selected for resistance to Fusarium wilt.
[d] Seed lots identified by two letters derived from landscape trees of unknown origin and wilt resistance.
[e] Seedlings of previous control group, inoculated with *Fusarium* on 21 May 1984 at time of potting in nematode-infested soil.

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