Screening for Resistance to *Sclerotinia trifoliorum* in Alfalfa by Inoculation of Excised Leaf Tissue

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I thank M. S. Stokes for technical assistance and D. L. Boykin, USDA, ARS, supervisory statistician, for assistance with statistical analysis.
Accepted for publication 28 May 1996.

ABSTRACT

Pratt, R. G. 1996. Screening for resistance to *Sclerotinia trifoliorum* in alfalfa by inoculation of excised leaf tissue. Phytopathology 86:923-928.

Excised leaf tissues of alfalfa were inoculated with *Sclerotinia trifoliorum* to determine whether leaf-inoculation techniques may be used to screen for heritable and effective resistance to the pathogen. Leaf tissues were placed on water agar in petri plates, inoculated with mycelia of *S. trifoliorum*, and incubated at 17°C. Host responses were evaluated according to the rate and extent of necrosis that developed during 1 week. One thousand plants of cultivar Delta were screened for resistance by successive inoculation of excised unifoliate leaves, first trifoliate leaflets, and disks of tissue from later leaves. The five plants considered most resistant were intercrossed, and progeny were compared with those of five plants selected for susceptibility, five selected at random, and the parent cultivar. Progeny of resistant plants had significantly ($P = 0.05$) less severe disease than did progenies of susceptible and random plants and the parent cultivar when evaluated by inoculation of unifoliate leaves, first trifoliate leaflets, leaf disks, excised stems, and whole plants. In field experiments with natural infection during two growing seasons, progeny of resistant plants had less severe disease and produced significantly ($P = 0.05$) higher yields of forage through the first or second harvests than did progenies of susceptible and random plants and the parent cultivar. These results establish that inoculation of excised leaf tissue is an effective and efficient means to screen for resistance to *S. trifoliorum* in alfalfa.

Sclerotinia crown and stem rot, caused by *Sclerotinia trifoliorum* Eriks., is an important disease of forage legumes in temperate climates throughout the world. On alfalfa (*Medicago sativa* L.) in North America, Sclerotinia disease is especially important in the southeastern and south-central United States where the crop is normally planted in late summer or early autumn (21,23). Primary infection by *S. trifoliorum* occurs in late autumn from ascospores that are deposited on leaves of young seedlings. Growth of mycelia, from lesions initiated by ascospores, and rotting of host tissue begin after several weeks or months, and disease development continues during favorable conditions from winter through early spring (23,27).

Severity of Sclerotinia disease varies greatly from year to year. After summers with excessive rainfall or dry winters with hard freezes, disease may occur at only trace levels (7,10). However, with abundant primary inoculum and favorable environmental conditions (moderate temperature and prolonged high humidity), fall-seeded stands may be almost completely destroyed (16,23,27, 29). High disease severity also is favored in conservation-tillage planting systems in which sclerotia are retained near the soil surface (23). In the northern United States and Canada, Sclerotinia disease is less severe, because alfalfa is planted in the spring and plants are 10 to 12 months old when the rotting phase of the disease occurs. These older plants are more resistant to *S. trifoliorum* and sustain less damage than do seedlings from fall plantings (23,26).

No cultivars of alfalfa or any other forage legume have been developed for resistance to *S. trifoliorum* in North America. However, existing cultivars of alfalfa vary in susceptibility or tolerance to the disease (2,19,26,28,29). Recent reports of progress in breeding for resistance, from both public organizations (16,20) and private industry (13), indicate that resistant cultivars can be developed and are likely to become the major form of disease management in the future.

Several techniques for screening for resistance to *S. trifoliorum* in alfalfa have given favorable results in progeny evaluations. Significant responses to selection were obtained by spraying plants with comminuted mycelia (16), by stem-tip inoculations (8,18), and with natural epidemics in the field (9,15). Recently, the first germ plasm of alfalfa developed for resistance to *S. trifoliorum*, "Mississippi *Sclerotinia*-Resistant" (MSR), was released (20). This germ plasm was selected from cultivar Delta by leaf- and stem-inoculation techniques, and it was significantly more resistant than 23 other cultivars (R. G. Pratt and D. E. Rowe, *unpublished data*).

Although progress has been made in breeding for resistance to *S. trifoliorum* in alfalfa, there is still a need for new techniques that can be used effectively and efficiently in large-scale screening programs. It is doubtful whether any techniques described to date can be used in this manner. Foliar inoculations with comminuted mycelia or infested grain enable comparisons of overall levels of host resistance in cultivars or populations (16,19,21). However, these techniques may not be satisfactory for identifying the most resistant individuals at low frequencies in a population, since results can be variable or inconsistent because of nonuniform application of inoculum and other causes (16,19,21). Also, these whole-plant-inoculation techniques allow only a single determination of the host response of each plant, and this does not enable checks on the possible selection of susceptible escapes. The stem-inoculation technique allows for multiple determinations of the host response for each plant (2,8,17), but this technique is time-consuming and impractical for use in large-scale screening programs (16,18). Other artificial screening techniques have been described for *S. trifoliorum* on alfalfa, but their effectiveness has not been proven by progeny testing (24,28). Selection following

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Publication no. P-1996-0617-01R
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natural epidemics in the field has given increased resistance (9,15), but natural epidemics do not occur consistently and often are not severe enough for effective selection.

Assays based on inoculation of excised leaf tissue have been used to evaluate disease interactions for a wide range of fungal pathogens and host plants. Most leaf assays are used for rapid identification of host resistance and susceptibility (4,6,11,14), but some have also been used to evaluate virulence of pathogens (5) and fungicide activity (30). Most assays involve incubation of whole leaves or disks of leaf tissue on agar, liquid, or saturated substrates and inoculation with spores or mycelia of a fungal pathogen. Results are compared by the extent of symptom development (primarily necrosis) in leaf tissue (4,5,14) or by sporulation of the pathogen (6,11,30).

Excised leaf-inoculation techniques have been described for *S. trifoliorum* in two reports. Mousset-Declas et al. (12) applied ascospore suspensions to excised leaves of red clover; differences in disease symptoms were observed between cultivars after 6 days, and these correlated with field ratings. Arseniuk (1) inoculated excised leaves of red clover with mycelia; significant differences between genotypes were observed in rotting and in electrolyte loss from tissue after 72 h.

In development of the MSR alfalfa germ plasm (20), plants were screened for resistance by an array of leaf-inoculation techniques. Several techniques were considered efficient and effective, but details of their use were not described and their effectiveness was not evaluated objectively. Therefore, this study was undertaken to evaluate the inoculation of excised leaf tissue as a sole approach to screening for heritable and effective resistance to *S. trifoliorum* in alfalfa.

MATERIALS AND METHODS

Experimental approach. One thousand plants of alfalfa cultivar Delta were screened for responses to *S. trifoliorum* by successive inoculations of excised unifoliate leaves, first trifoliate leaflets, and disks of tissue from later leaves. Five plants with low scores of disease severity were selected as resistant parents, five with high scores were selected as susceptible parents, and five were selected at random. Plants of each group were polycrossed, and seed were harvested separately from each maternal plant to give five half-sib families of progeny per group. The three groups of progeny (resistant, susceptible, and random) and the parental cultivar then were evaluated for resistance to *S. trifoliorum* by inoculation of unifoliate leaves, first trifoliate leaflets, leaf disks, stems, whole plants, and with natural infection in the field. For leaf and stem inoculations, the half-sib families of each group were evaluated separately. For whole-plant inoculations and natural infection in the field, families were composited and comparisons were made between groups of progeny.

Growth, increase, and crossing of plants. Alfalfa seed were germinated on inverted plates of water agar for 2 days and planted in plastic cones containing a greenhouse potting mixture (19) when radicles were 1 cm long. Commercial inoculum of *Rhizobium meliloti* was dusted over seedlings and watered into soil after planting. After 6 to 8 weeks, selected plants were transplanted to clay pots (574-cm^3 capacity) with potting mixture, fertilized as described (18), and cloned by rooting stem cuttings. For each group (resistant, susceptible, and random) of five plants, equal numbers of cloned plants were grown to flowering and cross-pollinated in a cage with honey bees (*Apis mellifera* Linnaeus). Half-sib families of seed were harvested separately for each maternal genotype.

Storage and growth of *S. trifoliorum*. Sclerotia of a moderately virulent isolate of *S. trifoliorum* used in previous studies (8,18,19) were air-dried and stored at room temperature. Sclerotia were surface-disinfested and plated on agar to initiate growth (19). Colonies were grown on 20% V-8 juice agar at room temperature (23 to 25°C) in alternate light and darkness (laboratory bench). Transfers were made only from growing colony margins to edges of 9-cm plates, and colonies were transferred at approximately 4-day intervals before they reached opposite sides of the plates, so that the isolate was kept in a continual phase of active vegetative growth. Whenever decreases in growth rate were observed, cultures were discarded, and new colonies were initiated from sclerotia.

Inoculation of leaves and leaf disks. At 10 days after planting, unifoliate leaves were excised at the tip of the petiole and inverted individually in petri plates (35-mm diameter) of water agar. Disks, 4-mm diameter, were cut with a cork borer from the margins of growing colonies of *S. trifoliorum* on V-8 agar and bisected parallel to the colony radius (Fig. 1A). One half of each disk was inverted on the unifoliate leaf near the middle of the distal edge (Fig. 1B).

When plants were 17 days old, terminal leaflets of first trifoliate leaves were collected and inoculated in the same manner as for unifoliate leaves, except that leaflets were not inverted on agar (Fig. 1C).

To assay older leaves, a disk (12-mm diameter) was cut with a cork borer from a leaflet of the second fully expanded trifoliate leaf distal from the tip of an actively growing stem. Each leaf disk was placed on water agar and inoculated by inverting a 6-mm-diameter disk of agar, cut from a growing colony margin of *S. trifoliorum* on V-8 agar, over the center (Fig. 1D).

Plates with unifoliate leaves, trifoliate leaflets, and leaf disks were incubated on a growth bench at 17 to 20°C under fluorescent growth lights (80 $\mu E\ m^{-2}\ s^{-1}$ intensity) with a 12-h photoperiod (19) and observed daily for disease symptoms. Visual scores were assigned inversely according to the time at which tissue became completely necrotic (Fig. 2A, B, and C): e.g., leaves that became completely necrotic at 2 days after inoculation were scored "6", and those completely necrotic at 7 days were scored "1". Scores of "5" to "2" were assigned on intermediate days. Leaves not completely necrotic at 7 days after inoculation were scored "0.00 to 0.99" according to percentages of tissue that visibly appeared necrotic (Fig. 2D, E, and F).

In screening experiments, plates with leaf tissue were completely randomized on the growth bench. Selections were made based on symptoms or mean scores and without statistical analysis. In experiments to evaluate groups of parent plants (resistant, susceptible, and random) prior to crossing, four replicate plates of leaf tissue from each plant (three disks per plate) were arranged in a completely random design. Mean scores of disks in each plate were used as replicate values, and groups were compared by analysis of variance and the least significant difference (LSD) test at $P = 0.05$. In experiments to evaluate progeny for disease responses in unifoliate leaves, first trifoliate leaflets, and disks of leaf tissue, 20 replicate plates (one plate per plant) from each treatment (half-sib family or the parent cultivar) were arranged in a randomized complete-block design. Because of treatment structure (four groups of treatments, one to five treatments per group), the sum of squares was partitioned into contrasts for groups and treatments within groups. Additional comparisons between group means were performed with the LSD test at $P = 0.05$.

Inoculation of stems. Half-sib families of progeny and the parent cultivar were evaluated for responses to stem inoculations with *S. trifoliorum* as in previous studies (8,18). Growing stems 23 to 25 cm long were excised from plants, inoculated by sealing infested cotton around tips with masking tape, placed in flasks of water, and maintained in complete humidity for the first 4 days after inoculation (8,18). Each flask contained one replicate stem from each experimental entry and was considered a randomized complete block. The length of necrosis induced by *S. trifoliorum* in each stem was measured at 2 weeks after inoculation as done previously (8,18). Results were compared by analysis of variance with contrasts as for leaf inoculations, and differences between

groups of progeny and the parent cultivar were determined by the LSD test at $P = 0.05$.

Inoculation of whole plants. Survival of progeny and the parent cultivar after whole-plant inoculations was determined as in previous studies (18,19). Plants were grown in plastic cones for 25 days in the greenhouse. For each group of progeny (resistant, susceptible, and random), equal numbers of plants of available half-sib families were combined, randomized, set in pots (eight per pot), and held erect with sand. Plants were inoculated by dusting comminuted wheat and oat grain infested with *S. trifoliorum* over foliage (1.875 g per pot) and incubated at 17°C in alternate periods of complete humidity and ambient air (18,19). The three groups of progeny and cultivar Delta were arranged in a randomized complete-block design with 14 replicate pots. Numbers of surviving plants were counted at 24 days after inoculation (18,19). Data were compared by analysis of variance, and significant differences were determined by the LSD test at $P = 0.05$.

Field experiment. For each group of progeny (resistant, susceptible, and random), equal quantities of seed of available half-sib families were combined. Cultivar Delta and the three groups of progeny were each grown in five replicate 0.61 × 0.61-m plots (1.25 g seed per plot = 30 lb/acre) in a randomized complete-block design. The experiment was performed twice during each of two winter growing seasons (1992 to 1993 and 1993 to 1994) in naturally infested soils on the Leveck Animal Research Center at Mississippi State University. Plots were seeded in September, and stands were established by irrigation. To augment natural inoculum and ensure primary infection in all plots, 100 sclerotia of *S. trifoliorum* collected from naturally infected plants the previous spring were added to surface soil in each plot in early October. Stand counts were made for all plots in November. Forage was harvested and dry-matter yields were determined in April and May after disease development ceased.

RESULTS

Selection, evaluation, and crossing of parent plants. Of 1,000 plants inoculated, 50 plants with severe disease symptoms in unifoliate leaves were selected as potentially susceptible, and 50 with slight symptoms were selected as potentially resistant. First trifoliate leaflets of the 100 plants then were inoculated, and 20 plants of each group were retained based on severity of symptoms. From the remaining 960 plants, five plants also were selected at random without regard to disease scores. The 45 selected plants were

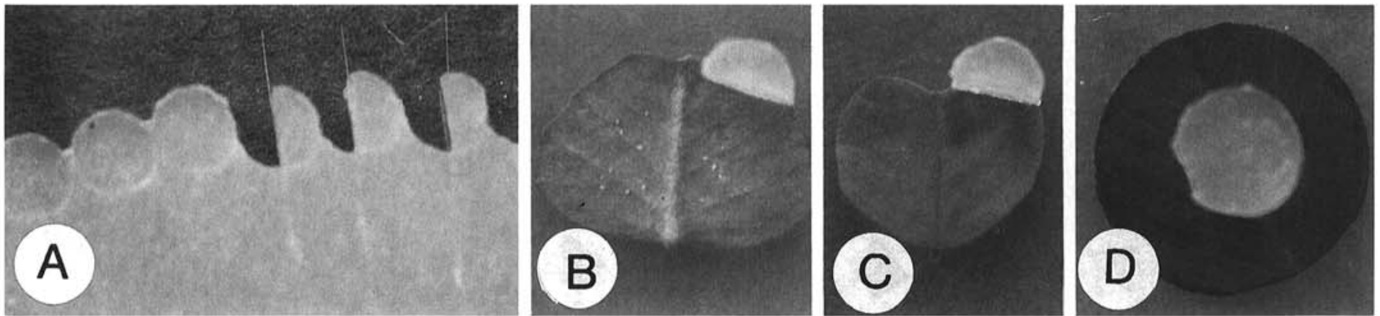

Fig. 1. Inoculation of leaves and leaf disks with mycelia of *Sclerotinia trifoliorum*. **A,** Circular disks (4-mm diameter) cut from the colony margin of *S. trifoliorum* on V-8 juice agar and bisected parallel to the radius of the colony. **B,** Half of disk from **A** inverted over the terminal margin of a unifoliate leaf inverted on a small (35-mm diameter) plate of water agar. **C,** Half of disk from **A** inverted over the terminal margin of the middle leaflet of a first trifoliate leaf on plate of water agar. **D,** Disk (6-mm diameter) cut as in **A** and inverted over disk of tissue (12-mm diameter) cut from the middle leaflet of a second fully expanded leaf proximal to the tip of a growing stem.

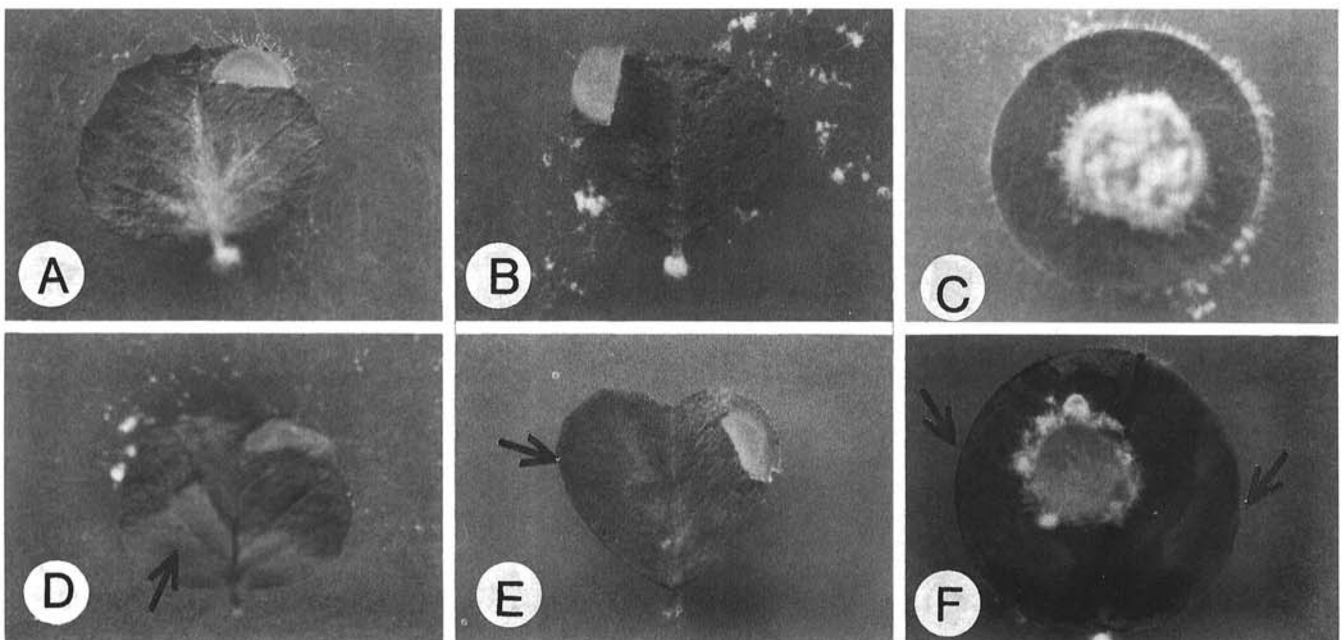

Fig. 2. Susceptible and resistant phenotypic responses in excised alfalfa leaf tissues inoculated with *Sclerotinia trifoliorum*. Complete necrosis of **A,** unifoliate leaf; **B,** first trifoliate leaflet; and **C,** leaf disk at 2 days after inoculation (susceptible phenotypes). Incomplete necrosis of **D,** unifoliate leaf; **E,** first trifoliate leaflet; and **F,** leaf disk, respectively, at 5 to 7 days after inoculation (potential resistant phenotypes). Arrows indicate green, nonnecrotic tissue.

transplanted to pots (16-cm diameter) and grown for 7 weeks. Final screening of the 20 susceptible and 20 resistant plants was performed in nine runs with one leaf disk per plant. The five plants of each group with the highest and lowest mean scores, respectively, were retained as susceptible and resistant parents.

Before crossing, host responses of resistant, susceptible, and random parent plants were compared in two leaf-disk-inoculation experiments. Mean scores were highest for susceptible plants, intermediate for random plants, and lowest for resistant plants, and all differences between groups were significant at $P = 0.05$ (Table 1).

Five susceptible, five random, and four resistant parents produced sufficient seed for progeny evaluations. One resistant parent flowered but set little seed; progeny of this plant, therefore, were not included in evaluations.

Evaluation of progeny by leaf and stem inoculations. Progeny were evaluated in two experiments each with unifoliate leaves, first trifoliate leaflets, and leaf disks. Different plants were used for each experiment. Significant differences occurred between groups of progeny and between families within groups (Table 2).

TABLE 1. Disease severity in leaf disks of resistant, random, and susceptible groups of alfalfa parent plants following inoculation with mycelia of *Sclerotinia trifoliorum*[y]

Parental group	Experiment 1	Experiment 2
Resistant	0.75 A[z]	1.03 A
Random	3.05 B	3.43 B
Susceptible	3.72 C	4.60 C

[y] Disks (12-mm diameter) were incubated on plates of water agar (three per plate) and inoculated with agar disks from colony margins of *S. trifoliorum*. Disks were scored for disease severity depending on times when tissue became necrotic; 7.00 = most severe and 0.00 = no disease after 7 days. Values for plates were mean scores of the three disks.

[z] Means of four plates per plant and five plants per group. Means within columns not followed by the same letter differ significantly at $P = 0.05$ according to the least significant difference test.

In all experiments, disease severity was significantly less in resistant progeny than in all other groups (Table 3). In four of six experiments, disease severity was significantly greater in susceptible progeny than in random progeny and the parent cultivar. Random progeny did not differ from the parent cultivar in four experiments, but they were more susceptible in two experiments. In both stem-inoculation experiments, resistant progeny differed significantly from all other groups, and no other differences were significant (Table 4).

Evaluation of progeny by whole-plant inoculations. Survival of whole plants after inoculation was significantly greater ($P = 0.05$) for resistant progeny than for other populations in both experiments (Table 5). Survival of susceptible progeny was significantly lower ($P = 0.05$) than for 'Delta'. Survival of random progeny and 'Delta' did not differ significantly from either 'Delta' or susceptible progeny.

Evaluation of progeny by natural infection in the field. Stand counts in early November revealed no significant differences between entries in any experiment. Apothecia developed from sclerotia and primary infection occurred in November and December. Secondary disease development (rotting of leaves and stems) commenced in all plots of both experiments in December of 1992 and January of 1994. By February of each year, resistant progeny manifested visibly less rotting of stems and stronger canopy development than did the other entries; resistant plots could be identified visually on this basis in nearly all blocks. Overall disease development was greater in 1993 than in 1994, and it ceased in both years with the onset of warm temperatures and dry conditions in early April. First harvests of forage from the four experiments were taken in April and May after flowering commenced. Second harvests were taken in June of 1993, but not in 1994 because of drought.

First-harvest yields of resistant progeny were significantly greater ($P = 0.05$) than for all other entries in three of four experiments (Table 6). Second-harvest yields were greater than for all other entries in one of two experiments in 1993. No significant differences in yield occurred between 'Delta', random progeny, and susceptible progeny in any experiment (Table 6).

TABLE 2. Summary of analyses of variance with contrasts for differences between groups of alfalfa populations (parental cultivar and resistant, susceptible, and random progeny) and populations within groups in inoculation experiments with *Sclerotinia trifoliorum* on excised leaf and stem tissues[y]

		Groups of populations[z]		Populations within groups[z]	
Tissue inoculated	Experiment	*F* value	*P* > *F*	*F* value	*P* > *F*
Unifoliate leaf	1	92.21	<0.01	2.91	<0.01
	2	14.58	<0.01	17.70	<0.01
First trifoliate leaflet	1	135.95	<0.01	3.06	<0.01
	2	105.70	<0.01	1.77	<0.05
Leaf disk	1	65.95	<0.01	2.58	<0.01
	2	20.57	<0.01	3.70	<0.01
Stem	1	18.22	<0.01	1.27	>0.10
	2	13.07	<0.01	2.36	<0.01

[y] Corresponding data and differences between groups are given in Tables 3 and 4.

[z] Degrees of freedom for groups = 3, degrees of freedom for populations within groups = 11.

TABLE 3. Disease severity in excised unifoliate leaves, first trifoliate leaflets, and leaf disks of alfalfa cultivar Delta and resistant, susceptible, and random progeny following inoculation with *Sclerotinia trifoliorum*[y]

	Unifoliate leaves		First trifoliate leaflets		Leaf disks	
Alfalfa population	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Resistant	2.76 A[z]	3.88 A	2.91 A	3.36 A	3.28 A	0.68 A
'Delta'	4.20 B	4.75 B	4.19 B	4.75 B	4.35 B	2.12 B
Random	4.52 B	5.00 B	4.72 C	4.75 B	4.75 BC	2.94 C
Susceptible	4.81 C	5.21 C	4.88 C	4.97 C	4.91 C	3.47 D

[y] Leaf tissues were incubated individually in small plates (35-mm diameter) of water agar and inoculated with agar disks from colony margins of *S. trifoliorum*. Leaves and disks were assigned scores for disease severity (7.00 = most severe, 0.00 = least severe) depending on times until tissue became necrotic.

[z] Means for 'Delta' based on 20 replicate leaves per experiment. Means for resistant, random, and susceptible populations based on four, five, and five half-sib families, respectively, with 20 leaves or leaflets per family per experiment. Means within columns not followed by the same letter differed significantly at $P = 0.05$ by the least significant difference test.

DISCUSSION

Results of this study demonstrate that inoculation of excised leaf tissue is an effective and efficient means of screening alfalfa for resistance to *S. trifoliorum*. With one cycle of selection for resistance at the 0.5% level (five plants per 1,000), progeny of resistant selections were significantly more resistant than the parent cultivar and susceptible and random progeny when assayed with unifoliate leaves, first trifoliate leaflets, leaf disks, excised stems, whole plants, and natural infection in the field. Therefore, these results indicate that inoculation of excised leaf tissue could be used as the sole means to screen for resistance to *S. trifoliorum* in alfalfa.

The most important aspect of the results is that resistance selected by detached leaf inoculations in the laboratory was also effective against disease that developed naturally in the field. In three of four experiments conducted during two growing seasons, the resistant population manifested significantly higher plant survival and dry-matter yield in the presence of natural disease than did the parent cultivar, the susceptible population, and the random population. In one experiment in which the resistant population did not give significantly higher yields, overall disease incidence and severity were relatively low and may not have limited yields sufficiently to reflect significant differences in susceptibility of populations. These results appear to represent only the second instance in which selection for resistance to *S. trifoliorum* in alfalfa by artificial inoculations in the laboratory was shown to enhance resistance to disease in the field (18). However, Pierson et al. (15), in opposite sequence, showed that selection for resistance in the field significantly increased resistance expressed following artificial inoculations with comminuted mycelia in the laboratory. This suggests that use of comminuted mycelia to screen for resistance in the laboratory could also give increased resistance in the field. A second important feature of leaf inoculations is that they allow multiple assays of the host response of individual plants. Multiple assays enable strong responses to selection, because they increase the likelihood of selecting the most resistant plants in a population (2). This consideration is especially important for alfalfa, because host response to *S. trifoliorum* appears to represent a multigenic characteristic with continuous variation between susceptible and resistant extremes (2,8,17). Differences in resistance between individual plants, families, populations, or cultivars may be slight (2,8,17,18,19,22,29), and variability in the results of whole-plant-inoculation experiments (19,21) may preclude their use as single assays to identify the most resistant plants in a population. Failure to identify accurately the most resistant plants in screening experiments will reduce gains from selection and increase the number of cycles required to attain a high level of resistance. The stem-inoculation technique also allows multiple assays, but this is not practical for use in large screening programs, because plants must be grown for several months to obtain stems and inoculations are time-consuming to perform (8,16,18).

Because resistance to *S. trifoliorum* in alfalfa is not a qualitative characteristic, screening is most effectively accomplished by the progressive removal of susceptible plants from a population during repeated assays until only the most resistant individuals remain. If disease severity is not too strong, as can occur with a highly virulent isolate or succulent tissue (19), then high credibility should be given to "susceptible" host responses, and plants may be discarded when these appear early in screening. Low credibility should be given to apparent "resistant" host responses until these have been confirmed repeatedly in subsequent assays.

In practical screening for resistance with relatively susceptible populations such as 'Delta' (19), 80 to 90% of the most susceptible-appearing plants can be eliminated by the unifoliate leaf assay. Approximately 50% of remaining plants can be further eliminated by assay of the first trifoliate leaflets. Therefore, 90 to 95% of the most susceptible-appearing plants can be discarded by 25 days after planting. The remaining 5 to 10% of plants from the original population can be further reduced to 1% or less on the basis of six or more independent assays of disks from large leaves. Plants finally retained for crossing are those with the lowest mean scores for leaf disks, and none of these are likely to represent relatively susceptible escapes. In fact, there appears to be a high likelihood that plants retained following eight or more independent assays, even at a low frequency of selection, will represent

TABLE 4. Lengths of necrotic lesions on excised stems of alfalfa cultivar Delta and resistant, susceptible, and random progeny following inoculation with *Sclerotinia trifoliorum*

	Mean length of lesion (cm)[z]	
Alfalfa population	Experiment 1	Experiment 2
Resistant	3.13 A	4.00 A
'Delta'	4.55 B	4.93 B
Random	3.91 B	5.31 B
Susceptible	4.30 B	5.40 B

[z] Means for 'Delta' based on 20 replicate stems per experiment. Means for resistant, random, and susceptible populations based on four, five, and five half-sib families, respectively, with 20 stems per family per experiment. Means within columns not followed by the same letter differ significantly at $P = 0.05$ according to the least significant difference test.

TABLE 5. Survival of plants of alfalfa cultivar Delta and resistant, susceptible, and random progeny following whole-plant inoculations with *Sclerotinia trifoliorum*

	Mean percentage of surviving plants[z]	
Alfalfa population	Experiment 1	Experiment 2
Resistant	53 A	52 A
'Delta'	28 B	26 B
Random	20 BC	20 BC
Susceptible	10 C	9 C

[z] Means based on 14 replicate pots per treatment with eight plants per pot. Analysis of variance was performed on numbers of plants alive in each pot at 24 days after inoculation. Means within columns not followed by the same letter differ significantly at $P = 0.05$ according to the least significant difference test.

TABLE 6. Yields of alfalfa cultivar Delta and resistant, susceptible, and random progeny following disease development by *Sclerotinia trifoliorum* in field plots at Mississippi State, MS, during two growing seasons

	Year, experiment, harvest date, and mean dry-matter yield					
	1992 to 1993				1993 to 1994	
	Experiment 1		Experiment 2		Experiment 1	Experiment 2
Alfalfa population	27 April 1993	4 June 1993	6 May 1993	10 June 1993	14 April 1994	19 April 1994
Resistant	155 A[z]	301 A	138 A	241 A	216 A	203 A
'Delta'	99 B	247 B	82 B	207 AB	169 B	162 A
Random	78 B	206 B	65 B	167 B	164 B	172 A
Susceptible	80 B	211 B	64 B	168 B	149 B	163 A

[z] Mean dry-matter yield (grams per plot) of five replicate plots. Means within columns not followed by the same letter differ significantly ($P = 0.05$) according to the least significant difference test.

some of the most resistant individuals that were present in the original population.

An important requirement for the successful use of leaf-inoculation techniques to screen for resistance is that the isolate of *S. trifoliorum* not be highly virulent. A moderately virulent isolate is more desirable, because it allows expression of the broadest range of differences in host susceptibility and resistance (19). Weakly virulent isolates do not cause sufficient symptoms to fully reveal host susceptibility, and highly virulent isolates may override and obscure moderate levels of resistance that have to be selected initially in breeding programs. The isolate used here was also used previously to inoculate stems (2,8,18) and whole plants ("*S. trifoliorum* #1" [19]). With whole-plant inoculations, this isolate revealed a significantly broader range of differences among cultivars than did more weakly or highly virulent isolates (19).

In some host plants, resistance to Sclerotinia disease is attributed partly to differences in plant morphology or architecture. Cultivars of dry bean most susceptible to *S. sclerotiorum* have a viny growth habit that favors disease development; more resistant or tolerant cultivars often have a bushy growth habit that retards disease development (25). In soybean, canopy height is correlated with disease severity, and reduced severity in shorter cultivars represents significant disease escape (3). No similar differences in plant architecture or height are apparent in populations of alfalfa selected for resistance to *S. trifoliorum* by leaf and stem techniques (18). Resistance expressed in these populations represents physiological host-plant resistance rather than tolerance due to modifications in plant architecture or morphology.

The stem-inoculation technique was first used in this program to identify resistant plants and produce a moderately resistant population termed "STR" (18). This population expressed significant resistance to *S. trifoliorum* with stem inoculations, whole-plant inoculations, and natural infection in the field. The STR population then was used as a known resistant control to aid in development of the leaf-inoculation techniques described here. Possibly these techniques, or germ plasms developed with them (20), may, in turn, contribute to development of more efficient and effective procedures in the future.

LITERATURE CITED

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