Evaluation of Virulence of *Fusarium oxysporum* f. sp. *medicaginis* and Fusarium Wilt Resistance in Alfalfa

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ABSTRACT

Emberger, G., and Welty, R. E. 1983. Evaluation of virulence of *Fusarium oxysporum* f. sp. *medicaginis* and Fusarium wilt resistance in alfalfa. Plant Disease 67:94-98.

Isolates of *Fusarium oxysporum* f. sp. *medicaginis* from Minnesota, Pennsylvania, Maryland, and North Carolina did not differ in virulence to alfalfa (*Medicago sativa* cv. Narragansett). Conidia of four isolates from North Carolina were combined to inoculate wilt-resistant (Moapa, Liberty, and NCMP 2) and wilt-susceptible (Apalachee and Narragansett) cultivars of alfalfa in the field and greenhouse. Field and greenhouse results were highly correlated ($P = 0.01$). Wilt resistance in 10 cultivars and four breeding lines adapted to the southeastern United States was evaluated at two locations. Entries with the highest level of wilt resistance were germ plasms NCMP 9, NCMP 11, and NCMP 13 and cultivars Cimarron and Saranac AR; Apalachee was highly wilt-susceptible and the remaining entries were moderately wilt-resistant. The disease reaction of entries common to wilt nurseries in North Carolina and Minnesota were similar.

Additional key words: disease losses.

Alfalfa, *Medicago sativa* L., is one of the world's most valuable forages, yet its full production potential is often not realized due to diseases, poor management, and insects. Fusarium wilt of alfalfa is caused by *Fusarium oxysporum* Schlecht. f. sp. *medicaginis* (Weimer) Snyd. & Hans. and two other formae speciales of *F. oxysporum* (1,9) and is one of several diseases implicated in reducing yield and stand persistence (5–7).

In a natural stand, the infected shoots wilt, take on a bleached appearance, and eventually the entire plant is killed (5,7). A distinctive characteristic of the disease is dark brown discoloration of the root and stem xylem, which corresponds to external symptom severity. Cultivars resistant to wilt offer the only current economically feasible means of control.

Part of the strategy to improve yield and persistence of alfalfa is to develop multiple pest-resistant cultivars (3). Frosheiser and Barnes (4) developed effective procedures for screening alfalfa for Fusarium wilt resistance. They recommended that alfalfa cultivars being developed for specific areas should be screened against isolates from those areas until pathogenic variability within *F. oxysporum* is more thoroughly investigated. Otherwise cultivars evaluated for resistance to the pathogen in one region may not perform as expected in other regions.

The objectives of this study were to compare 1) the virulence of isolates of *F. oxysporum* f. sp. *medicaginis* from North Carolina with those from Maryland, Minnesota, and Pennsylvania, 2) the effectiveness of disease evaluation in the greenhouse and field using cultivars resistant and susceptible to *F. oxysporum* f. sp. *medicaginis*, and 3) the disease reaction of several breeding lines and cultivars in field nurseries in two locations.

MATERIALS AND METHODS

Pathogenicity test. Twenty-two isolates of *F. oxysporum* f. sp. *medicaginis* were evaluated for virulence by inoculating seedlings of wilt-susceptible cultivar Narragansett. Isolates tested were from stems or roots of wilting alfalfa obtained from Granville, Scotland, Guilford, Lincoln, Iredell, and Rockingham counties in North Carolina and from cooperators in Minnesota, Maryland, and Pennsylvania. All isolates were from single conidia and were lyophilized and are maintained as lyophilized cultures at the USDA Tobacco Research Laboratory, Oxford, NC, and the Fusarium Research Center, Pennsylvania State University, University Park.

Inoculum was prepared from lyophilized cultures by growing single conidia from each isolate in test tubes of potato-dextrose agar (PDA) at 21–23 C for 2 wk under fluorescent lights (12-hr on/off cycle) (8). Conidia were washed from the culture surface with distilled water, filtered through two layers of cheesecloth and diluted to a suspension of 1×10^6 conidia per milliliter as estimated by hemacytometer counts.

Seeds of cultivar Narragansett were inoculated with *Rhizobium meliloti* Dangeard and planted in 350-ml plastic cups (8 cm in diameter) containing a 1:1 (v/v) mixture of Metro Mix 220 (W. R. Grace & Co., Cambridge, MA 02140) and pasteurized (aerated steam at 80 C for 30 min) coarse sand. Plants were thinned to one per cup and grown at 25–30 C in a greenhouse where they were watered and fertilized to maintain vigorous growth.

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Paper 8214 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh. Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, North Carolina State University.

Accepted for publication 30 May 1982.

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Plants were lifted 12 wk after planting (first-bloom stage), roots were washed, clipped to 7 cm below the crown, and soaked for 45 min in a conidial suspension. The foliage was removed 7 cm above the crown and plants were potted in 10-cm clay pots containing pasteurized soil and sand in a 1:1 (v/v) mixture. Control plants were treated the same except they were soaked in distilled water. The study was arranged as a randomized complete block with 10 replicates. A single plant per isolate was inoculated in each replicate.

Five weeks after inoculation, roots were washed and the plants were evaluated for dry matter, number of dead roots, and the severity of xylem discoloration. Discoloration was rated on a 1–6 scale adapted from that of Frosheisner (4) where 1 = no discoloration, 2 = one to three small dark areas of discoloration, 3 = more than three small isolated areas of discoloration, 4 = a partial ring of discoloration, 5 = a complete ring of discoloration, and 6 = xylem completely discolored or plant dead. Roots were cut transversely below the crown and the cut surface was rated according to the above scale. The average rating for a given treatment comprised the disease severity index (DSI). Isolations from plant materials confirmed the presence of the pathogen.

Linear growth of 15 representative isolates was measured from 5-mm disks of mycelium placed in the centers of PDA plates. The cultures were replicated and incubated in the dark for 6 days at temperatures from 8 to 36 C.

Field and greenhouse evaluation for wilt resistance. Disease development in the field and the greenhouse was compared for four alfalfa cultivars and one breeding line. Moapa and Narragansett are resistant and susceptible to the pathogen, respectively, and are cultivars used for comparison when evaluating levels of Fusarium wilt resistance (4) (F. Frosheiser, *personal communication*). Preliminary results indicated that Liberty and Apalachee (cultivars adapted to southeastern United States) might substitute for these standard cultivars. NCMP 2 was included to evaluate wilt resistance in this breeding line. Uninoculated seedlings of Liberty and Apalachee were included in field and greenhouse tests.

Seedlings were grown in the greenhouse in wooden flats (50 × 38 × 14 cm) filled with a 1:1 (v/v) mixture of Metro Mix 220 and pasteurized sand. Plants were inoculated with *Rhizobium meliloti*. In mid-April, half of the seedlings in each entry were lifted when about 11 wk old for the first field study (840 plants = 6 reps × 7 entries × 20 plants); the remaining seedlings (840 plants) were used for the first greenhouse study. Seedlings of equivalent age but planted a year later were used in the second field study. Inoculum was prepared by combining equal proportions of conidia of four isolates of *F. oxysporum* f. sp. *medicaginis* highly virulent on cultivar Narragansett (designated 0-979, 0-981, 0-982, and 0-984) and diluting the conidial suspension to 1×10^6 conidia per milliliter. Plants were inoculated as previously described. Plants of the same treatment were bundled together, wrapped in moist paper towels, and stored overnight at 6 C before transplanting.

The first of two field studies consisted of a randomized complete block with six blocks (1980). There were seven treatments (entries) per block with 20 plants per treatment. The second study (1981), done at two locations (Oxford and Reidsville, NC) consisted of four randomized complete blocks with 14 entries (10 cultivars and four breeding lines). Uninoculated plants were transplanted first to minimize contamination in both studies. Plants were transplanted 30 cm apart in rows spaced 1.2 m apart. Soil at Oxford was a Helena Series with a 2–6% slope (2) and at Reidsville an Appling Series was used.

Numbers of surviving plants were recorded 2 wk after transplanting and at 2-wk intervals throughout the growing season. Yield data were recorded over five clippings. Surviving plants were uprooted in early October when the roots were washed and taproots cut through approximately 2 cm below the crown. The degree of xylem discoloration was rated according to the 1–6 scale previously described. Roots scored 1 and 2 were considered resistant to the pathogen and, from 3 to 6, susceptible to the pathogen. Isolations were made from plants that died during the growing season.

The remaining half of the seedlings not used for the field study were used for the first of two greenhouse studies. The second greenhouse study used seedlings of equivalent age but planted several months after the first study. In both experiments, the alfalfa populations, inoculum preparation, and inoculation were as previously described for the field studies. Plants were replanted into a 1:1 mixture of Metro Mix 220 and pasteurized sand for the first study and a 1:1 mixture of pasteurized sand and soil for the second study. Both experiments, terminated 7 wk after inoculation when plants first flowered, were arranged in four randomized complete block designs. The first and second experiments consisted of 25 and 20 plants per treatment, respectively. Isolations were

Table 1. Relative virulence of 22 isolates of *Fusarium oxysporum* f. sp. *medicaginis* on alfalfa cultivar Narragansett

Isolate source and identification	Xylem DSI[a] (1–6)	Dry matter (g)	Dead[b] (no.)
Minnesota			
7F-2 (0-958)[c]	4.5	1.94	2
31F-3 (0-959)	4.3	1.79	1
Maryland			
55 (0-955)	3.7	2.40	2
258 (0-957)	3.2	2.65	0
255 (0-967)	5.0	1.26	5
Pennsylvania			
762 (0-960)	1.3	2.67	0
900 (0-961)	5.0	1.32	4
933 (0-963)	5.0	1.74	2
969 (0-964)	4.5	1.78	0
1236 (0-965)	4.9	1.46	4
North Carolina[d]			
GR-1 (0-978)	4.8	1.35	1
GR-2 (0-982)	5.0	1.30	1
GR-3 (0-983)	4.8	1.60	3
GR-4 (0-984)	5.4	1.25	3
GR-5 (0-985)	5.3	1.57	4
S-1 (9-972)	2.0	2.28	0
G-1 (0-973)	1.7	2.22	0
I-1 (0-975)	1.6	2.88	0
L-1 (0-977)	1.9	2.11	0
I-2 (0-976)	1.6	2.14	0
R-2 (0-979)	4.6	1.27	2
R-9 (0-981)	4.5	1.99	1
Check	1.6	2.72	0
Mixture (0-972, 0-975, 0-977, 0-981)	4.0	1.64	1
FLSD[e] ($P = 0.05$)	1.03	0.68	

[a] Disease severity index based on degree of root xylem discoloration where 1 = no disease and 6 = highest disease rating.
[b] Number dead out of 10 plants.
[c] First identification number supplied by cooperator, second number was assigned by Fusarium Research Center, Pennsylvania State University, University Park.
[d] Isolates listed by county with Granville (GR), Guilford (G), Scotland (S), Lincoln (L), Iredell (I), and Rockingham (R) counties represented.
[e] Fisher's (protected) LSD.

made from inoculated and uninoculated roots.

RESULTS

Pathogenicity test. Virulence on Narragansett among the isolates ranged from 1.3 to 5.4 DSI (Table 1). Isolates 0-960, 0-972, 0-973, 0-975, 0-976, and 0-977 were judged nonpathogenic. Other isolates were considered highly virulent and four (0-979, 0-981, 0-982, and 0-984) were used as inocula in the field and greenhouse tests. Linear growth of representative isolates on PDA was similar (Fig. 1). Virulent isolates from North Carolina were originally obtained from wilting or dead stems of plants with wilt symptoms; nonpathogenic isolates were obtained from roots of plants with wilt symptoms. The DSI (4.0) for the mixture of isolates that contained three nonpathogenic isolates was only slightly less than the DSI (4.5) for the single highly virulent isolate (0-981). Highly virulent isolates 0-979, 0-981, 0-982, and 0-984 were deposited as ATCC 46584, ATCC 46585, ATCC 46586, and ATCC 46587, respectively, in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

Field and greenhouse evaluation for wilt resistance. Results from two greenhouse experiments and the 1980 field experiment were highly correlated ($P = 0.01$) in ranking the level of resistance (DSI) for the five alfalfa entries (Table 2). Entries with the most resistance in the 1981 field evaluations (Table 3), averaged for locations, were NCMP 11 > NCMP 13 > Vangard > Cimarron > Arc > Classic > NCMP 9 > Saranac AR > WL 311 > NCMP 6 > WL 318 > Apollo > Liberty > Apalachee. Percent wilt-resistant plants and DSI for an entry between locations were highly correlated ($P = 0.01$) ($r = 0.93$ and $r = 0.79$, respectively).

The increase in disease (percent dead plants) was similar for both growing seasons (Fig. 2). The rate of plant death was most rapid during the first half of the season, but plants continued to die until they were lifted for disease scoring. Differences in resistance to Fusarium wilt were evident within 2 mo of inoculation.

In both seasons, yields for the entries

Table 2. Correlation of DSI ratings from two greenhouse studies with a field plot study for five alfalfa populations inoculated with *Fusarium oxysporum* f. sp. *medicaginis*[a]

	DSI		
		Greenhouse	Greenhouse
Entry	Field	#1	#2
Inoculated			
Moapa	2.99	2.32	3.00
Liberty	3.22	4.29	3.50
NCMP 2	3.22	3.74	3.23
Narragansett	4.37	4.71	4.31
Apalachee	5.45	5.32	5.23
Uninoculated			
Liberty	1.97	1.82	1.85
Apalachee	2.18	1.81	2.05
Correlation (r)[b]		(0.91**)[b]	(0.99**)[b]

[a] Disease severity index (DSI) based on degree of root xylem discoloration where 1 = no disease and 6 = highest disease rating.
[b] The correlation of DSI scores for greenhouse #1 and #2 with DSI scores from the field experiment was significant at $P = 0.01$.

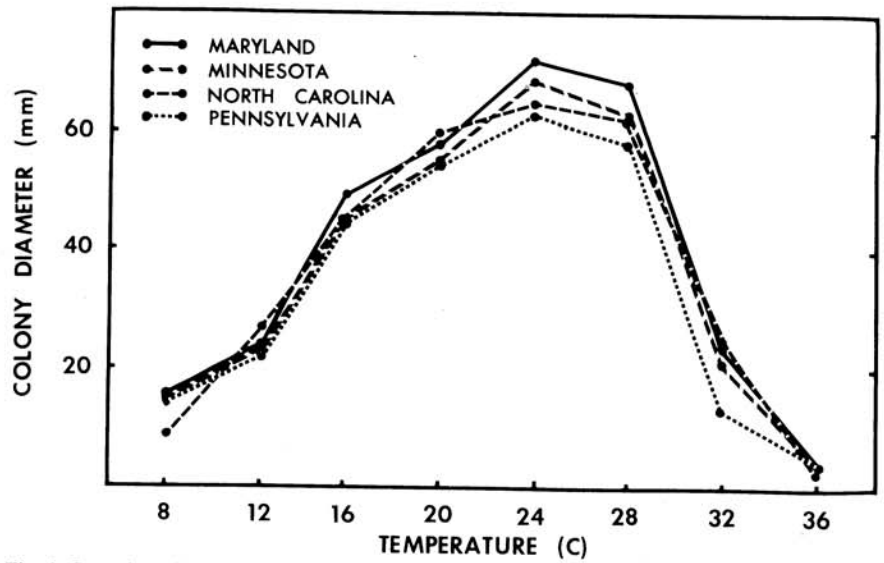

Fig. 1. Growth on PDA of representative isolates of *Fusarium oxysporum* f. sp. *medicaginis* (all virulent on Narragansett alfalfa) from Maryland, Minnesota, North Carolina, and Pennsylvania; means from four replicates derived from two, two, seven, and four isolates, respectively.

Table 3. Yield and disease reaction of 10 alfalfa cultivars and four breeding lines to *Fusarium oxysporum* f. sp. *medicaginis*[v]

	Dry matter (g)[w]				Disease severity[x] index (1–6)		Resistant plants[y] (%)	
	Inoculated		Uninoculated					
Entry	Oxford	Reidsville	Oxford	Reidsville	Oxford	Reidsville	Oxford	Reidsville
Apalachee	929 e	746 e	2,161 b	1,573 a	5.55 g	4.93 g	3 d	7 g
Apollo	1,708 cd	1,409 bcd	3,063 a	1,899 a	4.69 ef	3.43 bcde	21 c	31 ef
Arc	2,197 abc	1,305 bcd	...	...	4.10 bcdef	4.15 ef	23 c	22 f
Cimarron	2,478 ab	1,708 ab	2,670 ab	2,210 a	3.90 abcd	3.26 bcd	29 abc	48 bcd
Classic	1,499 de	1,180 cde	...	...	4.59 def	4.34 fg	14 cd	22 f
Liberty	1,856 bcd	1,104 de	2,263 b	1,568 a	4.80 f	4.02 ef	14 cd	27 ef
Saranac AR	1,628 cd	1,438 bcd	...	...	4.35 cdef	3.27 bcd	25 bc	47 bcd
Vangard	2,586 a	1,409 bcd	3,051 a	1,985 a	4.13 bcdef	3.11 abc	25 bc	39 cde
WL 311	2,033 abcd	1,457 bcd	...	...	4.05 bcde	3.71 cdef	27 bc	36 de
WL 318	1,840 bcd	1,175 cde	3,058 a	2,050 a	4.39 cdef	3.95 def	25 bc	30 ef
NCMP 6	1,795 bcd	1,572 bc	...	...	4.70 ef	3.61 cde	15 cd	36 de
NCMP 9	2,287 abc	1,724 ab	...	...	3.28 a	3.08 abc	46 a	52 abc
NCMP 11	2,173 abcd	1,588 bc	...	...	3.51 ab	2.49 a	41 ab	63 a
NCMP 13	2,313 abc	2,091 a	...	...	3.70 abc	2.74 ab	41 ab	56 ab
cv[z]	(20%)	(18.8%)	(15.9%)	(25.2%)	(12.7%)	(16.5%)	(41.4%)	(13.8%)

[v] Seedlings (12 wk old) were inoculated and transplanted to field plots at two locations in North Carolina. Each number is based on 20 plants (Oxford) and 22 plants (Reidsville) per row per entry and is an average of four replicates. Means followed by the same letter in a column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[w] Total from five harvests.
[x] Average disease severity index based on a 1–6 scale: 1 = no discoloration and 6 = stele completely discolored or plant dead.
[y] Plants with a disease score of 1 or 2 were considered resistant.
[z] cv = Coefficient of variability.

with the most wilt resistance always exceeded those with the least resistance (Tables 3 and 4). Yield data for 1980 were highly correlated (r = 0.97) with DSI scores. Yield data for 1981 for the entries at Oxford and Reidsville were highly correlated with DSI (r = 0.80 and 0.83, respectively). Entries with high DSI scores yielded the least, reflecting the high number of dead plants.

In both years, some uninoculated plants died late in the season. Samples were taken and cultured; *F. oxysporum* f. sp. *medicaginis* was never isolated from their root tissue nor did these plants have the stem or root xylem discoloration characteristic of the disease. In contrast, *F. oxysporum* f. sp. *medicaginis* was consistently isolated from inoculated plants that died throughout the season. Anthracnose, caused by *Colletotrichum trifolii* Bain, was sporadic in the planting in both years and caused a few inoculated and uninoculated plants to die, but its incidence was not considered severe enough to alter the results of these tests.

DISCUSSION

Isolates of *F. oxysporum* f. sp. *medicaginis* tested from North Carolina did not differ in relative virulence on Narragansett with isolates from Maryland, Minnesota, or Pennsylvania. Isolates from North Carolina were used as inocula in our field tests because isolates from other areas might have reacted differently when tested under climatic or edaphic conditions different from their area of origin.

Since the results from the greenhouse and field experiments were highly correlated, wilt resistance in inoculated seedlings can be evaluated by either test or in combination. Evaluations in the greenhouse, however, preclude identifying plants that may possess a usable level of field resistance to wilt. For this reason, we believe that if only one test is to be done, populations should be evaluated in the field rather than the greenhouse.

Cultivars classified as wilt resistant or susceptible in field studies in Minnesota (4; F. I. Frosheiser, *personal communication*) were included in both years of field studies. Although disease scores were higher in North Carolina than in Minnesota, cultivar rankings in wilt resistance were similar. For example, the percentage of plants resistant to wilt in Minnesota and North Carolina nurseries, respectively, was Arc 33 and 23, WL 318, 34 and 28, Liberty, 55 and 33 (averaged for years and locations), and Apalachee, 10 and 4 (averaged for years and locations). This is probably because plants remained in the field in North Carolina about 2 mo longer than in Minnesota and because soil temperatures were generally warmer in North Carolina. Plants continued to die throughout the season in both years (Fig. 2). This does not explain, however, why DSI were

Table 4. Yield and disease reaction of four alfalfa cultivars and one germ plasm line to *Fusarium oxysporum* f. sp. *medicaginis*[w]

Entry	Dry matter (g)[x]		DSI (1–6)[y]		Resistant (%)[z]
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Apalachee	782	2,059	5.45	2.18	3
Liberty	1,748	2,068	3.22	1.97	44
Moapa	1,673	···	2.99	···	57
Narragansett	1,099	···	4.37	···	22
NCMP 2	1,853	···	3.22	···	47

[w] Seedlings (about 11 wk old) were inoculated and transplanted into field plots in Oxford, NC, in 1980. Each number is based on 20 plants per row per entry and is the average from six replicates.
[x] Total from five harvests.
[y] Average disease severity index based on 1–6 scale; 1 = no discoloration and 6 = stele completely discolored or plant dead; LSD (P = 0.05) = 0.43.
[z] Plants with a disease score of 1 or 2 were considered resistant to the pathogen.

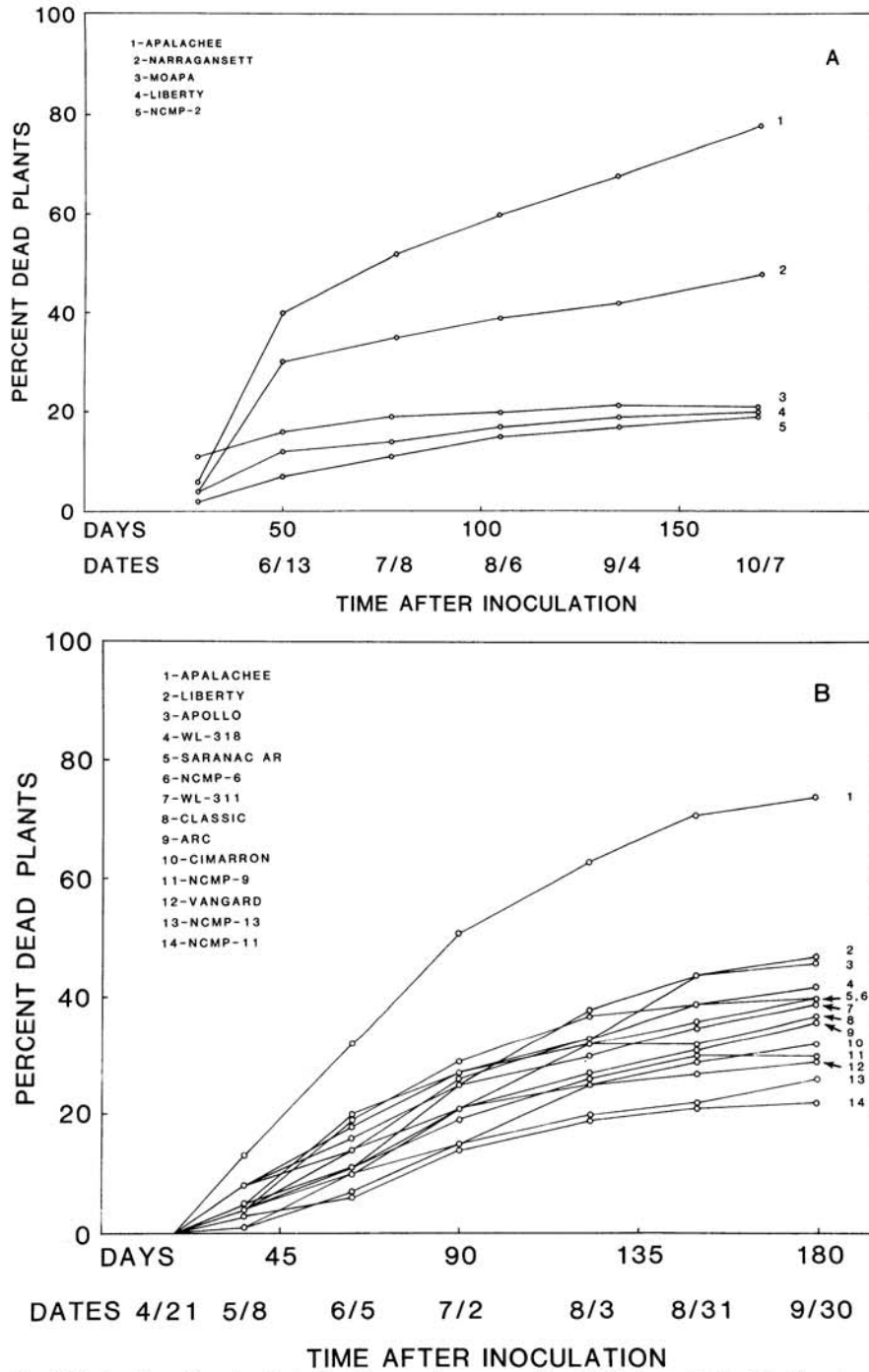

Fig. 2. Rate of seedling death during the growing season for alfalfa inoculated with *Fusarium oxysporum* f. sp. *medicaginis*: **(A)** 1980, **(B)** 1981.

usually larger at Oxford than Reidsville. In view of this, we agree that it seems desirable to maintain wilt nurseries in one or more geographical areas where adapted cultivars are grown (4). Based on our results and those reported elsewhere (4), susceptibility and resistance of an alfalfa cultivar to *F. oxysporum* f. sp. *medicaginis* might be evaluated in the field in one growing season.

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