

# Sources of Resistance to Anthracnose in the Annual *Medicago* Core Collection

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## ABSTRACT

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The annual genus *Medicago* core collection, consisting of 201 accessions, represents the genetic diversity inherent in 3,159 accessions from 36 annual *Medicago* species. This germ plasm was evaluated for resistance to anthracnose caused by *Colletotrichum trifolii*. Anthracnose is a major disease in perennial alfalfa (*Medicago sativa* L.) grown in North America and disease control is based principally on the use of resistant varieties. Evaluation of the core collection was conducted using standardized environmental conditions in growth chambers, and included the *M. sativa* standard reference cvs. Arc (resistant) and Saranac (susceptible). The degree of resistance found among accessions within species was highly variable; however, most annual species and accessions were susceptible. Only 14 accessions from seven species exhibited resistance greater than 40% seedling survival. These included accessions of *M. murex*, *M. muricoleptis*, *M. polymorpha* var. *brevispina*, *M. polymorpha* var. *polymorpha*, *M. radiata*, *M. soleirolii*, *M. truncatula*, and *M. turbinata*. Of the 12 accessions of *M. polymorpha* var. *polymorpha*, 4 exhibited more than 50% resistance, but 3 accessions were 100% susceptible. Most of the *M. truncatula* and *M. turbinata* accessions exhibited significantly more resistance than accessions of other species. Plant introduction (PI) accession number PI 495401 of *M. muricoleptis* exhibited 90.3% resistance. Accessions of *M. scutellata* were uniformly susceptible. Histological examinations of 14 of the most anthracnose-resistant accessions revealed that *C. trifolii* spores germinated and produced typical appressoria, but failed to penetrate and produce the primary and secondary hyphae characteristic of susceptible interactions. Resistant reactions were similar to those found in incompatible interactions with *C. trifolii* and alfalfa, which have been associated with specific genes leading to the production of isoflavonoid phytoalexins. The large genetic variability in annual *Medicago* spp. offers potential for locating and utilizing disease resistance genes through breeding or genetic engineering that will enhance the utilization of *Medicago* spp. as a forage crop.

Additional keywords: alfalfa, *Colletotrichum trifolii*, disease resistance, lucerne

Recent interest in the use of annual species of *Medicago* as a cover and forage crop for use in sustainable agriculture systems in the United States has prompted both an evaluation of the diversity of germ plasm that exists and the development of an annual genus *Medicago* core collection. This collection represents the genetic diversity inherent in 3,159 accessions from 36 species of annual *Medicago* contained in the United States National Plant Germplasm System (8,10). A subset of 1,240 accessions has been evaluated for morphological and agronomic traits, and accessions were chosen within a species to represent the greatest diversity in geographical origin (9). The selected core collection of 201 accessions from 33 species was re-evaluated at seven locations across the

United States and found to remain stable across environments and to represent the variability of the germ plasm collection (9). Annual medics have potential use in North America as weed-suppressing smother crops, cover crops in row-crop production, and for short-season forage crops.

There is little information regarding sources and levels of resistance in annual *Medicago* spp. to the most important indigenous foliar diseases of alfalfa (1,2,29), which include anthracnose caused by *Colletotrichum trifolii* Bain & Essary, spring black stem and leaf spot caused by *Phoma medicaginis* Malbr. & Roum. in Roum., and *Leptosphaerulina* leaf and stem spot caused by *Leptosphaerulina briosiana* (Pollacci) J. H. Graham & Luttrell (19,28). Annual *Medicago* spp. are native to regions surrounding the Mediterranean Sea, and commercial cultivars are important forage crops in Australia and South Africa. The species that are most widely grown and from which commercial varieties have been developed are *M. littoralis* Rohde ex Lois., *M. murex* Willd., *M. polymorpha* (L.), *M. rugosa* Desr., *M. scutellata* (L.) Mill., and *M. truncatula* Gaertn. (6). Foliar diseases frequently oc-

cur in these species and cause significant yield losses (1-3,19). The same foliar diseases cause significant losses in forage and seed yield in *M. sativa* (5,12,28-30). The availability of disease-resistant accessions in the annual medic collection will enhance the utilization of these species in North American agro-ecosystems, and may also serve as a potential source of new or novel resistance genes amenable for incorporation into adapted *M. sativa* genotypes.

Anthracnose, caused by *C. trifolii*, is a major disease in perennial alfalfa grown in North America and is characterized by premature leaf drop, crown and stem blight, and foliar and stem necrosis (29). Losses in yield and quality can be high, and disease control is based principally on the use of resistant varieties (12,13). Variation in virulence found among isolates of *C. trifolii* showed the need for more durable sources of resistance because not all genotypes are resistant to all pathogen races (22,24). Anthracnose resistance has been identified in a limited number of annual *Medicago* accessions, but the mechanisms for such resistance have not been investigated (13,18,27). Defense interactions in legume-*Colletotrichum* pathosystems may differ widely, involving phytoalexins, structural cell-wall components such as hydroxyproline-rich glycoproteins, and plant defense proteins such as glucanases and chitinases (14). In the case of *C. trifolii* and alfalfa, resistance has been associated with the production of pterocarpin and isoflavonoid phytoalexins following infection in germ plasm with genes for resistance (11,23,25,26). Fungitoxic phenolic compounds have been identified from several annual *Medicago* spp., but their role in disease resistance has not been investigated (17). Annual *Medicago* spp. exhibiting disease resistance may contain novel disease-resistance genes or produce fungitoxic secondary metabolites which could be exploited for use in adapted perennial alfalfa. It has been shown that the transfer of only a single gene is sufficient to confer the capacity to produce an inducible phytoalexin (15), and good progress is being made towards engineering this type of disease resistance in alfalfa (11).

Alfalfa is an autotetraploid that suffers from inbreeding depression, resulting in self-sterile accessions after a few generations of self-pollination. Cultivars are very heterogeneous and are usually developed as synthetics. Because most annual medics are diploids ( $2n = 14$  or  $16$ ), autogamous,

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and self-fertile, these species are genetically simpler to study and easier to manipulate than alfalfa. Some species, such as *M. truncatula*, are transformable and are being used as a model for legume systems to study symbiosis-related plant genes and to manipulate and enhance resistance to foliar pathogens based on inducible or altered levels of phytoalexins (4,11,16,26).

There is a need to evaluate populations of annual *Medicago* spp. for resistance to foliar diseases, and to determine whether or not resistance is based on altered levels or different kinds of flavonoids and isoflavonoids or new, potentially more fungitoxic, phytoalexins. The objective of the present study was to evaluate resistance to anthracnose in the annual genus *Medicago* core collection, and to compare fungal development and host response in resistant and susceptible accessions.

## MATERIALS AND METHODS

Seedlings (2 weeks old) of 201 accessions from 33 species were evaluated in growth chambers for resistance to anthracnose caused by *C. trifolii*. *M. sativa* anthracnose standard reference cvs. Arc (resistant) and Saranac (susceptible) were included (13,29). The seedling reaction has been demonstrated to predict field performance in natural epidemics with *M. sativa*. Cultivars occasionally appear more resistant in the field than indicated by seedling tests but, generally, good correlations are observed between greenhouse and field tests. Growth conditions, inoculation, and evaluation of accessions for anthracnose resistance were conducted by modifying standardized procedures used to evaluate anthracnose resistance in alfalfa (22,24). Seed were scarified by abrasion against sand paper and 35 seeds per pot were planted in pasteurized field soil in 0.2-cm pots. Each accession was replicated three times and arranged in a completely random design in a growth chamber at 23°C with a 16-h fluorescent light photoperiod for 14 days. The number of seedlings per pot was determined 10 days after planting. At 14 days after planting, seedlings were spray-inoculated to just prior to run-off (approximately 2 ml/pot) with a spore suspension of *C. trifolii* race 1 isolate 2sp2 ( $2 \times 10^6$  spores/ml) and incubated in a mist chamber at 23°C for 48 h. Isolate 2sp2 was isolated from *M. sativa* in Maryland and has shown consistently high virulence to susceptible cultivars (22–25,29). Inoculum was prepared by suspending spores in sterile distilled water containing two drops of Tween 20/liter. Spores were obtained from 7-day-old cultures grown on half-strength oatmeal agar (36 g of Difco oatmeal agar, 1 liter of distilled water, 7.5 g of agar) at 21°C under 12 h of fluorescent light. Plants were returned to the growth chamber to allow disease development, and evaluated for resistance (percent survival) after 8 days. A

**Table 1.** Evaluation of the annual genus *Medicago* core collection for resistance to anthracnose caused by *Colletotrichum trifolii* race 1

Species and ascension <sup>a</sup>	Origin	No. tested	Resistance (% survival) <sup>b</sup>
<i>M. arabica</i>			
PI 495200	France	103	0.0
PI 495212	Hungary	68	5.6
<i>M. blanchena</i>			
PI 495215	Unknown	135	5.6
PI 495216	Turkey	91	0.0
PI 495222	Lebanon	130	4.6
PI 495223	Lebanon	81	4.4
PI 495227	Hungary	99	0.0
PI 505415	Spain	135	0.0
PI 505416	Spain	130	10.3
<i>M. ciliaris</i>			
PI 368928	California, USA	114	0.0
PI 442645	Turkey	127	7.5
PI 498731	Czechoslovakia	148	0.0
PI 498750	Lebanon	119	15.6
PI 498784	Tunisia	125	0.8
PI 498785	Morocco	154	0.0
<i>M. constricta</i>			
PI 495240	Greece	167	9.4
PI 534177	Bulgaria	85	0.0
PI 534182	Cyprus	71	0.0
<i>M. coronata</i>			
PI 498790	Greece	137	0.7
PI 498805	Lebanon	133	8.2
<i>M. disciformis</i>			
PI 487317	Bulgaria	98	0.0
PI 487321	Greece	124	0.0
PI 487322	Italy	157	0.0
PI 487333	Cyprus	149	12.9
<i>M. doliata</i>			
PI 495278	Lebanon	55	33.3
PI 505420	Spain	60	13.8
<i>M. doliata</i> var. <i>muricata</i>			
PI 534202	Lebanon	113	10.9
PI 534211	Algeria	84	13.6
<i>M. granadensis</i>			
PI 498810	Israel	157	0.0
PI 498812	Turkey	122	0.0
PI 498813	Turkey	154	5.9
PI 498817	Turkey	119	0.0
<i>M. heyriana</i>			
PI 537136	Greece	85	7.1
<i>M. intertexta</i>			
PI 498824	Portugal	120	8.2
PI 498828	Italy	133	0.0
PI 516649	Morocco	95	0.0
PI 516650	Morocco	68	0.0
PI 535606	Tunisia	107	10.0
PI 535607	Tunisia	164	1.9
<i>M. italica</i>			
PI 384640	Morocco	135	10.3
PI 385014	Tunisia	152	3.4
PI 459188	Turkey	96	5.0
PI 566864	Spain	135	7.8
PI 566865	Georgia, USA	127	8.7
PI 566866	Italy	129	0.0
PI 566867	Morocco	141	31.8
PI 566868	Sweden	87	0.0
<i>M. laciniata</i>			
PI 498839	United Kingdom	132	0.0
PI 498841	Israel	105	0.0
PI 498842	Czechoslovakia	138	0.0
PI 498853	Spain	117	0.0
PI 498864	Spain	195	0.0
PI 498890	Spain	187	0.0

(continued on next page)

<sup>a</sup> Six-digit numbers are plant introduction (PI) numbers used by the National Plant Germ Plasm System of the United States Department of Agriculture.

<sup>b</sup> Percentages represent the means of three replicates. Plants were placed in a mist chamber for 48 h and percent survival was determined 10 days after inoculation. The means were arcsine transformed for analysis of the data. Least significant difference ( $P = 0.05$ ) = 13.7.

<sup>c</sup> Standard reference cultivars for anthracnose resistance in *M. sativa*.

Table 1. (continued from preceding page)

Species and ascension	Origin	No. tested	Resistance (% survival)
<i>M. laciniata</i> (continued)			
PI 498902	Morocco	141	0.0
PI 498918	Iraq	151	2.5
PI 535738	Libya	104	4.0
<i>M. lanigera</i>			
PI 498930	Former USSR	173	1.7
<i>M. lesinsii</i>			
PI 534233	Israel	142	0.0
PI 537259	Australia	94	12.9
<i>M. littoralis</i>			
PI 385006	Tunisia	122	3.1
PI 517206	Australia	149	0.0
PI 537168	Cyprus	72	0.0
PI 537171	Lebanon	97	2.3
PI 537201	Italy	96	8.3
PI 537207	Spain	86	0.0
PI 537222	Morocco	77	0.0
<i>M. lupulina</i>			
PI 189128	Denmark	96	0.0
PI 202038	Argentina	126	4.4
PI 215245	Nebraska, USA	164	5.0
PI 227452	Iran	67	0.0
PI 234821	Switzerland	59	1.6
PI 251834	Italy	45	0.0
PI 269926	Pakistan	117	0.0
PI 290723	United Kingdom	63	0.0
PI 304527	Turkey	87	0.0
PI 308059	Czechoslovakia	65	0.0
PI 314538	Former USSR	168	4.3
PI 319026	Spain	156	0.0
PI 452459	Canada	117	6.2
PI 532942	Nepal	77	0.0
PI 566869	Netherlands	115	0.0
<i>M. minima</i>			
PI 227032	Iran	139	9.2
PI 499022	Lebanon	160	0.0
PI 499072	Italy	143	2.8
PI 499080	Turkey	102	17.4
<i>M. murex</i>			
PI 308062	Czechoslovakia	50	100.0
PI 495350	Italy	65	7.3
PI 495379	France	84	13.6
PI 534231	Canada	72	0.0
PI 516720	Morocco	83	0.0
<i>M. muricoleptis</i>			
PI 495401	Italy	103	90.3
<i>M. noeana</i>			
PI 495407	Turkey	135	3.5
PI 495414	Unknown	126	0.0
<i>M. orbicularis</i>			
PI 210425	Greece	129	0.0
PI 251474	Turkey	152	0.0
PI 283645	Morocco	129	0.0
PI 292421	Israel	151	4.2
PI 505425	Spain	85	3.0
PI 566870	Romania	111	0.0
PI 566871	Italy	122	5.1
PI 566872	Italy	163	0.0
<i>M. polymorpha</i>			
PI 478531	Peru	86	5.8
PI 493292	Portugal	101	9.8
PI 493293	Portugal	142	0.0
PI 566873	Italy	70	1.7
PI 566874	Greece	113	0.0
PI 566875	Cyprus	110	24.4
PI 566876	California, USA	90	8.6
PI 566877	Italy	89	7.4
PI 566878	Georgia, USA	112	0.0
PI 566879	Germany	89	0.0
PI 566880	Belgium	76	0.0
PI 566881	Lebanon	79	7.7

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preliminary experiment was conducted with all accessions and, to conserve seed, only 10 seeds per pot, three replications per accession, were planted and evaluated. Some of the accessions exhibited poor germination in the preliminary experiment; therefore, two sets of pots of these accessions were planted, evaluated, and combined for analysis in the reported experiment. Due to space limitations for inoculation and plant growth chambers, approximately 50 accessions, including the standard cvs. Arc and Saranac, were evaluated per experiment. Data from five experiments were combined for analysis. The percent survival data was arcsine transformed to stabilize variance of the data, and subjected to analysis of variance with the SAS general linear models procedure (SAS release 6.11, SAS Institute, Cary, NC). Means were compared by the least significant difference test at  $P = 0.05$ .

Accessions exhibiting more than 40% seedling survival were evaluated histologically to observe fungal development and determine the stage at which resistance was expressed. For comparison, compatible disease interactions were observed either in susceptible accessions of the same species or in a susceptible portion of seedlings within a resistant accession. Seeds were planted in pots, grown for 2 weeks, inoculated, and incubated 48 h in a mist chamber as described above. Two of the trifoliate leaves from at least three seedlings were removed from each accession and examined 24, 48, and 72 h after inoculation. Disease reactions exhibited in leaves excised from inoculated seedlings have been shown to be a reliable indicator of host resistance phenotype in alfalfa (24). Each remaining plant was evaluated for susceptibility 10 days after inoculation to determine whether the excised leaves originated from a resistant or susceptible plant. The histology events were recorded for resistant individuals on all but three of the accessions. Leaves were examined on a microscope slide as whole mounts in Trypan blue (0.05%) in lactic acid: glycerol:distilled water (1:2:1 by volume). Slides were heated briefly to clear tissue. The developmental condition of at least 50 conidia/leaf surface was observed, and measurements included spore germination, appressorial development, presence of germ pores, production of primary and secondary hyphae, and tissue collapse (7).

## RESULTS

Accessions in the annual genus *Medicago* core collection were highly susceptible to anthracnose caused by *C. trifolii* (Table 1). Only 14 accessions representing seven species exhibited more than 40% resistance as determined by percent seedling survival (Fig. 1). These included accessions of *M. murex*, *M. muricoleptis*, *M.*

*polymorpha* var. *brevispina*, *M. polymorpha* var. *polymorpha*, *M. radiata*, *M. soleirolii*, *M. truncatula*, and *M. turbinata*. Disease severity among accessions and within species was highly variable. With few exceptions, resistance was not generally consistent among accessions within species. In one of the most resistant species, *M. polymorpha* var. *polymorpha*, 4 of the 12 accessions exhibited higher levels of survival, but 3 accessions were 100% susceptible. Most of the *M. truncatula* and *M. turbinata* accessions exhibited significantly more resistance than accessions of other species. Plant introduction (PI) accession number PI 495401 of *M. muricoleptis* exhibited 90.3% resistance, and one of the five accessions of *M. murex* (PI 308062) was completely resistant. Seedling survival of check cultivars was consistent between five experiments. The susceptible reference cv. Saranac ranged from 0 to 2% resistant, and resistant cv. Arc ranged from 64 to 70% resistant.

Histological examinations of 14 anthracnose-resistant accessions revealed that resistance was not associated with the failure of conidia to germinate or to form appressoria. Spores of most accessions adhered to leaves, germinated, and produced typical mature appressoria within 24 h (Table 2). The fungus failed to produce secondary hyphae in resistant tissues, and fungal development was restricted to the initially infected cell. The cell collapse observed in the resistant annual *Medicago* sp. 72 h after inoculation is characteristic of a hypersensitive response, which suggests the presence of dominant resistance genes (7,14). Disease resistance was expressed at or near the time of epidermal penetration, which is similar to *M. sativa* accessions exhibiting anthracnose resistance (7,20,21). *C. trifolii* appeared to develop at different rates among the resistant *Medicago* accessions (Table 2). Germ pores were observed in three resistant accessions (PI 197340, PI 56687, and PI 292436) but did not progress to produce primary or secondary hyphae during the next 24 h. Most of the resistant accessions exhibited primary hyphae in a penetrated epidermal cell after 72 h.

The early infection events also occurred in susceptible accessions, but the disease continued to progress and kill the seedlings. Primary hyphae, secondary hyphae, or tissue collapse were evident 72 h after inoculation of susceptible seedlings of *M. polymorpha* var. *brevispina* PI 197530, *M. polymorpha* var. *polymorpha* PI 206695, *M. truncatula* PI 566889, *M. turbinata* PI 566894 and PI 441943, and *M. sativa* cv. Saranac. Thin, secondary hyphae were observed in three susceptible accessions, and tissue collapse was observed in these tissues within 96 h. The fungus attacked all above-ground parts, causing shriveling and death of susceptible seedlings.

**Table 1.** (continued from preceding page)

Species and ascension	Origin	No. tested	Resistance (% survival)
<i>M. polymorpha</i> (continued)			
PI 566882	Greece	60	3.3
PI 566883	France	64	23.7
PI 566884	Syria	66	11.1
PI 566885	Morocco	68	11.1
<i>M. polymorpha</i> var. <i>brevispina</i>			
PI 186329	Australia	123	0.0
PI 197340	Australia	55	88.1
PI 197530	Algeria	67	0.0
PI 226648	Iran	76	1.4
PI 368949	Chile	61	0.0
PI 385017	Tunisia	44	87.5
<i>M. polymorpha</i> var. <i>polymorpha</i>			
PI 206695	Turkey	106	0.0
PI 244312	Spain	16	0.0
PI 250782	Afghanistan	51	0.0
PI 253448	Yugoslavia	35	62.0
PI 283657	Sweden	126	22.7
PI 286534	Ethiopia	74	67.1
PI 292427	Israel	51	39.8
PI 302926	Spain	79	23.7
PI 308055	Czechoslovakia	73	10.3
PI 319036	Spain	32	50.6
PI 404795	Uruguay	76	50.0
PI 459130	Turkey	72	8.5
<i>M. praecox</i>			
PI 495429	Greece	123	0.0
PI 495434	France	145	0.0
<i>M. radiata</i>			
PI 340800	Hungary	104	0.0
PI 459142	Turkey	56	23.3
PI 459145	Turkey	59	49.3
PI 459146	Turkey	77	64.3
<i>M. rigidula</i>			
PI 230350	Iran	77	0.0
PI 233250	Israel	44	28.2
PI 319048	Spain	18	33.3
PI 495517	Greece	63	0.0
PI 534236	Turkey	44	5.7
PI 534250	Turkey	33	0.0
<i>M. rotata</i>			
PI 292430	Israel	26	0.0
PI 495576	Unknown	17	0.0
PI 495577	Italy	11	4.8
PI 495583	Turkey	34	0.0
PI 495586	Lebanon	25	0.0
PI 537236	Czechoslovakia	117	0.0
<i>M. rugosa</i>			
PI 368962	Greece	71	0.0
PI 442893	Australia	39	7.7
PI 487363	Portugal	44	16.5
PI 487374	Lebanon	16	0.0
PI 487377	Spain	102	0.0
PI 487379	Italy	41	27.6
PI 487382	Italy	67	0.0
PI 487386	Tunisia	94	0.0
PI 308061	Czechoslovakia	68	0.0
PI 535534	Tunisia	37	0.0
PI 535537	Tunisia	79	0.0
<i>M. sauvagei</i>			
PI 499152	Morocco	106	0.0
PI 499153	Unknown	93	0.0
<i>M. scutellata</i>			
PI 161415	Uruguay	93	0.0
PI 197806	Australia	88	6.6
PI 197817	United States	58	0.0
PI 197821	Cyprus	100	0.0
PI 292432	Israel	76	0.0
PI 487392	Sweden	102	0.0
PI 487393	Cyprus	80	0.0
PI 487394	Germany	72	0.0

(continued on next page)

Table 1. (continued from preceding page)

Species and ascension	Origin	No. tested	Resistance (% survival)
<i>M. scutellata</i> (continued)			
PI 487396	Hungary	52	0.0
PI 487403	Turkey	90	0.0
PI 487409	Lebanon	90	0.0
PI 487411	Italy	74	0.0
PI 516907	Morocco	91	0.0
PI 517255	Ethiopia	56	0.0
PI 535643	Tunisia	94	0.0
PI 535644	Tunisia	58	0.0
PI 535645	Tunisia	62	0.0
<i>M. soleirolii</i>			
PI 537242	Algeria	71	26.2
PI 537243	Australia	50	97.4
<i>M. tenoreana</i>			
PI 499161	Italy	159	0.0
<i>M. truncatula</i>			
PI 292436	Israel	75	97.6
PI 384648	Morocco	111	67.4
PI 566886	Italy	75	6.3
PI 566887	Greece	84	87.2
PI 566888	Australia	65	95.6
PI 566889	Turkey	76	19.6
PI 566890	Greece	90	53.7
PI 566891	Czechoslovakia	17	91.1
PI 566892	Spain	93	7.4
<i>M. turbinata</i>			
PI 441943	Syria	136	0.0
PI 535555	Tunisia	70	54.3
PI 566893	Lebanon	31	80.0
PI 566894	Turkey	83	28.7
PI 566895	Czechoslovakia	91	96.2
<i>M. sativa</i> <sup>c</sup>			
Arc	United States	482	66.0
Saranac	United States	598	0.0

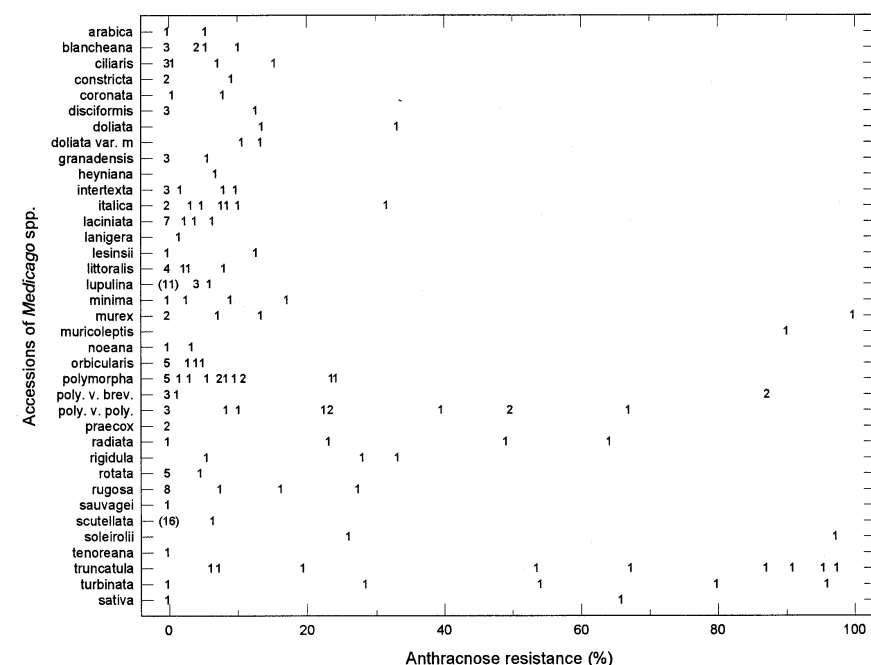


Fig. 1. Anthracnose susceptibility in 201 accessions from 33 species in the annual genus *Medicago* core collection. Each figure indicates the number of accessions exhibiting anthracnose resistance at the percent indicated. Parentheses are used to distinguish two-digit numbers. Standard reference cultivars for anthracnose resistance in alfalfa are Arc (resistant) and Saranac (susceptible).

## DISCUSSION

Little resistance to anthracnose was found in the core collection, and efforts should be made to use or incorporate the resistance that is available into species of

annual *Medicago* intended for use in areas where anthracnose is severe. Accessions within most species had markedly different reactions to anthracnose; thus, few generalizations can be made regarding suscepti-

bility within a species. For example, *M. murex* resistance among five accessions ranged from 0 to 100% survival in this study, while two other accessions evaluated previously exhibited 100% resistance, indicating that resistance may be all or nothing with the single isolate used in this study (13). Likewise, resistance in six accessions of *M. rigidula* ranged from 0 to 33.3% survival, while in an earlier study one accession was 100% resistant (13). There is some consistency in anthracnose resistance among accessions of *M. scutellata* because accessions in this species appear to be uniformly susceptible (13,18). Seven of the nine accessions within *M. truncatula* exhibited significant anthracnose resistance, and the level of resistance in two of these were confirmed as reported earlier by Elgin and Ostazeski (13) and Lamprecht (18). Only one highly virulent isolate of *C. trifolii*, race 1, was used to evaluate the resistance in the study reported here. Reactions to other strains of the pathogen may vary, and differences in reactions to race 1 and 2 have been reported (13). The heterogeneity in resistance phenotypes within an individual PI accession was unexpected because annual *Medicago* spp. are self pollinated. This variation may be explained by the limited amount of out-crossing that does occur, the method in which the seed is increased, and the possibility of infection escapes.

The entire process of pathogenesis from spore deposition and differentiation of appressoria and infection hyphae was similar in annual *Medicago* spp. to that observed in *M. sativa*. Limitation of fungal growth at the time of appressorial maturation and penetration of the epidermal cell suggests that resistance may be based on a phytoalexin response.

Resistance to major diseases in alfalfa increases quality, yield, and adaptability. However, traditional methods of breeding alfalfa for resistance are limited by the autotetraploid nature of alfalfa and the severe effects of inbreeding depression. Annual *Medicago* spp. may be a source of new or different phytoalexins for disease control, with the added advantage that they are genetically simpler to study and manipulate. Resistance is the most desirable type of control for alfalfa pathogens because of the large acreage grown and the perennial character of the crop. Chemical control is not feasible because it is environmentally undesirable or economically unsound. Crop rotations do not reduce many alfalfa pathogen populations, especially the persistent pathogens or those that have a broad host range. Resistance to several foliar pathogens in alfalfa is currently needed. New resistance genes, development of broad-spectrum biological control agents, or application of an inducible disease defense system to control alfalfa diseases would enhance benefits derived from genetic resistance, chemical

treatments, and cultural control methods. New sources of disease-resistance genes should result from screening diverse sources of annual *Medicago* spp. and *M. sativa* germ plasm, and represents the best means of reducing losses. The reliance on present control measures, especially plant breeding, could be reduced by identifying new plant defense mechanisms, and facilitate the development of genetically engineered plants with broad-spectrum disease resistance.

Detailed biochemical and genetic studies are needed to determine whether the resistance to *C. trifolii* observed in different species and accessions of *Medicago* is attributable or related to genes or gene products functioning as receptors to pathogen-induced signals with subsequent elaboration of secondary fungitoxic compounds. We are currently evaluating resistant annual *Medicago* accessions for the presence of a constitutive or inducible defense mechanism as expressed by the production of fungitoxic secondary compounds. Such metabolites or genes may provide new, effective sources of disease resistance to foliar pathogens.

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**Table 2.** Histopathological observations of fungal development in annual medics inoculated with *Colletotrichum trifolii*

<i>Medicago</i> sp. and accession <sup>b</sup>	Resistance (%) <sup>c</sup>	Fungal development <sup>a</sup>		
		24 h	48 h	72 h
<i>M. murex</i> PI 308062	100.00	...	G,A	A,H
<i>M. muricoleptis</i> PI 495401	90.3	G	G,A	G,A
<i>M. polymorpha</i> var. <i>brevispina</i> PI 197530	0.0	...	G	A,P,H
PI 197340	88.1	G,A	A,P	A,P
PI 385017	87.5	G,A	A	A,P,H
<i>M. polymorpha</i> var. <i>polymorpha</i> PI 206695	0.0	G	A	A,H,S
PI 253448	62.0	G,A	A	A,P,H
PI 286534	67.1	G,A	G,A	G,A
<i>M. radiata</i> PI 459146	64.3	...	G	G,A
<i>M. soleirolia</i> PI 537243	97.4	G	G	A,H,S
<i>M. truncatula</i> PI 566889	19.6 <sup>d</sup>	G	G,A,H	A,P,H,S
PI 566887	87.2	G,A	A,P	A,P
PI 566888	95.6	G,A	A	A,P
PI 292436	97.6	G,A	A,P	A,P
PI 384648	67.4	G,A	A	A
<i>M. turbinata</i> PI 566894	28.7 <sup>d</sup>	G	G,A	A,P,H,S
PI 566893	80.0	G,A	A	A
PI 441943	0.0	G,A	A,P,H	A,P,H,C
<i>M. sativa</i> <sup>e</sup> Arc	66.0	G,A	A,P	A,P
Saranac <sup>d</sup>	0.0	G,A	A,P,H	A,P,H,C

<sup>a</sup> Development of *C. trifolii* spores on leaf tissue 24, 48, and 72 h after inoculation. Letters indicate more than 50% of spores per sample exhibited the event. Events are: G = germinated spore, A = mature appressoria, P = germ pore, H = primary hyphae, S = secondary hyphae, C = tissue collapse.

<sup>b</sup> Six-digit numbers are plant introduction (PI) numbers used by the National Plant Germ Plasm System of the United States Department of Agriculture.

<sup>c</sup> Plants were evaluated for percent survival 10 days after inoculation with *C. trifolii*. Least significant difference among accessions in the annual genus *Medicago* core collection ( $P = 0.05$ ) = 13.7 (arcsine transformed).

<sup>d</sup> Observations were taken on susceptible seedlings of this accession.

<sup>e</sup> Arc and Saranac are perennial *M. sativa* anthracnose reference cultivars. Arc carries the An<sub>1</sub> gene for resistance to race 1 anthracnose. Saranac is a susceptible cultivar.

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