Pathogenic Variability and Host Resistance in the *Colletotrichum trifolii/Medicago sativa* Pathosystem

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ABSTRACT

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Two hundred and fifty alfalfa cultivars currently grown in the U.S. were evaluated for their reaction to anthracnose caused by race 2 of *Colletotrichum trifolii.* Resistance was present in 30 cultivars, with seedling survival ranging from 20 to 68%. Three unusually highly virulent isolates of *Colletotrichum* collected from three locations were identified to species and race by morphological and cultural characteristics, and by reactions on host differential cvs. Arc, Saranac, and Saranac AR. Isolates Arl-NW and SB-2 were characterized as *C. trifolii* isolates of races 1 and 2, respectively. The morphology of isolate 57RR was distinct from that of *C. trifolii* and *C. destructivum,* and most closely conformed to the composite group *C. gloeosporioides.* Resistance to these isolates was evaluated in 40 selected and race differential cultivars. Resistance to isolate Arl-NW was highly variable, with seedling survival ranging from 0 to 77.8%. The isolate was classified as race 1 based on reactions with standard check cultivars. Isolate 57RR was highly virulent to anthracnose-resistant alfalfa clones and cultivars in needle inoculation assays, and was highly virulent to Saranac AR, Arc, and several race 2 resistant cultivars in seedling tests. *Colletotrichum gloeosporioides* is reported as a new pathogen of alfalfa and should be considered a potential disease problem. The variability of resistance reactions in commercially grown alfalfa cultivars to diverse isolates of *Colletotrichum* suggests that these cultivars will be useful sources for different types of anthracnose resistance.

Alfalfa (*Medicago sativa* L.) anthracnose caused by *Colletotrichum trifolii* Bain & Essary causes a foliar, stem, and crown disease. *C. trifolii* exists as two physiological races which exhibit distinct reactions on differential alfalfa cultivars (5,8,13,14). This disease decreases plant vigor and reduces growth and forage yield (3,6). Resistant cultivars developed by multiple cycles of phenotypic selection for anthracnose resistance have been used to reduce losses caused by this disease (8). Race 1 is present wherever alfalfa is grown, and race 2 has been reported from only Maryland, Virginia, and North Carolina (18,25,26). In addition to cultural differences, considerable variation in aggressiveness has been found among isolates within a race (10,14,27).

In a study with other species of *Colletotrichum* from alfalfa in the mid-Atlantic states, Graham et al. (10) found that isolates of *C. destructivum* O'Gara and *C. dematium* (Pers.) Grove f. *truncata* (Schw.) v. Arx. were less pathogenic to alfalfa than *C. trifolii.* In Ontario, anthracnose is caused primarily by *C. destructivum* (5). Graham et al. (10) isolated a different *Colletotrichum* sp. from infected alfalfa plants growing in a greenhouse and this isolate was pathogenic to cultivars resistant and susceptible to race 1. Conidia from this isolate were intermediate in size between *C. destructivum* and *C. trifolii,* and based on von Arx's classification (23) they placed this isolate in the composite group *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz.

In 1982, three isolates from Oklahoma were designated *C. trifolii* race 3 based on reduced virulence to cultivars susceptible to race 1, and lack of virulence to cv. Arc (1). The authors noted that these isolates appeared different from race 1 in culture. Isolates or herbarium specimens of these fungi are not available for examination, and spore measurements and morphology were not reported to permit confirmation of fungus identification. Examination of photographs of this material suggests that these fungi may have been isolates of *C. destructivum.*

Alfalfa is a cross-pollinated, autotetraploid crop, and cultivars are heterogeneous genetic mixtures with only a percentage of seed carrying gene(s) for resistance. Therefore, assessment of disease resistance in alfalfa cultivars is based on a percentage of the population exhibiting resistance. Because this type of resistance is relative to the amount of inoculum employed in tests, this variable as well as other sources of variation are evaluated by comparison with a specific set of check differential cultivars (13,17). In these cultivars, resistance to races 1 and 2 is conditioned independently by different, single dominant genes, designated An_1 and An_2, respectively. These genes are inherited tetrasomically in cvs. Arc (RS phenotype, indicating the presence of An_1 and lack of An_2), Saranac AR (RR phenotype, indicating presence of An_1 and An_2), and Saranac (SS phenotype, susceptible to both races) (7,16). Approximate expected resistance ranges for percent survival in standard check cultivars inoculated with race 1 are 65 to 70% (Arc), 45% (Saranac AR), and 1% (Saranac) (13).

An evaluation of 33 cultivars for resistance to race 2 was conducted in 1982, and only one source of resistance was found, tracing to Saranac AN 4 and Vernal AN 4 (8,25). An evaluation of alfalfa cultivars currently grown in the U.S. is needed to determine the sources and availability of race 2 resistance. Cultivars with resistance to race 2 may also carry race 1 resistance.

The present investigation was conducted to (i) evaluate alfalfa cultivars currently grown in the U.S. for resistance to race 2 of anthracnose, (ii) assess virulence of highly aggressive isolates on commercial alfalfa cultivars, and (iii) identify the species and race specificity of highly aggressive *Colletotrichum* isolates. Aggressiveness is defined as the relative ability to cause damage to a host without regard to resistance genes (20). It is synonymous with parasitic fitness, and here represents the number of plants killed under a given set of environmental conditions. The term aggressiveness is used in this paper when the resistance phenotype of the alfalfa cultivars (except differential check cultivars) is unknown. Virulence is considered the genetic ability of a pathogen race to overcome a genetically determined host resistance, which is effective against other races of that pathogen and causes a compatible interaction (20). The term relative virulence has been used to describe the number of plants of a known resistance genotype killed by an isolate under a given set of environmental conditions (14).

MATERIALS AND METHODS

Fungal isolates. Cultures of field isolates were obtained and maintained as described earlier (14). Isolates 57RR and 47RR originated from lesions found on stems of alfalfa clones resistant to race 2 growing in the greenhouse at Beltsville,

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MD. (14). They were similar culturally and morphologically, and identified as *C. gloeosporioides*. These isolates were virulent to Arc and Saranac in seedling inoculation tests (14). The isolates were evaluated for virulence to clones of known phenotypes, 4SS, 31SS, 19RS, 16RS, 54RS, 1RR, and 24RR (9) by means of a needle inoculation method (15). Four stems of each clone were inoculated with each isolate and evaluated for symptoms after 6 days.

Earlier observations indicated that cvs. Saranac AR and Arc were highly susceptible to isolate Arl-NW (Craig Grau and Sharie Nygaard, personal communications). In North Carolina tests, race 2 isolate SB-2 was found to be highly virulent on alfalfa cv. Shenandoah, which is highly resistant to races 1 and 2 (T. H. Busbice, personal communication). *Colletotrichum* isolates 57RR, Arl-NW, and SB-2 were identified by classical criteria (21), including cultural characteristics and spore morphology (conidial shape and size, setae, sclerotia, appressoria). Spore measurements were determined for at least 200 spores of each isolate. A Zeiss Universal microscope equipped with an image analysis system (Loats Associates, Inc., Westminster, MD) was used to observe and measure spores.

Resistance of commercial germ plasm to race 2 and highly virulent isolates. Race 2 resistance was evaluated in 250 alfalfa cultivars, which included all of the cultivars currently grown in North America. Standard seedling inoculation and evaluation methods, using race 2 isolate SB-2, were conducted as described previously (13,14). Those cultivars characterized as having some degree of resistance to race 2 were re-tested using *C. trifolii* isolates 57RR and Arl-NW. Saranac, Arc, and Saranac AR were included as differential check cultivars throughout the study, and experiments were conducted twice. (Data from the second experiment are presented.)

RESULTS AND DISCUSSION

Characterization of fungi. Spore morphology and cultural characteristics from 57RR and five other isolates collected from noninoculated alfalfa from the greenhouse appeared to conform most closely with isolates in the composite group *C. gloeosporioides*. The conidia were longer than most *C. trifolii* isolates and smaller than *C. destructivum* (Table 1). Conidia were oblong and germinating spores formed appressoria prior to penetration. Unlike *C. trifolii*, these isolates rarely formed setae in culture. Pinkish spore masses and abundant white mycelia were produced in culture on PDA (potato dextrose agar) and on stem lesions. Cultural appearance was similar to other *C. gloeosporioides* isolates but distinctly different from the cultural characteristics of *C. trifolii* and *C. destructivum*. *Colletotrichum destructivum* produced black subsurface sclerotia in culture, and spores were significantly longer than those of *C. trifolii* and *C. gloeosporioides*. In previous seedling inoculation tests reported by O'Neill et al. (14), isolate 57RR was found to be pathogenic to cvs. Arc (RS phenotype) and Saranac (SS phenotype). Six days after inoculation, all other alfalfa clones (SS, RS, and RR phenotypes) needle-inoculated with 47RR and 57RR had characteristic anthracnose lesions, stem wilting, and leaf chlorosis. Acervuli were evident on most stems, especially on succulent tissue at the edge of lesions.

On the basis of cultural characteristics and spore morphology, race 1 and 2 isolates could not be distinguished from each other or from Arl-NW and SB-2. Spores of Arl-NW were shorter and narrower than spores of races 1 and 2, but within ranges determined for *C. trifolii* isolates (Table 1) (2,11,19,21,23). Arl NW and SB-2 could not be distinguished morphologically or culturally from other *C. trifolii* isolates. Appressoria formation, cultural characteristics, and production of setae were similar.

Unfortunately, authoritative and uniform systems for identification in *Colletotrichum* have not been developed. Species concepts in *Colletotrichum* are very broad, and physiological host specialization continues to be important. Morphological characters derived from growth of isolates in culture are not consistent and cultural conditions have rarely been standardized. Combined with the inherent phenotypic plasticity of individual isolates, authoritative identifications are difficult, especially within the composite species *C. gloeosporioides*.

Resistance of commercial cultivars to races 1 and 2 and high virulence isolates. The aggressiveness of races 1 and 2 of *C. trifolii* is variable. In a study comparing inoculations with 12 race 2 isolates, survival of seedlings in the resistant cv. Saranac AR ranged from 11.7 to 68.0% (14). Aggressiveness among race 1 isolates was less variable, with percent survival in the resistant cv. Arc ranging from 52.3 to 79.2%. Isolates of *C. trifolii* frequently lose aggressiveness and sometimes the capacity to sporulate while in culture (N. R.

Table 1. Host, source, and spore dimensions of straight-spored isolates of *Colletotrichum* pathogenic to alfalfa

Species and isolate designation	Host	Location	Spore measurements (μm)[a]			Source or reference
			Length	Width	Ratio	
C. trifolii	Red clover	...	11.0-13.0	3.0-4.0	...	Bain and Essary (2)
C. trifolii (J371)	Alfalfa	Madison, WI	10.5-12.9	3.5-4.0	...	Tiffany and Gilman (22)
C. trifolii (3017)	Alfalfa	Beltsville, MD	10.5-11.5	3.5-4.0	...	Tiffany and Gilman (22)
C. trifolii (3020)	Alfalfa	Beltsville, MD	10.5-11.5	3.5-4.2	...	Tiffany and Gilman (22
C. trifolii	Sweet clover	Madison, WI	10.5-11.9	3.5-4.0	...	Tiffany and Gilman (22)
C. trifolii (race 1)	Alfalfa	Pennsylvania	6-(11.6)-14	3-(5.5)-7	0.47	Welty (24)
C. trifolii (race 2)	Alfalfa	North Carolina	9-(13.3)-22	4-(5.4)-7	0.41	Welty (24)
C. trifolii (race 1, 2sp2)	Alfalfa	Clarksville, MD	8.6-(10.9)-13.2	3.8-(4.2)-5.4	0.39	O'Neill
C. trifolii (race 1, 3-5)	Alfalfa	Clarksville, MD	8.4-(10.9)-12.6	3.7-(4.6)-5.6	0.42	O'Neill
C. trifolii (race 1, TpRe1)	Red clover	Beltsville, MD	7.8-(9.2)-11.8	3.4-(4.4)-5.4	0.48	O'Neill
C. trifolii (race 1, Vertus Beck)	Alfalfa	Maryland	9.6-(10.3)-11.7	3.2-(4.0)-5.2	0.39	O'Neill
C. trifolii (race 2, SB-1)	Alfalfa	Beltsville, MD	9.2-(11.3)-12.4	3.4-(4.4)-6.1	0.39	O'Neill
C. trifolii (race 2, SB-2)	Alfalfa	Beltsville, MD	7.1-(9.9)-11.0	3.9-(4.6)-6.2	0.46	O'Neill
C. trifolii (race 2, S2-4)	Alfalfa	Cambridge, MD	8.8-(9.6)-12.3	4.2-(5.0)-6.3	0.52	O'Neill
C. trifolii (race 2, H4-2)	Alfalfa	Cambridge, MD	8.2-(9.8)-13.4	3.4-(4.6)-6.0	0.47	O'Neill
C. trifolii (Arl-NW)	Alfalfa	Wisconsin	7.8-(8.3)-9.8	2.9-(3.4)-4.8	0.41	Craig Grau
C. gloeosporioides (C129)	Tomato	Virginia	13.8-(16.9)-18.2	2.2-(3.1)-4.0	0.18	O'Neill
C. gloeosporioides (57RR)	Alfalfa	Beltsville, MD	10.6-(11.9)-13.4	3.2-(4.5)-5.6	0.38	O'Neill
C. trifolii (race 1, SM)	Alfalfa	Wisconsin	10.8-(13.4)-14.6	3.0-(3.1)-5.7	0.23	Craig Grau
C. trifolii (race 1, Mag)	Alfalfa	Wisconsin	10.6-(13.7)-15.2	3.4-(4.8)-6.1	0.35	Craig Grau
C. trifolii (race 1 Wupp)	Alfalfa	Wisconsin	10.8-(13.6)-14.3	3.8-(4.8)-6.3	0.35	Craig Grau
C. destructivum	Red clover	Utah	14-22	3.5-5.5	...	O'Gara (13)
C. destructivum	Alfalfa	Ames, IA	14.0-19.2	3.5-4.5	...	Tiffany and Gilman (22)
C. destructivum (J351)	Alfalfa	Wisconsin	17.5-21.0	3.0-3.5	...	Tiffany and Gilman (22)
C. destructivum	Alfalfa	Wisconsin	14.0-17.5	3.0-3.5	...	Tiffany and Gilman (22)

[a] Minimum–(mean)–maximum value.

O'Neill, unpublished). Occasionally, highly virulent *C. trifolii* isolates are identified when tests with resistant cultivars exhibit severe disease (T. H. Busbice, S. L. Nygaard, C. R. Grau, and L. H. Rhodes, personal communications). One of these isolates, Arl-NW, was characterized as highly pathogenic to race 1 and 2 resistant germ plasm, including the differential check cv. Saranac AR (Sharie Nygaard and Craig Grau, personal communications). Formerly resistant cultivars have been reported to be susceptible to race 2, in particular isolate SB-2 (T. H. Busbice, personal communication).

In 1990, 50 commercial alfalfa cultivars were tested for race 1 and race 2 resistance. Most cultivars were resistant to race

Table 2. Evaluation of resistance in commercial cultivars of *Medicago sativa* to *Colletotrichum trifolii* race 2

Cultivar No.	Cultivar Name	Experimental designation	NAVRB year passed[a]	No. plants tested	Resistance (% survival)[b]
1	Elevation	LL 3110 LL3110A	1984	215	6.02
2	AgriBOSS	LL 3387 RS-3387	1987	179	17.05
3	VS 663			174	6.16
4	GH 747	LL/RS 3510	1988	220	8.73
5	VS 872			208	7.18
6	Champ	LL 3309 RS-3309	1987	153	0.76
7	120	LL 159	1978	134	8.76
8	Blazer	P455 & P455A	1978	187	7.82
9	VIP	LL/RS 7890	1988	196	8.07
10	Sparta	LL 3018	1984	205	0.00
11	Cimarron			192	9.35
12	Cimarron VR		1989	262	31.60
13	Belmont			83	30.27
14	Shenandoah	Shen L980		188	59.21
15	5333			218	25.62
16	5331	XAR 53 et al.	1987	247	14.20
17	5373	XAM73 et al.	1989	237	1.80
18	5364	XAR64 et al.	1988	223	1.78
19	555	PZ-2 & 75Z-1	1979	137	1.37
20	531	MB-2	1977	219	28.71
21	532	75 Y-1	1979	269	20.02
22	5444	XAR21,80V-1, YAR21,UV-L	1984	214	8.21
23	5432	XAR 32	1985	270	2.69
24	5472	XAL 72 et al.	1988	216	2.69
25	526	76E-1	1981	247	0.00
26	572	72N-1 & MN	1975	121	0.83
27	5262	XAF62 et al.	1988	239	4.29
28	581	74X-1 & DX	1977	182	0.00
29	545	PF-1 & 540	1977	218	0.75
30	5683	XAS61,YAS61, et al.	1989	199	2.55
31	5929	XAN21,80MNQ-1,UMNQ-1	1983	124	4.75
32	5311			191	11.05
33	Anstar	Syn 7905 MPDR-III	1985	206	19.41
34	Haymark al			123	39.14
35	Hi-Phy	Syn DH	1978	202	10.69
36	Resistar			182	5.02
37	WAMPR	85WAMPR	1988	180	5.71
38	Ok 49			173	1.70
39	Ok 08			177	0.66
40	Alfagraz	GA-GC	1989	184	1.11
41	Cayuga	N.Y.B.	1962	22	0.00
42	Honeoye	SYN L N.Y.	1975	195	0.00
43	Ht55	Reselect Saranac	1985	205	0.00
44	Iroquois	WRN N.Y.	1966	204	0.48
45	Mark II	HSN N.Y.	1965	162	0.00
46	Mohawk	Iroquois AR	1985	182	20.85
47	Multileaf	ML N.Y.	1980	184	0.00
48	Oneida	IROQUOIS PR & SYN I	1980	176	0.00
49	Saranac	WRF N.Y.	1963	67	1.19
50	Saranac AR	Anth ResSaranac	1975	218	45.69
51	Majestic			186	0.74
52	Oneida VR	NY8301,NY OV	1986	229	0.00
53	Pinnacle			221	7.57
54	Victory			225	15.13
55	Deseret	U5045 & KAYSERI	1974	231	0.00
56	Uinta	U SYN C	1962	239	0.00
57	Renegade	Wi & AGR-72	1989	172	0.63
58	Ladak 65	WR LADAK	1964	245	0.00
59	5715			181	0.00
60	5888			165	1.00

(*continued on next page*)

[a] Year the cultivar was approved by the National Alfalfa Variety Review Board.
[b] Numbers indicate percent seedling survival of cultivars inoculated with race 2 isolate SB-2. Least significant difference ($P = 0.05$) = 8%.
[c] No release date. Seed was not available.
[d] Standard check cultivars.

1; however, only three had some resistance to race 2 (N. R. O'Neill, unpublished). In the current study, 40 of 250 cultivars tested exhibited some degree of resistance to race 2 (Table 2). The most resistant cultivars were WL225 (68.0%), Shenandoah (59.2%), Condor (55.6%), Promise (47.7%), Atlas (41.4%), and Saranac AR (55.5%). The Saranac AR seed lot received with the other cultivars (#50) exhibited 45.7% resistance. The standard check cvs. Saranac AR, Arc, and Saranac exhibited percent survivals expected from a race 2 inoculation, indicating that the test should give a valid indication of genetic resistance to race 2 in the other cultivars (13,14). Isolate SB-2 was not exceptionally virulent to Shenandoah in standardized greenhouse tests (Table 2).

When virulence of isolates Arl-NW was evaluated against the standard differential

Table 2. (*continued from preceding page*)

Cultivar No.	Name	Experimental designation	NAVRB year passed[a]	No. plants tested	Resistance (% survival)[b]
61	Echo	83-10	1988	179	3.32
62	Royalty	86-142 (WL-)Cargill Exp420	1988	225	7.76
63	Af 31			222	2.08
64	Af21			199	4.99
65	Allegiance	857(Uagrs)ALLIANCE 84-27 W-L	1987	174	9.01
66	Break-thru	84-16 & CFS Exp 1000 W-L	1987	142	17.98
67	Eagle	CA 760	1983	143	12.35
68	Empress	85-141	1990	117	13.03
69	New Era 90	84-26/85-135	1988	195	1.36
70	Premier	84T34	1988	180	3.39
71	Pro-Cut	84-14 W-L	1987	150	30.71
72	Promise	84-25/85-136	1988	231	47.67
73	Thrive	86-124 W-L	1990	256	34.27
74	WL 222	Ca 838, GH715	1986	191	41.82
75	WL 225	84-11	1987	226	68.01
76	WL 316	78 T 4	1981	145	50.21
77	WL 317	85-126	1988	180	23.23
78	WL 320	CA 7931-32	1983	151	9.51
79	WL 516	B-10	1985	155	28.57
80	WL 605	B 57	1985	176	17.49
81	WL Southern Special	77T2	1982	165	29.60
82	Quest			132	3.25
83	WL 322 HQ			132	1.00
84	77-8 CaB	77-8-CAB	1980	156	0.00
85	86-295	86-295	1989	205	0.00
86	Allstar	84-19 W-L	1987	191	0.00
87	Chief	S-39-84 & 84-24	1987	215	1.55
88	Crusader	82-5	1986	220	1.87
89	GH 737	S-W9 W-L	1985	132	9.38
90	Kingstar	83-3	1986	187	9.38
91	WL 457	86-215	1989	186	5.80
92	WL 515	73 CA A & 73 CA A-2	1981	154	7.79
93	WL 610			159	5.65
94	82 CaB	82 CaB	1989	161	1.13
95	82T46	82 T46	1989	157	14.24
96	83T63	83T63	1989	216	1.36
97	Commandor	NK 82503	1985	177	6.79
98	Condor	83585	1986	180	55.58
99	Crockett			188	2.57
100	Drummor	80335	1983	188	0.00
101	Fortress	83632 N-K	1987	178	21.54
102	Maxidor	K7-706 & PCC-77-127	1978	147	11.92
103	Meteor	83580	1986	195	1.15
104	Pierce	79176	1982	149	0.00
105	Pike	78115 & PCC-77-122	1981	142	0.00
106	Spredor II	K6-11	1980	186	0.00
107	Sundor	83587	1986	183	0.00
108	Trumpetor	78015	1981	239	6.81
109	Vancor	K7-29	1980	183	0.45
110	Arrow	NAPB 20	1985	166	8.06
111	Admiral	NAPB 110	1985	175	11.31
112	Answer			155	0.69
113	AP8623B			270	11.94
114	ABI700			208	0.40
115	Archer	AP 8640	1988	180	1.58
116	Aggressor	AP 8743	1989	191	2.40
117	Apollo Supreme	NAPB 32	1986	182	1.11
118	Advantage	NAPB 87	1981	131	0.00
119	Atlas	NAPB 41	1976	141	41.42
120	Apollo II	NAPB 109	1981	174	5.41
121	Anchor	RP 38	1971	152	3.65
122	Armor	NAPB 89	1981	131	7.14
123	Apollo	NAPB 44	1975	171	1.42

(*continued on next page*)

check cultivars, the percent survival in these cultivars indicated that Arl-NW was a highly aggressive race 1 isolate of *Colletotrichum trifolii* (Table 3). Survival rate of Saranac, which has little or no resistance to either race, was low. Survival rate of Saranac AR (57.3%), a race 1 and 2 anthracnose resistance selection, was similar to that obtained with SB-2 (55.51%) and with other race 2 evaluations with Saranac AR (13,14,17). Survival rate in Arc (54.2%) was somewhat lower than the expected 65 to 75% obtained with typical race 1 inoculations with this cultivar. Typical race 2 isolates permit less than 5% survival in Arc (14). These results indicate that Arl-NW is an aggressive race 1 isolate.

Table 2. (*continued from preceding page*)

Cultivar No.	Cultivar Name	Experimental designation	NAVRB year passed[a]	No. plants tested	Resistance (% survival)[b]
124	Baron	NAPB 92	1982	139	0.83
125	Cutter	AP 8742	1989	217	0.00
126	Comet	AP 8322	1988	141	0.00
127	Citation	RP 103	1974	197	2.01
128	Clipper	NAPB 31	1986	200	1.96
129	Diamond	NAPB 27	1985	198	2.78
130	Dart	NAPB 22	1985	203	1.83
131	Duke	NAPB 86	1981	177	1.46
132	Endure	NAPB 108	1983	217	1.89
133	Envy	AP 41	1988	164	3.66
134	Expo	NAPB 74	1981	141	0.00
135	Gt. 58	IH 135	1986	170	0.53
136	Granada	NAPB 91	1982	163	11.43
137	Garst 645			198	8.54
138	Garst 636	NAPB 24	1985	170	8.05
139	GT 13R plus	ND 124	1986	239	7.81
140	G 7730	NAPB 73	1980	212	0.00
141	Impact	NAPB 26A	1985	201	1.54
142	Maverick	NAPB 53	1981	146	0.61
143	Mercury	NAPB 93	1981	144	2.03
144	Nordic			153	31.10
145	Profit	NAPB 21	1985	188	0.00
146	StarMaster			188	3.06
147	Surpass	NAPB 23	1985	193	0.68
148	Sapphire	NAPB 29	1985	159	1.14
149	Thunder	NAPB 90	1981	143	9.15
150	Trident	NAPB 61	1978	119	6.60
151	Tridant II	AP 8610B	1989	235	0.44
152	Voris A-77	NAPB 51 & FAME	1978	163	0.00
153	Buffalo	Kansas AES	1943	126	1.92
154	Cody	Kansas AES	1959	124	0.71
155	Kanza	KS 12	1968	177	2.62
156	Riley	KS 43	1977	173	3.95
157	Anik	Canada	1975	400	0.30
158	Barrier	Canada		211	5.28
159	Beaver	Canada	1961	198	0.57
160	Drylander	Canada	1971	200	0.99
161	Grimm	Ag Canada	1900	150	0.00
162	Heinrichs	Canada		231	0.00
163	Peace	Canada		165	11.11
164	Rambler	Canada	1955	169	4.87
165	Rangelander			195	11.07
166	Roamer	Canada	1966	191	0.00
167	Trek	Canada	1975	245	0.40
168	AC Caribou	Canada	1975	193	0.00
169	Advance	Canada	1966	191	1.58
170	Algonquin	Canada	1974	137	0.00
171	Alouette	Canada		198	2.14
172	Angus	Canada	1974	164	0.00
173	Apica	Canada		227	6.96
174	Comsel	Canada		177	5.69
175	Hunter[c]	Canada			
176	OAC	Minto	Canada	182	0.00
177	Olinda	Canada		104	0.00
178	Riel	Canada		206	8.78
179	98	C/W 540 VS-540	1988	227	0.00
180	2833	VS-533	1988	155	0.61
181	2980	VS-635	1988	126	0.00
182	Asset	VS-655	1988	225	30.06
183	Bronco	VS-627	1988	119	32.24
184	Blazer XL			147	1.39
185	Centurion	C/W 349, Agway exp 12	1985	244	1.79
186	Crown	C/W 339, Exp 12	1985	191	0.00
187	Crown II	VS-545 RS-545 Exp 545	1988	218	0.00
188	DK 122	C/W-623, VS-623,	1989	77	3.57

(*continued on next page*)

When 40 commercial cultivars selected for race 2 resistance were evaluated for susceptibility to Arl-NW and compared with results from inoculation with a race 2 isolate, a race designation for isolate Arl-NW became more problematic. About half of the cultivars exhibiting some resistance to race 2 were more susceptible to Arl-NW. Among these are Condor, WL Southern Special, WL 222, Garst 630, WL 516, WL 225, and Thorobred. About half the cultivars were more resistant to Arl-NW than to the race 2 isolate, a reaction typical of *C. trifolii* race 1. These results suggest that race 2 resistance may be more complex in cultivars other than Saranac AR, in which resistance is attributed to a single gene.

Table 2. (*continued from preceding page*)

Cultivar No.	Name	Experimental designation	NAVRB year passed[a]	No. plants tested	Resistance (% survival)[b]
189	DK 125	C/W 327, DK 2000, DKExp	1985	130	0.00
190	DK 189			230	5.76
191	Express			169	4.46
192	G2841	C/W 541 VS 541	1987	225	0.00
193	G2852	C/W 252	1986	185	0.00
194	Legacy			164	3.07
195	Legend	C/W 464 VS-464	1987	136	21.42
196	Mede			191	24.18
197	Multi-plier	VS-622	1988	157	2.95
198	Precedent			179	0.00
199	PROCUT 2	VS-639	1988	123	2.27
200	Sure	C/W 341	1985	143	6.12
201	Ultimate	VS-531	1987	215	0.00
202	Zenith			142	0.00
203	Armona	83C65/84C72	1986	222	8.27
204	Arroyo	83B36	1987	116	0.00
205	Bell Ringer	ICB-31	1985	152	2.14
206	High Prairie			230	0.00
207	Husky	ICO-16	1984	148	0.00
208	Inca	LS 79-1,4	1985	219	0.00
209	Madera	83C61/84C70	1986	173	12.53
210	Maricopa	83C62/84C71	1986	220	20.05
211	Mecca	83B24/84B23	1987	255	0.00
212	Milkmaker	ICO-2	1984	150	4.21
213	Sutter	83C63/84C69	1987	181	0.00
214	Thorobred	ICO-3	1985	211	28.09
215	Vortex	83B27	1987	205	0.00
216	Yolo	83C64/84D91	1986	186	1.63
217	84B36	84B36	1988	171	1.62
218	84B47/8533	84B47/8533C	1988	138	0.00
219	84S37/8548N	84S37/8548N	1988	216	0.45
220	Florida 77	FLORIDA 66A	1979	105	0.00
221	African	Egypt	1924	169	0.00
222	Agate	MNP-A2	1972	183	0.00
223	Alta	Sweden	1955	223	7.34
224	Baker	N.S. 68	1976	218	0.00
225	Cossack	Russia	1907	120	0.00
226	Dawson	N.S. 27	1966	179	10.77
227	DuPuits	N-K & Tourneur Freres	1947	210	13.88
228	Grimm (Mn)	Germany (Mn Grimm)		184	0.00
229	Indian	Indian	1913	163	0.00
230	Joaquin II	SC-111	1968	184	2.62
231	Narragansett	Rhode Island AES	1946	234	2.08
232	Nitro	AMN UC X Swm NOVA mn5887	1985	177	0.00
233	Nomad	Burlingham	1941	223	2.16
234	Norseman	NORSEMAN EXP A	1964	107	0.00
235	Perry	N.S. 82	1979	173	0.00
236	Ramsey	MN SYN N	1972	199	0.00
237	Ranger	Nebraska AES	1942	197	0.00
238	Wrangler	N.S. 79 P2	1984	215	1.74
239	Magnum	DS 7406 & DS-2 & MLM 2015	1979	177	11.44
240	Magnum plus	DS 305	1984	218	15.21
241	Magnum III	DS 503	1986	185	0.00
242	Jade	MSB2822	1989	202	0.00
243	Crystal	MSB2821	1990	156	0.00
244	Alpine	DS531, BioPlaRes	1988	209	6.24
245	Voyager	DS 512	1987	130	3.88
246	RamRod	DS 504	1988	184	1.67
247	Target II	DS702	1988	95	18.35
248	Patriot	DS707	1989	166	15.14
249	Good As Gold	MSB2946	1989	230	0.00
250	Garst 630	DS 309	1985	166	44.41
	Arc[d]	MSHP6F-AN4W4	1973	249	3.02
	Saranac[d]	WRF N.Y.	1963	912	0.21
	Saranac AR[d]	Anth Res Saranac	1975	674	55.51

Isolate Arl-NW was more virulent to Saranac AR and Arc (less than 5% seedling survival) when it was first isolated (Craig Grau, personal communication). It is not unlikely that Arl-NW has lost some of its aggressiveness by annual transfers over the last 6 to 7 years. Because of the generally recognized problem of loss of aggressiveness in *C. trifolii* cultures over time, it is recommended that breeders use mixtures of isolates of one race for anthracnose resistance evaluations.

Seedling survival in tests with isolate 57RR was very high (Table 3). This isolate exhibited low virulence to most of the 40 cultivars tested. Standard check cvs. Arc and Saranac AR, and cv. Dawson, however, were highly susceptible. In seedling evaluations conducted with 57RR shortly after it was placed in culture, it was even more virulent (14). The pathogen's origin is unknown, but it probably came from alfalfa seed or plants brought into the greenhouse. It is not likely to be an artifact from other experiments because, to our knowledge, no research studies with isolates of this species have ever been conducted in these greenhouses.

Table 3. Virulence of isolates Arl-NW and 57RR to Alfalfa differential check cultivars and cultivars selected for resistance to *Colletotrichum. trifolii* race 2

Cultivar		*C. trifolii* (Arl-NW)		*C. gloeosporioides* (57RR)	
No.	Name	No. seedlings tested	Resistance[a] (% survival)	No. seedlings tested	Resistance[b] (% survival)
80	WL 605	143	0.00 (17.49)[c]	173	86.71
81	WL Sou.Special	148	0.00 (29.60)	191	86.91
95	82T46	149	0.00 (14.24)	177	96.61
98	Condor	139	0.00 (55.58)	120	38.33
102	Maxidor	153	0.00 (11.92)	193	27.98
209	Madera	181	0.00 (12.53)	153	26.80
210	Maricopa	151	0.00 (12.05)	203	88.18
214	Thorobred	152	0.00 (28.09)	217	91.24
79	WL 516	111	1.73 (28.57)	164	46.34
74	WL 222	154	2.33 (41.82)	197	56.85
21	532	165	2.34 (20.02)	199	45.73
136	Granada	128	2.43 (11.43)	174	92.53
165	Rangelander	151	2.72 (11.00)	185	87.03
20	531	187	3.20 (10.77)	189	93.12
226	Dawson	163	3.25 (10.77)	176	9.09
32	5311	187	3.26 (11.05)	216	87.50
247	Target II	145	6.74 (18.35)	137	18.98
250	Garst 630	183	12.56 (44.41)	208	64.90
163	Peace	167	13.86 (11.11)	204	32.35
77	WL 317	151	15.15 (23.23)	156	44.23
33	Anstar	141	15.20 (19.41)	155	83.24
248	Patriot	184	15.66 (15.14)	222	88.29
71	Pro-Cut	157	15.73 (31.71)	202	85.64
67	Eagle	154	15.73 (12.35)	195	60.51
240	Magnum plus	184	16.10 (15.21)	126	94.44
239	Magnum	189	22.14 (11.44)	201	69.65
101	Fortress	174	26.68 (21.54)	222	84.68
68	Empress	153	27.36 (13.03)	172	81.98
183	Bronco	108	31.27 (32.24)	130	65.38
72	Promise	203	34.35 (47.67)	199	72.86
182	Asset	151	34.48 (30.06)	179	60.89
75	WL 225	150	37.81 (68.01)	200	51.00
76	WL 316	164	41.57 (50.21)	186	67.20
119	Atlas	128	43.98 (41.42)	159	88.05
73	Thrive	160	49.13 (34.27)	174	64.94
54	Victory	169	49.14 (15.13)	191	80.63
34	Haymark al	169	50.74 (39.14)	194	71.65
14	Shenandoah	167	51.78 (59.21)	185	78.92
195	Legend	75	51.86 (21.42)	77	77.92
13	Belmont	113	57.77 (30.27)	153	71.90
144	Nordic	147	58.25 (31.10)	161	28.57
196	Mede	142	67.72 (24.18)	152	24.34
50	Saranac AR	189	68.69 (45.69)	225	14.22
15	5333	140	77.18 (25.62)	145	70.34
2	AgriBOSS	138	77.80 (17.05)	200	89.00
	Arc[d]	103	54.16 (3.02)	144	0.00
	Saranac[d]	128	0.72 (0.72)	178	51.69
	Saranac AR[d]	127	57.29 (55.51)	166	5.54

[a] Number given is the mean of four replicates. Least significant difference ($P = 0.05$) = 12.47 for cultivars in a column inoculated with isolate Arl-NW.

[b] Number given is the mean of four replicates. Least significant difference($P = 0.05$) = 14.34 for cultivars in a column inoculated with isolate 57RR.

[c] Resistance (percent survival) in cultivars inoculated with race 2 isolate SB-2, from Table 2. Percent survival in standard check cultivars is typical for inoculatons with race 2.

[d] Standard check cultivars.

New races are characterized when a pathogen becomes virulent to a differential set of cultivars with specific gene(s) for resistance. Race 1 and race 2 resistance in check cvs. Arc and Saranac AR, respectively, was effective against isolates Arl-NW and SB-2, respectively. These data support the conclusion that highly aggressive *C. trifolii* isolates Arl-NW and SB-2 are physiologically specialized for races 1 and 2, respectively (18,25). Isolate 57RR of *C. gloeosporioides* was highly pathogenic to cvs. Arc and Saranac AR. Although this pathogen is reported from the greenhouse and has not been reported in nature, its pathogenicity to standard check cultivars suggests that *C. gloeosporioides* should be considered a potential disease problem in alfalfa. The symptoms produced on alfalfa are identical to those produced by *C. trifolii*, so that the presence of this pathogen in the field could easily be mistaken for anthracnose caused by *C. trifolii.* Morphological and cultural characteristics are also not easily distinguishable. In cases of unusual outbreaks of anthracnose on resistant cultivars, breeders and growers should obtain definitive identification of the causal agent. A culture of *C. gloeosporioides* isolate 57RR has been deposited at the ATCC, Rockville, MD.

Thirty of the approximately 250 cultivars currently grown in the U.S. exhibit at least 20% resistance to race 2 anthracnose. This degree of resistance is sufficient to cause significant reductions in anthracnose severity in those cultivars in the field. These cultivars may represent a diverse source for race 2 resistant germ plasm because it is not known whether the resistance exhibited is due to one or many genes. The variation in resistance reactions to different *Colletotrichum* isolates suggests that different resistance mechanisms may be involved. Further investigations are needed to determine the cultural and biochemical parameters causing plasticity in fungal aggressiveness of isolates. Such information will be useful in developing cultivars with stable resistance.

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