Evaluation of *Amaranthus* Accessions for Resistance to Damping-Off by *Pythium myriotylum*

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ABSTRACT

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One hundred twenty-six accessions of species and cultivars of *Amaranthus* were screened for resistance to damping-off by *Pythium myriotylum*. Accessions ranged in resistance from very susceptible (> 100% weighted mortality) in a vegetable cultivar of *A. tricolor* from India to very resistant (1.8% weighted mortality) in *A. tricolor* 'Early Splendor'. Both cultivars and wild species included susceptible and resistant groups. Cluster analysis was used in an attempt to place the accessions into resistance clusters. However, examination of the resulting dendrogram and the results of a ranking procedure indicated that few, if any, natural clusters exist in these data. It was suggested that accessions that exhibited weighted percent mortalities of less than 20% be selected for use in breeding programs to breed in resistance to damping-off by *P. myriotylum*. Promising accessions for breeding programs included 13 cultivars of *A. tricolor*, four cultivars of *A. dubius*, a cultivar of *A. hybridus*, an accession of *A. spinosus*, and an accession of *A. palmeri*.

Several species of *Amaranthus* L. are ancient cultivated crops that have recently been rediscovered by western agriculturalists. Amaranth is a multifunctional crop. It is eaten as both a grain and a vegetable (12), used extensively as an ornamental (4), and grown as a source of red dyes (5). However, *Amaranthus* spp. are best known to American agricultural producers as weeds (7). Amaranths produce reasonable yields in even infertile soils and have few pest problems (2). Damping-off by species of *Pythium* Pringsheim is a problem that plagues amaranth producers and breeders (9,11). A primary research need in amaranth production is controlling damping-off (9). One method to accomplish this is to breed resistance to damping-off by *Pythium* spp. into susceptible production lines.

In screening lines of a plant taxon for resistance to a disease, it is desirable to use some method for allocating the assessed lines into groups according to levels of resistance to that disease. Cluster analysis is a numerical method that has been used extensively in taxonomy to group together related organisms and to indicate their degree of similarity (1). More recently, this method of analysis has been used to group varieties of wheat into clusters with differing levels of susceptibility to *Puccinia striiformis* Westend. (10). The similarities between susceptibility groups can be displayed in the form of a dendrogram (6).

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The objectives of this study were 1) to identify accessions of *Amaranthus* that are resistant to damping-off by *P. myriotylum* Drechsler, and 2) to determine if accessions could be grouped by resistance to damping-off for use in crop breeding programs.

MATERIALS AND METHODS

The 126 accessions of *Amaranthus* and the control line used in this study came from regional and international sources, with the majority from the Rodale Research Center (Box 323, R. D. 1, Kutztown, PA 19530) (Table 1). The strain of *P. myriotylum* used in this study was isolated from a diseased seedling of *Amaranthus cruentus* L. × *hybridus* L. The strain was isolated and maintained on a modified form of Mitchell's CPV agar (streptomycin was used in place of the usual vancomycin because of a chronic gram-negative, rather than gram-positive, bacterial contaminant) (8). Oospores were produced by mycelia of the fungus on oatmeal agar (OA) containing 20 g of Bacto agar and 20 ml of oatmeal infusion per liter of distilled water and incubating for a least 1 wk at 23–26 C in the dark. Free oospores were obtained using a modification of the isolation method of Ruben, Frank, and Chet (11). The final filtration step was deleted because our oospore preparation was quite free of debris without this step. Purified oospores suspended in sterile distilled water were stored at 12 C no longer than 1 wk. A concentration of 1×10^7 oospores per liter was used in the screening process. Preliminary experiments had shown that this oospore concentration resulted in 50% mortality of controls used. Oospores were counted using a standard hemacytometer (8).

Amaranthus accessions were screened in a completely randomized design. Because species of *Amaranthus* outcross readily in the wild there is a potential for genetic variability in any lot of seeds of an accession. To account for this, large samples were used in the screening process. Also, phenotypic variation among the seedlings was observed in less than 3% of the accessions used in this study. They were seeded in 10-cm-square plastic pots, five pots per accession, using Redi-Earth seeding mix (Wolff Wholesale Florists, P.O. Box 330, Waco, TX 76703), and watered daily. On the second day after emergence, the seedlings were thinned to 10 seedlings per pot. On the day following thinning, each seedling was challenged with 0.5 ml of the oospore suspension. The number of dead seedlings in each pot was counted daily for 6 days and were summed for each accession. Controls for each screen consisted of the inoculation of a known susceptible line of *Amaranthus* (Table 1) with the oospore suspension (positive control) or with sterile distilled water (negative control). Screening tests were conducted under ambient conditions. Greenhouse air temperature was measured using a standard thermometer, and incident radiation was measured using a Li-Cor radiometer (Li-Cor, Inc., 4421 Superior St., P.O. Box 4425, Lincoln, NE 68504).

The screening process was divided into three separate units and each unit was replicated twice. Screening was begun on 16 October, 1986, and was completed on 15 June, 1987. One hundred seedlings of each accession were tested. In addition, one each of a susceptible, intermediate, and resistant accession found in units 1 and 2 was included in unit 3 to monitor uniformity of performance of the inoculum and techniques.

Percent mortalities (MORT) were calculated for each accession screened. For additional analyses, a weighted percent mortality (WMT) was calculated for each accession using: $WMT = MORT \cdot [PCM_{MAX})/PCM]$, where WMT is the weighted percent mortality of a particular accession, MORT is the percent mortality of that accession, PCM_{MAX} is the maximum percent mortality of all positive controls, and PCM is the percent mortality of the positive control in that accession's replicate. Weighted percent mortality was used because of the environmentally-mediated variability seen with the positive control. Results were analyzed using the SAS ANOVA and Duncan programs (SAS Institute, Inc., Box 8000, Cary, NC 27511-8000). Attempts were made to cluster the accessions into susceptibility groups

Table 1. Accessions of *Amaranthus* challenged by *Pythium myriotylum*

Species	Code[a]	Cultivar or common name	Origin	Type
1. *A. blitum* L.	BLIINA	...[b]	India	v[c]
2. *A. blitum*	BLIINB	...	India	v
3. *A. blitum*	BLIINC	...	India	v
4. *A. blitum*	BLIIND	...	India	v
5. *A. blitum*	BLIINE	...	India	v
6. *A. blitum*	BLIINF	...	India	v
7. *A. blitum*	BLIHK	...	Hong Kong	v
8. *A. blitum*	BLIUP	...	United Provinces	v
9. *A. blitum*	BLI?[d]	...	?[d]	v
10. *A. caudatus* L.	CA	...	?	g
11. *A. caudatus*	CANE	...	Nepal	g
12. *A. caudatus*	CAPE	...	Peru	g
13. *A. caudatus*	CAUS	Love Lies Bleeding	USA	o
14. *A. caudatus*	CASAM		South America	g
15. *A. caudatus*	CAR	Ramdana	Good Seed Co.	g
16. *A. cruentus*	CRDA	Fotete	Dahomey	g
17. *A. cruentus*	CRTA	...	Taiwan	g
18. *A. cruentus*	CRET	Sierra Leon Spinach	Ethiopia	v, g
19. *A. cruentus*	CRMX	...	Mexico	v, g
20. *A. cruentus*	CRPE	...	Peru	o
21. *A. cruentus*	CRPEB	...	Peru	o
22. *A. cruentus*	CRGUAT	...	Guatamala	g
23. *A. cruentus*	CRAFR	...	Africa	g
24. *A. cruentus*	CRGHA	...	Ghana	g
25. *A. cruentus*	CRGHB	...	Ghana	g
26. *A. cruentus*	CRGHC	...	Ghana	g
27. *A. cruentus*	CRGHD	...	Ghana	g
28. *A. cruentus*	CRTAN	...	Tanzania	g
29. *A. cruentus*	CR?	...	?	g
30. *A. cruentus*	CRNIG	...	Nigeria	g
31. *A. cruentus*	CRNEP	...	Nepal	g
32. *A. cruentus*	CRR	...	Good Seed Co.	g
33. *A. cruentus*	CRSM	San Martin	Good Seed Co.	g
34. *A. cruentus*	CRK	...	Good Seed Co.	g
35. *A. cruentus*	CRGG	Golden Giant	Good Seed Co.	g
36. *A. cruentus*	CRRED	(Red)	?	g
37. *A. cruentus*	CRGR	(Green)	?	g
38. *A. cruentus*	CRXCA	× caudatus	Rodale Research	g
39. *A. dubius*	DUBTA	...	Taiwan	v
40. *A. dubius*	DUBLC	...	Lovango Cay	v
41. *A. dubius*	DUBSE	...	Seychelles	v
42. *A. dubius*	DUBIN	...	India	v
43. *A. dubius*	DUBGH	...	Ghana	v
44. *A. dubius*	DUBZAM	Ibondwe	Zambia	v
45. *A. dubius*	DUB	...	?	?
46. *A. hybridus*	HBUSA	...	USA	g
47. *A. hybridus*	HBUSB	...	USA	o
48. *A. hybridus*	HBUSC	...	USA	g
49. *A. hybridus*	HBA	Smooth pigweed	?	w
50. *A. hybridus*	HBB	Smooth pigweed	?	w
51. *A. hybridus*	HBC	Smooth pigweed	?	w
52. *A. hybridus*	HBBF	Biflorus	?	w(?)[d]
53. *A. hybridus*	HBPE	Sangoranche	Peru	o, i
54. *A. hybridus*	HBECA	Sangoranche	Ecuador	o, i
55. *A. hybridus*	HBECB	...	Ecuador	?
56. *A. hybridus*	HBECC	Sangoranche	Ecuador	o, i
57. *A. hybridus*	HBECD	Sangoranche	Ecuador	o, i
58. *A. hybridus*	HBIN	...	India	g
59. *A. hybridus*	HB?A	Smooth pigweed	?	w
60. *A. hybridus*	HB?B	Smooth pigweed	?	w
61. *A. hybridus*	HB?C	Smooth pigweed	?	w

(continued on next page)

[a] Code names were assigned to accessions for convenience in this study. The first two or three letters refer to the specific epithet and the last portion refers to place of origin, cultivar, or common name.
[b] No cultivar or common name is available.
[c] Symbols: g = grain, i = industrial (dye), o = ornamental, v = vegetable, w = weed.
[d] ? Indicates unknown.

using the UPGMA method of the BIOSTAT II (Biostat, 1430 Shalanwood, Placentia, CA 92670). Ranking was done using the SAS RANK program.

RESULTS AND DISCUSSION

The amount of disease development seen in this study was in large part dependent on the accession of *Amaranthus* tested (Table 2). In an ANOVA procedure making MORT entirely a function of the accession used, the accessions were significantly different (*P*

Table 1. (*continued from preceding page*)

Species	Code[a]	Cultivar or common name	Origin	Type
62. *A. hybridus*	HBDL	Smooth pigweed	Delaware	w
63. *A. hybridus*	HBIL	Smooth pigweed	Illinois	w
64. *A. hybridus*	HBMS	Smooth pigweed	Mississippi	w
65. *A. hybridus*	HBLA	Smooth pigweed	Louisiana	w
66. *A. hybridus*	HBMXA	Smooth pigweed	Mexico	w
67. *A. hybridus*	HBMXB	Quilete	Mexico	v
68. *A. hybridus*	HBGR	Vleta	Greece	v
69. *A. hybridus*	HBVL	Vleta	Greece	v
70. *A. hybridus*	HBTRMD	Smooth pigweed (triazine resistant)	Maryland	w
71. *A. hypochondriacus* L.	HYMX	(spike type)	Mexico	g
72. *A. hypochondriacus*	HYXHB	× hybridus	Rodale Research	g
73. *A. hypochondriacus*	HYXHBB	× hybridus	Rodale Research	g
74. *A. hypochondriacus*	HYBA	Black Alegria	Mexico	g
75. *A. hypochondriacus*	HYM	...	Morales, Mexico	g
76. *A. hypochondriacus*	HYT	...	Tulyehualco, Mexico	g
77. *A. hypochondriacus*	HYDM	...	del Milago, Mexico	g
78. *A. hypochondriacus*	HYA	...	Amecas, Mexico	g
79. *A. palmeri*	PAL	Palmer's pigweed	Texas	w
80. *A. retroflexus* L.	RETTA	Red root pigweed	Taiwan	w
81. *A. retroflexus*	RETJA	Red root pigweed	Jamaica	w
82. *A. retroflexus*	RETCA	Red root pigweed	Canada	w
83. *A. retroflexus*	RETPA	Red root pigweed	Pennsylvania	w
84. *A. retroflexus*	RETUS	Red root pigweed	USA	w
85. *A. retroflexus*	RETUSA	Red root pigweed	USA	w
86. *A. retroflexus*	RETTUR	Red root pigweed	Turkey	w
87. *A. retroflexus*	RET?	Red root pigweed	?	w
88. *A. retroflexus*	RETBER	Red root pigweed	(Bertram) Texas	w
89. *A. spinosus*	SPN	Spiny pigweed	(Shreveport) Louisiana	w
90. *A. tricolor*	TRIUSA	Tampala	USA	v
91. *A. tricolor*	TRIUSB	Summer Poinsettia	USA	o
92. *A. tricolor*	TRIINA	Tiger leaf	India	v
93. *A. tricolor*	TRIINB	(All Red)	India	v
94. *A. tricolor*	TRIINC	...	India	v
95. *A. tricolor*	TRIIND	...	India	v
96. *A. tricolor*	TRIINE	...	India	w
97. *A. tricolor*	TRIINF	...	India	w
98. *A. tricolor*	TRICHRED	Red	China	v
99. *A. tricolor*	TRICH	...	China	v
100. *A. tricolor*	TRICHA	...	China	v
101. *A. tricolor*	TRICHB	...	China	v
102. *A. tricolor*	TRITAI	...	Taiwan	v
103. *A. tricolor*	TRITAIA	Tiger leaf	Taiwan	v
104. *A. tricolor*	TRITAIB	White leaf	Taiwan	v
105. *A. tricolor*	TRITAIC	...	Taiwan	v
106. *A. tricolor*	TRITAID	...	Taiwan	v
107. *A. tricolor*	TRITAIE	...	Taiwan	v
108. *A. tricolor*	TRITAIF	...	Taiwan	v
109. *A. tricolor*	TRITAIG	...	Taiwan	v
110. *A. tricolor*	TRITHA	...	Thailand	v
111. *A. tricolor*	TRITHB	...	Thailand	v
112. *A. tricolor*	TRIPS	Tampala	Park Seed Co.	v
113. *A. tricolor*	TRIIL	Illumination	Park Seed Co.	o
114. *A. tricolor*	TRIES	Early Splendor	Park Seed Co.	o
115. *A. tricolor*	TRIPG	Purple Giant	Good Seed Co.	v
116. *A. tricolor*	TRIRWM	Red & White Mixed	?	o
117. *A. tricolor*	TRIAF	...	Africa	v
118. *A. tricolor*	TRIHK	...	Hong Kong	v
119. *A. tricolor*	TRIINDO	...	Indonesia	w
120. *A. tricolor*	TRIMAL	...	Malaysia	w
121. *A. tricolor*	TRIRED	(Red)	?	v
122. *A. tricolor*	TRITM	Tampala	?	v
123. *A. tricolor*	TRIHC	Hin Choy	?	v
124. *A. tricolor*	TRI?A	...	?	v
125. *A. tricolor*	TRI?B	...	?	?
126. *A. viridis* L.	VIRINDO	...	Indonesia	w
Controls	CRXHB	cruentus × hybridus	Rodale Research	g

Table 2. Weighted percent mortalities (WMT) of *Amaranthus* accessions challenged by *Pythium myriotylum*

Number[a]	Code[b]	WMT[c]	Number[a]	Code[b]	WMT[c]
114	TRIES	1.81	4	BLIIND	41.80
121	TRIRED	1.81	60	HB?B	44.13
117	TRIAF	7.26	49	HBA	44.28
106	TRITAID	9.07	10	CA	45.33
99	TRICH	9.68	90	TRIUSA	45.42
125	TRI?B	11.14	98	TRICHRED	47.19
93	TRIINB	11.21	32	CRR	47.37
89	SPN	12.04	45	DUB	48.61
40	DUBLC	12.71	17	CRTA	49.83
43	DUBGH	13.06	76	HYT	50.92
119	TRIINDO	14.52	30	CRNIG	51.24
120	TRIMAL	14.52	24	CRGHA	51.77
102	TRITAI	14.97	68	HBGR	52.00
41	DUBSE	15.26	31	CRNEP	52.09
110	TRITHA	15.49	91	TRIUSB	52.63
44	DUBZAM	16.33	88	RETBER	52.94
105	TRITAIC	18.15	81	RETJA	53.75
103	TRITAIA	18.37	86	RETTUR	54.42
79	PAL	18.67	3	BLIINC	56.54
69	HBVL	19.97	59	HB?A	56.85
112	TRIPS	20.20	63	HBIL	58.21
122	TRITM	20.46	35	CRGG	58.49
101	TRICHB	20.58	27	CRGHD	58.51
109	TRITAIG	21.43	61	HB?C	60.36
92	TRIINA	21.58	6	BLIINF	60.65
56	HBECC	21.78	51	HBC	60.92
22	CRGUAT	21.78	18	CRET	61.50
54	HBECA	23.47	13	CAUS	61.71
104	TRITAIB	23.83	115	TRIPG	62.46
65	HBLA	24.63	66	HBMXA	63.33
113	TRIIL	26.16	34	CRK	63.33
107	TRITAIE	26.95	46	HBUSA	64.18
62	HBDL	26.98	75	HYM	64.50
97	TRIINF	27.23	37	CRGR	64.81
126	VIRINDO	27.23	84	RETUS	65.34
39	DUBTA	28.66	16	CRDA	66.08
20	CRPE	29.04	9	BLI?	67.81
21	CRPEB	29.04	80	RETTA	68.65
94	TRIINC	29.04	7	BLIHK	69.31
48	HBUSC	29.13	52	HBBF	69.33
67	HBMXB	29.40	36	CRRED	72.13
108	TRITAIF	29.92	29	CR?	72.13
96	TRIINE	29.95	82	RETCA	73.50
100	TRICHA	30.25	1	BLIINA	73.63
123	TRIHC	30.87	50	HBB	75.42
53	HBPE	32.00	70	HBTRMD	75.42
111	TRITHB	33.00	25	CRGHB	76.49
12	CAPE	33.96	77	HYDM	79.17
71	HYMX	34.49	78	HYA	79.77
118	TRIHK	34.61	2	BLIINB	81.25
57	HBECD	35.33	87	RET?	82.29
74	HYBA	35.64	73	HYXHBB	84.16
55	HBECB	36.53	83	RETPA	84.79
124	TRI?A	36.54	26	CRGHC	85.34
47	HBUSB	36.67	14	CASAM	90.75
72	HYXHB	37.07	33	CRSM	91.89
8	BLIUP	37.13	28	CRTAN	95.37
64	HBMS	38.31	85	RETUSA	96.96
11	CANE	38.57	5	BLIINE	97.49
23	CRAFR	38.89	116	TRIRWM	101.90
42	DUBIN	39.55	38	CRXCA	107.41
19	CRMX	39.93	95	TRIIND	122.24
15	CAR	40.01	Controls[d]	CRXHB	
58	HBIN	41.75			

[a]Numbers refer to the species from Table 1.
[b]Code names refer to the codes from Table 1.
[c]WMT are adjustments to account for environmental variations when *Amaranthus* accessions are challenged by *Pythium myriotylum*. (WMT is calculated by multiplying the percent mortality of each accession by a factor found when dividing the maximum observed percent mortality of all positive controls by the percent mortality of the positive control in that accession's replicate.) Amaranth seedlings were inoculated with oospores of *P. myriotylum* before true leaf development and the number of dead seedlings was recorded after 6 days.
[d]Controls' WMTs: positive = 38.42, negative = 0.00.

= 0.0001). However, environmental factors such as duration and intensity of incident radiation and temperature seem to have an influence on the amount of disease development. For example, from mid-December to mid-June, greenhouse temperatures varied from 22 to 39 C, photoperiod from 10.2 to 14.1 hr, and light intensity from 440 $\mu E \cdot m^{-2} \cdot s^{-1}$ to 1,800 $\mu E \cdot m^{-2} \cdot s^{-1}$. Since variations in light intensity and temperature were the only variations observed between screens, such variations may have been the reason why the percent mortality of the positive control ranged from 30 to 52%. Such variability has been reported in other greenhouse studies of resistance to *Pythium*-caused disease (3). This environmentally induced variability required that a weighted percent mortality be used to compare the performance of varieties used in separate units. The utility of WMT is evidenced by the fact that, when using WMT of selected accessions in all three screening units, a Duncan's multiple range test shows no significant differences between screen units (Table 3).

Cluster analysis was applied to the data in an attempt to place the accessions into resistance groups. However, the clusters that resulted generally were highly related to their neighboring clusters, i.e., their dissimilarity quotients were very minute (e.g., 0.213 for cluster number=3) (Fig. 1). A ranking procedure further indicated that the accessions generally do not cluster into naturally distinct groups, but rather fall into a continuum of very susceptible to very resistant (Fig. 2). Figure 3 is a diagram of the accessions used displayed in a histogram to demonstrate the frequency in 10% interval mortality groups. Here, the only trend in concentration of number of accessions toward a particular mortality level is a tendency for accessions to exhibit intermediate levels of mortality to damping-off than to exhibit either extreme mortality level.

Table 3. Duncan's multiple range groupings of six accessions[a] of *Amaranthus* when challenged by *Pythium myriotylum* throughout the three screening units

Screen	Weighted percent mortality[b]	Screen number
A	2.01	1
A	1.99	2
A	1.93	3

[a]The accessions used were: 79 and 121—resistant; 39 and 63—intermediate; 83 and 116—susceptible. Numbers refer to the same designations used in Table 1.
[b]Percent mortality of each accession multiplied by a factor of the maximum percent mortality of positive controls divided by the percent mortality of the positive control in that accession's replicate.

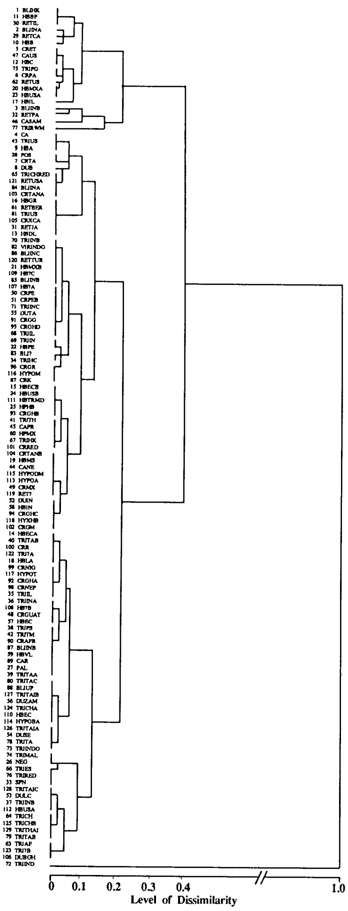

Fig. 1. Dendrogram depicting groupings among accessions of *Amaranth* evaluated by a hierarchical cluster analysis for weighted percent mortality when challenged by *Pythium myriotylum.* Weighted percent is calculated by multiplying the percent mortality of each accession by a factor found by dividing the maximum observed percent mortality of all positive controls by the percent mortality of the positive control in that accession's replicate.

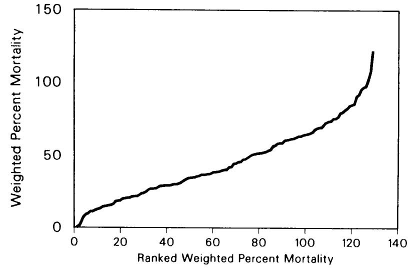

Fig. 2. Weighted percent mortality of *Amaranthus* accessions challenged by *Pythium myriotylum* plotted against ranked weighted percent mortality. Weighted percent is calculated by multiplying the percent mortality of each accession by a factor found by dividing the maximum observed percent mortality of all positive controls by the percent mortality of the positive control in that accession's replicate.

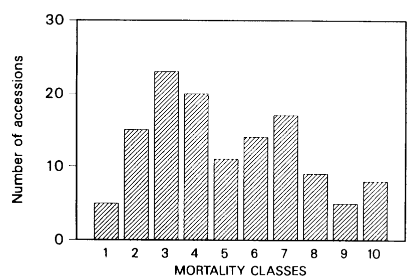

Fig. 3. Frequencies of *Amaranthus* accessions challenged by *Pythium myriotylum* in mortality classes. Numbers of *Amaranthus* accessions that fall into 10% interval mortality classes are plotted against these mortality classes. Mortality classes are: 1 = <10% mortality, 2 = ≥10% and <20%, 3 = ≥20% and <30%, 4 = ≥30% and <40%, 5 = ≥40% and <50%, 6 = ≥50% and <60%, 7 = ≥60% and <70%, 8 = ≥70% and <80%, 9 = ≥80% and <90%, and 10 = ≥90% mortality.

In a breeding program as new as that for amaranths, maintenance of a diverse gene pool is, of course, desirable. Therefore, we recommend that breeders would find it useful to include accessions that exhibit less than 20% mortality in breeding programs. These include several lines with divergent biochemical, growth, and use characteristics, but all with relatively high levels of resistance to damping-off. The recommended lines are 13 cultivars of *A. tricolor* L. (nine vegetable and one ornamental cultivar, two weed accessions, and one accession of undetermined use), four vegetable cultivars of *A. dubius* Mart. ex Thell., a vegetable cultivar of *A. hybridus* L., a weed accession of *A. spinosus* L., and a weed accession of *A. palmeri* Wats. Since *Amaranthus* spp. exhibit a great tendency for interspecific hybridization (9), one, or all, of these recommended lines might be included in most breeding programs aimed at decreasing losses from damping-off and increasing production.

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