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Screening for Resistance to Leaf Spot Diseases of Spinach

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***Abstract*. The entire U.S. Department of Agriculture (USDA) spinach (*Spinacia oleracea* L.) germplasm collection (338 accessions) and 22 commercial cultivars were evaluated for resistance to leaf spot caused by *Stemphylium botryosum* in a greenhouse trial with two replications in 2004. The resistant and susceptible accessions identified as well as the 22 commercial cultivars were included in a second test in 2005 with four replications to confirm the results. No genotype was completely resistant (immune) to the disease. However, there were significant differences in disease incidence (percent of plants with leaf spot) and severity (percent diseased leaf area) among the genotypes tested. Two accessions from Turkey, PI 169685 and PI 173809, consistently had low disease incidence and severity ratings. Two *Spinacia tetrandra* and four *Spinacia turkestanica* accessions screened in these public germplasm tests were all susceptible. None of the commercial cultivars tested consistently had low disease incidence or severity. There was no significant correlation between disease incidence/severity and leaf type (smooth, semisavoy, or savoy). In addition to the public germplasm evaluated, 138 proprietary spinach genotypes (breeding lines and cultivars) were obtained from seed companies and screened along with 10 accessions from the USDA germplasm collection for resistance to Stemphylium leaf spot and Cladosporium leaf spot (caused by *Cladosporium variabile*) in a greenhouse in both 2004 and 2005. Significant differences in severity of leaf spot were observed among the genotypes for both diseases. For each disease, there was a significant positive correlation in severity ratings of the genotypes between the 2004 and 2005 trials. Information on the relative resistance (or susceptibility) of the spinach germplasm evaluated in this study should be useful for plant breeders to develop leaf spot-resistant cultivars.**

Spinach is an important leafy vegetable crop with ≈22,000 ha grown in the United States for both fresh and processed markets at a crop value of $215 million (National Agricultural Statistics Service, 2007). Approximately 80% of U.S. spinach acreage is grown in California with approximately half of the state's crop produced in Monterey County's coastal Salinas Valley. Spinach production in California and Arizona involves exceptionally high seeding rates to ensure the leaves grow upright to facilitate mechanical harvest and to prevent soil from getting onto the leaves. Spinach grown for processed frozen, standard fresh market, and fresh market "baby leaf" (harvested at four to five true leaf stage) products is planted at 1.9 to 3.1, 6.2 to 9.1, and 9.1 to 18.5 million viable seeds per ha, respectively (Koike et al., 2001a). Such high plant densities create dense crop canopies, prolonged periods of leaf wetness, and high humidity that favor the development of foliar diseases such as downy mildew caused by *Peronospora farinosa* f. sp. *spinaciae* and Cladosporium leaf spot caused by *Cladosporium variabile* (Correll et al., 1994; Koike et al., 1992). This is particularly true for crops grown using overhead (sprinkler) irrigation, which is typical for most "baby leaf" and other fresh market spinach crops grown in California and Arizona (Koike, 2000).

In 1997, a new leaf spot disease was found in limited spinach acreage in the Salinas Valley (Koike et al., 2001a). Initial symptoms consisted of leaf spots that were 2 to 5 mm in diameter, circular to oval in shape, and gray–green in color. Over a period of time, leaf spots expanded and turned tan in color. Older leaf spots coalesced, dried up, became papery in texture, and sometimes resulted in necrosis of significant portions of the leaves. The pathogen was identified as *Stemphylium botryosum* Wallr (Koike et al., 2001a). The continued occurrence of the disease in spinach crops in the Salinas Valley indicates that the pathogen has become established in the state. During the past 7 years, *S. botryosum* has also been reported as a foliar pathogen in spinach seed crops in Washington (du Toit and Derie, 2001) and Oregon (du Toit and Hernandez-Perez, 2005) and in fresh market spinach crops in Delaware, Maryland (Everts and Armentrout, 2001), Florida, Quebec, Canada (Raid, 2004), and Arizona (Koike et al., 2005). Approximately 75% of the spinach produced in the Salinas Valley is for fresh market consumption (Monterey County Agricultural Commissioner's Office, 2001). The percentage of spinach acreage in the United States grown for fresh markets versus processing markets has increased significantly (Morelock and Correll, 2006). Quality standards for fresh market spinach are extremely high, so this newly emerging disease poses an additional threat for growers in California and other states who need to produce high-quality and defect-free products.

Hernandez-Perez and du Toit (2006) detected *S. botryosum* in 100% of 77 spinach seed lots produced in Denmark, The Netherlands, New Zealand, or the United States in 2000 to 2003. Hernandez-Perez (2005) recently demonstrated seed transmission of *S. botryosum* in greenhouse trials. Although seed transmission has not been proven under field conditions, outbreaks of Stemphylium leaf spot in baby leaf spinach crops in Florida suggested that the primary inoculum for the disease arose from infected or infested seeds (Raid, 2004). Chlorine or hot water seed treatment reduced the incidence of *S. botryosum* but could not eradicate the pathogen from the seed (du Toit and Hernandez-Perez, 2005).

To date, no resistance to *S. botryosum* has been reported in spinach. Koike et al. (2001a) inoculated 10 flat-leaf, eight semisavoy, and two savoy spinach cultivars each with four isolates of *S. botryosum* from California. All of the cultivars developed leaf spot symptoms similar to those observed in commercial fields after 2 weeks with only slight differences in disease severity among the cultivars. An additional series of spinach cultivars, including some with resistance to race 5 and race 6 of the downy mildew pathogen, were also susceptible to Stemphylium leaf spot (Koike et al., 2001b). In lettuce, for comparison, a source of resistance to

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Stemphylium botryosum f. sp. *lactucum* was found in a wild species (*Lactuca saligna*), for which the resistance is controlled by two genes, one dominant and one recessive (Netzer et al., 1985).

Because of the occurrence of Stemphylium leaf spot on spinach in numerous states, and persistence of the disease in California and Washington, we screened spinach germplasm for sources of resistance to the disease. The information obtained on resistance or susceptibility could be very useful in developing *S. botryosum*-resistant spinach cultivars.

Materials and Methods

Public germplasm collection. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, CA. The entire spinach collection from the USDA [seeds provided by the North Central Regional Plant Introduction Station (NCRPIS), Iowa State University, Ames, IA] plus 22 commercial cultivars were screened for Stemphylium leaf spot resistance in a greenhouse test. The USDA collection included 332 accessions of cultivated spinach (*Spinacia oleracea*), four accessions of *S. turkestanica*, and two accessions of *S. tetrandra*. The experimental design was a randomized complete block with two replications. In each replication, eight seeds from each genotype were planted in a plastic pot (10 cm × 10 cm × 10 cm) filled with Sunshine Mix #1 (Sun Gro Horticulture Canada Ltd., Seba Beach, Canada) on 5 Oct. 2004 and seedlings were thinned to five plants per pot after emergence.

An isolate of *Stemphylium botryosum* obtained from infected plants of the spinach cultivar Cheetah grown in Arizona was maintained for 4 weeks on plates of V8 juice (Campbells Soup Company, Camden, NJ) agar under a 12-h/12-h light/dark regime. A conidial suspension ($\approx 10^5$ conidia per mL) was prepared in water and applied using a handheld mister onto leaves of each genotype 5 weeks after seeding (at the four- to six-true-leaf stage). Inoculated plants were incubated in a humidity chamber maintained at 100% relative humidity for 72 h and then maintained in a greenhouse at 14/27 °C (night/day cycle). Three weeks after inoculation, the plants in each pot were evaluated for incidence (percent of plants with symptoms), and severity (percent of leaf area with symptoms) of Stemphylium leaf spot symptoms per plant. Reisolations for *S. botryosum* were conducted to confirm the presence of the pathogen in association with the leaf spots.

From this initial screening (Test 1), 24 putative resistant accessions were identified for further testing. These genotypes, plus eight susceptible accessions and 22 commercial cultivars included in Test 1, were planted on 4 Mar. 2005 for a second inoculation trial (Test 2), which was carried out as described previously for Test 1. The experimental design was a randomized complete block with four replications. Control plants of each genotype were sprayed with sterile distilled water and otherwise handled in the same manner as the inoculated plants.

Proprietary germplasm collection. To facilitate comparison of public versus proprietary spinach germplasm for resistance to Stemphylium leaf spot, a request was sent to commercial seed companies to contribute seed samples of proprietary spinach germplasm (cultivars or experimental breeding lines) to screen for resistance to Stemphylium leaf spot. The germplasm was evaluated in a greenhouse at the Washington State University (WSU) Mount Vernon Northwestern Washington Research & Extension Center (NWREC) in both 2004 and 2005. All entries were coded by the seed companies and again at the WSU Mount Vernon NWREC for anonymity of companies and cultivars or breeding lines. In addition, 10 accessions from the USDA NCRPIS that were included in Test 1 of the public germplasm screened in California were included in this test. Each of the three replications was arranged in a randomized complete block design with eight seeds per entry planted into Sunshine Mix #1 potting medium in a 10-cm diameter pot and thinned to five plants per pot after emergence.

At the four- to six-true-leaf stage, plants were inoculated with a conidial suspension of *S. botryosum* ($\approx 10^5$ conidia per mL) as described previously for the public germplasm trials and using the same Arizona isolate of the pathogen. Inoculated plants were immediately enclosed in a polyvinyl chloride frame that was covered with plastic and white cloth for 3 d to maintain near 100% humidity. After 3 d, the plants were uncovered and maintained in the greenhouse for 3 weeks. Severity of Stemphylium leaf spot (percent of total leaf area per pot with symptoms) was rated visually 3 weeks after inoculation. The resistance screen was carried out from February to Apr. 2004 and repeated from January to Mar. 2005 when daylength in northwestern Washington (48°27′ N) was short enough to minimize bolting (conversion from vegetative to reproductive growth) of the diversity of genotypes of this daylength-sensitive species. This was necessary to reduce the chance of pollen production, because spinach pollen increases the aggressiveness of *S. botryosum* on spinach (du Toit and Derie, 2002) and could confound severity and incidence ratings for Stemphylium leaf spot.

Based on requests from participating seed companies as well as the common occurrence of Cladosporium leaf spot and Stemphylium leaf spot in spinach seed crops (du Toit and Derie, 2002; Hernandez-Perez and du Toit, 2006), the proprietary spinach genotypes evaluated in this study were also screened for resistance to Cladosporium leaf spot. The Cladosporium leaf spot trial was carried out from February to Apr. 2004 and repeated from January to Apr. 2005 at the WSU Mount Vernon NWREC using the same methods described for the Stemphylium leaf spot trials. Each trial consisted of a randomized complete block design with three replicates of five plants per spinach line. The trials were carried out using a known pathogenic isolate of *C. variabile* (MV96) obtained from a plant with symptoms of Cladosporium leaf spot in a spinach seed crop grown in Washington state in 1996. The fungus was grown for 3 weeks on potato dextrose agar (PDA) in petri plates under natural light and used to prepare a spore suspension ($\approx 10^5$ spores per mL) for inoculum. Four- to 6-week-old plants of each spinach line were inoculated with a spore suspension of the fungus, incubated for 3 d at high humidity, uncovered, and then rated 3 weeks after inoculation for incidence and severity of Cladosporium leaf spot as for the Stemphylium leaf spot trials.

Five seed companies (A, B, C, D, and E) with headquarters in Denmark, the United States, or the Netherlands submitted proprietary spinach entries for the Stemphylium and Cladosporium leaf spot trials. In 2005, 148 entries were screened for resistance to Stemphylium leaf spot, including 27, 10, 27, 70, and four entries from companies A, B, C, D, and E, respectively; as well as 10 accessions from the USDA NCRPIS. Seeds of the entries from company B were only received after the 2004 trial was completed. Therefore, only 138 entries were screened for Stemphylium leaf spot resistance in 2004. A total of 118 entries were screened for resistance to Cladosporium leaf spot, including 10 USDA accessions and the proprietary cultivars and breeding lines from each of companies A, B, C, and E that were included in the Stemphylium leaf spot trials plus 40 entries from company D. The 40 entries from company D for the Cladosporium leaf spot trial were different from the entries that company submitted for the Stemphylium leaf spot trials. Seed of the company B entries were only received after the 2004 trial was completed, so only 108 entries were screened for resistance to Cladosporium leaf spot in 2004.

Statistical analysis. Disease severity values for the public germplasm screens were averaged for all plants in each pot, and statistical analyses were conducted on the basis of pot means. Data were analyzed by analysis of variance (ANOVA) using the general linear models (GLM) procedure of JMP Version 5 (SAS Institute, Cary, NC). Genotype was considered a fixed effect, and replication was considered a random effect. For comparisons among genotypes, least significant differences (LSD) were calculated with a Type I (α) error rate of $P = 0.05$.

For each trial of the proprietary germplasm collection, incidence and severity ratings per pot were subjected to ANOVAs using the GLM procedure of SAS Version 9.1 (SAS Institute). Spinach genotype and replication were considered fixed and random effects, respectively, in the linear model. Friedman's nonparametric rank test was then completed for each trial to compare the mean ranking of genotypes using Fisher's protected LSD (with a Type I error rate of 0.05) because of heterogeneous variances and nonnormal residuals for each dependent variable (Steele and Torrie, 1980). In addition, an ANOVA was carried out for each trial to determine if there were significant differences in severity of leaf spot for spinach entries from the five seed companies and the USDA NCRPIS. Source (companies and USDA NCRPIS) and replications were considered fixed and random effects, respectively, in the linear model. Pearson's correlation coefficients were calculated for the four leaf spot trials to determine if there might be an association between resistances of the genotypes to *S. botryosum* versus *C. variable* for the spinach germplasm evaluated.

Results and Discussion

Public germplasm collection. No spinach genotype was completely resistant (immune) to the disease. However, there were significant differences in both incidence and severity of the disease among the genotypes tested. Of the 360 genotypes evaluated in 2004 (Test 1), disease incidence ranged from 10% to 100% and averaged 53.4% ± 7.3% (mean ± SE), whereas disease severity had a range of 0.1% to 7.3% with a mean of 2.2% ± 0.1%. For the 54 selected genotypes tested in 2005 (Test 2), the disease incidence ranged from 5% to 100% with an average of 52.3% ± 7.2%, whereas disease severity ranged from 0.3% to 12.8% and averaged 5.0 ± 0.3% (Table 1). *Stemphylium botryosum* was consistently isolated from the leaf spots. None of the control plants treated with sterile distilled water developed symptoms of leaf spot. Two accessions from Turkey, PI 169685 and PI 173809, consistently had low disease incidence and severity ratings (Table 1). PI 169685 has a semiflat leaf surface, whereas PI 173809 is a flat leaf type. However, there appeared to be no significant correlation between disease incidence or severity ratings and leaf type (Table 1). The results showed both flat leaf and savoy accessions with high incidence and/or severity ratings as well as both types with low disease ratings.

The two resistant PI lines are open-pollinated accessions, and both showed some variation among plants for resistance to Stemphylium leaf spot in this study. PI 169685 had a mixture of smooth and prickly seeds, indicating some heterogeneity in the accession. This suggests an opportunity for further selection to increase the level of resistance to Stemphylium leaf spot in these accessions. Alternatively, individual resistant plants of these entries could be self-pollinated to generate resistant inbred lines for hybrid production. However, the nature of inheritance of the resistance trait is unknown. These Stemphylium leaf spot-resistant genotypes are both *Spinacia oleracea*. The two *S. tetrandra* and four *S. turkestanica* accessions screened in Test 1 were all susceptible to *S. botryosum* and were not included in Test 2.

None of the 22 commercial cultivars tested in the public germplasm screen consistently showed low disease incidence and severity ratings (Table 1). This is consistent with the results of Koike et al. (2001a, 2001b) who found no significant resistance to Stemphylium leaf spot in more than 24 commercial and developmental spinach cultivars evaluated. This suggests spinach breeders may need to start breeding for resistance using germplasm resources, which will take considerable time and effort to transfer resistance traits into commercially acceptable spinach cultivars. The two resistant lines identified in this study (PI 169685 and PI 173809) may provide a starting point for such efforts.

Proprietary germplasm collection. Significant differences in severity of Stemphylium leaf spot were observed among proprietary entries screened at the WSU Mount Vernon NWREC (data not shown). In 2004, severity of Stemphylium leaf spot averaged 4.6% ± 0.3% (mean ± SE) with a range from 0 (entry A65 from company A) to 33.3% ± 8.8% (D29 from company D) for the 138 genotypes. Ten lines had relatively severe Stemphylium leaf spot ratings of greater than 10%. Three lines had mean severity ratings less than 1%, including a *Spinacia turkestanica* line from Uzbekistan (PI 494751 with 0.6% ± 0.3% leaf spot severity), A65, and D59. Thirty lines had severity ratings less than 2.0%, including *S. oleracea* 'Dikenli' from Turkey (PI 171863). Unfortunately, there was not enough seed remaining to retest PI 494751 in 2005. In 2005, severity of Stemphylium leaf spot averaged 5.3% ± 0.3% for 148 entries, ranging from 0 (D3) to 28.3% ± 7.3% (A60). Fourteen entries had mean severity ratings greater than 10%, five of which also had high severity ratings in 2004 (D4, D5, D29, D34, and D45, all from company D). Two entries (D3 and D13) had mean ratings less than 1%, both of which had severity ratings less than 2.8% in 2004.

Similar to the Stemphylium leaf spot trials, significant differences in severity of Cladosporium leaf spot were observed among the 108 proprietary entries screened (data not shown). In 2004, severity of Cladosporium leaf spot averaged 10.9% ± 0.5% with a range from 2.7 ± 0.3 (entry D93) to 40.0 ± 0.0 (A54) for individual entries. Thirteen lines had mean Cladosporium leaf spot ratings of at least 20%. Eight lines had mean severity ratings less than 5%, including the *S. turkestanica* line from Uzbekistan (PI 494751 with 4.0% ± 1.0% Cladosporium leaf spot severity) that also had a low severity rating for Stemphylium leaf spot in 2004. There was insufficient seed remaining to evaluate this accession in 2005. In the 2005 trial, Cladosporium leaf spot was not as severe as in 2004, although the same inoculation protocol and isolate of the pathogen were used in both trials. The difference in severity may reflect differences in some of the proprietary lines included in the 2004 versus 2005 trials. Severity of Cladosporium leaf spot in the 2005 trial averaged 5.4% ± 0.2% for 117 entries, and ranged from 1.7% ± 0.7% (D106) to 23.3% ± 13.3% (D90). Twelve entries had mean ratings less than 3%, only one of which (D103) had a low mean Cladosporium leaf spot rating in 2004 and ranked in the top 20 entries in both years.

Although the order in which entries were ranked for severity of Stemphylium leaf spot varied between 2004 and 2005, there was a significant positive correlation between Stemphylium leaf spot severity ratings in 2004 and 2005 (0.2786 at $P < 0.0001$). Consistency in severity ratings of entries among trials suggests high heritability for the reactions of the entries to a particular pathogen; thus, the trait may be reliably selected in breeding for resistance. There was a similar significantly positive correlation for Cladosporium leaf spot ratings in 2004 and 2005 (0.26105 at $P < 0.0001$). Stemphylium leaf spot ratings for the proprietary germplasm collection evaluated in 2004 were positively correlated with Cladosporium leaf spot ratings in 2004 and 2005 (0.3980 at $P < 0.0001$ and 0.42267 at $P < 0.0001$, respectively), but correlations were not significant for the 2005 Stemphylium leaf spot trial. Some major inconsistencies were observed in severity ratings and rankings of the genotypes evaluated in the proprietary germplasm trials for each of the two leaf spot diseases. This may be attributed, in part, to the fact that many of the genotypes were segregating breeding lines, but a single leaf spot severity rating was assigned for all five plants in each pot to facilitate evaluating three replicates of each genotype for the two leaf spot diseases. In addition, some of the inconsistency in results may be associated with limited capacity to control relative humidity as precisely as desired in the "dew chamber" system used for these trials, which affected disease pressure among and within trials.

The Stemphylium leaf spot trials with the proprietary germplasm collection identified genotypes highly susceptible to *S. botryosum* as well as genotypes with putative resistance to this pathogen. Unfortunately, the two PI lines that consistently had low severity ratings for Stemphylium leaf spot in the public germplasm trials (PI 169685 and PI 173809) were not included in the proprietary germplasm trials. The *S. tetrandra* and *S. turkestanica* accessions evaluated as part of the proprietary germplasm screen showed opposite reactions to Stemphylium and Cladosporium leaf spots. The *S. tetrandra* accession (PI 608712) was susceptible to both leaf spot diseases in 2004 and in 2005, but the *S. turkestanica* accession (PI 494751) appeared to have partial resistance to both leaf spot diseases in the 2004 trial in which this accession was evaluated (ranked 17 out of 138 accessions screened for resistance to Stemphylium leaf spot and 8 out of 108 accessions screened for resistance to Cladosporium leaf spot).

Each of the seed companies that participated in the proprietary germplasm evaluation had individual spinach breeding programs. Overall, the genotypes received from company A had a significantly lower mean severity rating for Stemphylium leaf spot in 2004 (2.9%) compared with those of the genotypes received from companies C, D, and E (3.2%, 6.1%, and 4.4%, respectively)

Table 1. Means for incidence (percent of plants with symptoms) and severity (percent diseased leaf area) of Stemphylium leaf spot on 54 spinach genotypes evaluated in two inoculated tests.

Genotype	Source[z]	Leaf type	Incidence (%)		Severity (%)	
			Test 1	Test 2	Test 1	Test 2
PI 249920	USDA (Spain)	Semisavoy	90.0	83.8	2.5	12.8
Seven R	Seminis	Semisavoy	80.0	81.7	4.7	11.1
PI 179590	USDA (Belgium)	Semiflat	100.0	71.7	7.3	10.5
Bossanova	Seminis	Semiflat	75.0	66.7	1.8	9.5
NSL 4657	USDA (U.S.)	Semisavoy	100.0	80.0	3.6	9.4
PI 419004	USDA (China)	Semiflat	90.0	78.8	4.3	9.4
Whale	Rijk Zwaan	Semiflat	80.0	50.0	2.9	9.0
PI 169673	USDA (Turkey)	Semiflat	22.5	61.3	7.0	8.0
PI 171865	USDA (Turkey)	Semiflat	20.0	22.5	0.1	7.5
NSL 6082	USDA (U.S.)	Savoy	20.0	67.1	0.1	7.5
NSL 6085	USDA (U.S.)	Semiflat	80.0	88.8	2.0	7.3
PI 169671	USDA (Turkey)	Semisavoy	30.0	70.0	2.1	7.3
Springfield	Gowan Seed	Semiflat	70.0	55.8	1.2	7.2
PI 164965	USDA (Turkey)	Semiflat	43.3	19.6	0.5	6.8
Indian Summer	JSS	Semisavoy	40.0	45.0	2.4	6.7
Cheetah	Gowan Seed	Semiflat	54.2	70.4	1.5	6.7
PI 274057	USDA (unknown)	Semiflat	77.5	81.7	2.2	6.5
PI 173129	USDA (Turkey)	Flat	30.0	33.8	1.2	6.3
PI 205234	USDA (Turkey)	Semiflat	10.0	60.0	0.1	6.1
PI 175929	USDA (Turkey)	Flat	20.0	68.8	0.2	5.6
PI 262161	USDA (Spain)	Semiflat	100.0	65.0	6.4	5.5
Bolero	Seminis	Semiflat	75.0	81.3	7.0	5.3
PI 222749	USDA (Iran)	Semiflat	80.0	56.3	2.2	5.2
ASR6710362	Seminis	Semiflat	77.5	53.8	4.0	5.0
PI 171864	USDA (Turkey)	Flat	25.0	47.9	0.2	4.9
Eagle	Rijk Zwaan	Semiflat	62.5	35.0	4.9	4.8
Symphonie	Gautier Graines	Semiflat	10.0	62.5	2.5	4.5
PI 176775	USDA (Turkey)	Semiflat	90.0	83.8	2.8	4.5
Unipack 144	Seminis	Semiflat	83.3	100.0	0.4	4.1
Alrite	American Takii	Flat	70.0	56.3	0.8	4.1
Hellcat	Seminis	Semiflat	37.5	32.5	0.8	4.1
PI 165012	USDA (Turkey)	Semiflat	30.0	50.0	0.4	4.0
Polka	Seminis	Semiflat	63.3	63.3	3.8	3.9
Unipack 12	Seminis	Semiflat	50.0	36.3	3.7	3.8
PI 183246	USDA (Egypt)	Semiflat	12.5	38.8	0.1	3.7
PI 165043	USDA (Turkey)	Flat	37.5	35.4	0.6	3.4
NSL 6090	USDA (U.S.)	Semisavoy	36.7	14.6	0.3	3.1
PI 103063	USDA (China)	Flat	40.0	52.1	0.7	3.0
Melody	Seminis	Savoy	100.0	41.7	0.9	3.0
Unipack 277	Seminis	Semiflat	62.5	28.3	6.9	3.0
Space	Gowan Seed	Semiflat	70.0	48.8	3.4	2.8
PI 361127	USDA (U.K.)	Savoy	16.7	45.8	0.1	2.6
PI 174385	USDA (Turkey)	Semiflat	30.0	27.5	0.1	2.6
NSL 6782	USDA (Holland)	Semisavoy	100.0	81.3	4.0	2.6
PV 0063	Gowan Seed	Semiflat	67.5	67.5	5.3	2.5
NSL 4659	USDA (U.S.)	Semisavoy	20.0	26.3	0.3	2.3
Nordic IV	Gowan Seed	Semiflat	63.3	30.0	0.6	2.2
NSL 22149	USDA (U.S.)	Semisavoy	60.0	39.6	0.6	1.9
PI 164966	USDA (Turkey)	Flat	47.5	45.8	0.4	1.9
PI 173130	USDA (Turkey)	Flat	40.0	32.5	0.7	1.7
Lion	Rijk Zwaan	Semiflat	70.0	59.6	2.8	1.3
Tyee	JSS	Semisavoy	65.0	21.7	4.3	0.8
PI 173809	USDA (Turkey)	Flat	20.0	5.0	0.4	0.5
PI 169685	USDA (Turkey)	Semiflat	12.5	6.3	0.3	0.3
$LSD_{0.05}$[y]			52.6	25.3	2.4	2.8

[z]The countries from which the spinach seeds were collected are shown in parentheses for the USDA collection. Seed companies from which seeds were obtained include American Takii, Salinas, CA; Gautier Graines, Eyragues, France; Gowan Seed, Salinas, CA; Johnny's Selected Seeds (JSS), Winslow, ME;.Rijk Zwaan, De Lier, Holland; and Seminis Vegetable Seeds, Woodland, CA.

[y]Least significant differences at $P < 0.05$.

(Table 2). The 10 USDA NCRPIS accessions had a mean severity rating of 3.1% for Stemphylium leaf spot in 2004, which was not significantly different from that of the genotypes from company A. In 2005, the USDA accessions and the proprietary genotypes from company C had the lowest overall severity ratings for Stemphylium leaf spot (2.9% and 3.3%, respectively). For the Cladosporium leaf spot trials, the genotypes from company C had the lowest mean severity rating (6.2%) and the ones from company A had the highest (20.3%) in 2004; and in 2005, genotypes from companies B (not included in the 2004 trial) and C had the lowest leaf spot severity (2.9% and 3.7%, respectively), whereas entries from company A again showed the highest rating (6.5%). These results demonstrate the variation in resistance or susceptibility to Stemphylium and Cladosporium leaf spots that exists among spinach germplasm collections and the potential for developing spinach cultivars with greater resistance to the two diseases than what is currently available commercially.

The most economical means of disease control is through the use of genetic resistance. This is especially true for organic growers who must rely on a combination of plant resistance, organically certified fungicides, and cultural practices to control diseases. The use of resistant cultivars may

Table 2. Mean severity of Stemphylium leaf spot for proprietary spinach genotypes screened for resistance to *Stemphylium botryosum* in greenhouse trials in 2004 and 2005 based on the source of the germplasm.

Source[z]	No. of genotypes[y]	Stemphylium leaf spot	
		2004	2005
Company A	26 and 27	2.88 b	6.48 a
Company B	10	—	6.60 a
Company C	27	3.21 a	3.33 b
Company D	70	6.08 a	5.72 ab
Company E	4	4.42 a	3.40 ab
USDA NCRPIS	10	3.11 b	2.85 b
LSD ($P < 0.05$)[x]		Rank	Rank

[z]Seeds of proprietary spinach genotypes from five seed companies (A, B, C, D, and E, coded for anonymity) and from the USDA ARS North Central Regional Plant Introduction Station (NCRPIS). Refer to the text for details of genotypes and methods used to screen for resistance.

[y]One genotype from company A was not evaluated in 2004.

[x]LSD = Fisher's protected least significant difference (Steele and Torrie, 1980) based on analyses of variance. "Rank" indicates original means are shown, but means separation was based on Friedman's nonparametric rank test because of heterogeneous variances and/or non-normal distribution of the data.

reduce expenses for chemicals, energy, and labor associated with pesticide applications. In this study, a wide range of genetic variability in response to Stemphylium and Cladosporium leaf spot was found in the spinach germplasm evaluated. With the finding of potential sources of resistance, genetic improvements for leaf spot resistance seem feasible in spinach. Indeed, research is currently in progress to incorporate the public sources of resistance to Stemphylium leaf spot identified in this study into elite cultivars in a USDA spinach breeding program.

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