ABSTRACT

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

Project Title: Spinach Breeding and Genetics

Project Investigator: Beiquan Mou, Agricultural Research Service, U.S. Dept. of Agriculture, Salinas, CA

Summary:

Our emphasis is on problems facing the spinach industry in California, including coastal, desert, and interior valley. New or existing diseases, insects, or pathogens continue to appear or evolve to pose new challenges for growers, shippers, researchers, and the industry. Changes in production practices and marketing approaches also demand new genetic solutions. The spinach breeding and genetics program aims to incorporate valuable traits into spinach cultivars including resistances to downy mildew, Verticillium wilt, and Stemphylium leaf spot diseases, leafminer insect, and herbicides, and nutritional improvement in oxalic acid content. Spinach is known to have greater amount of oxalic acid than most crops, which may combine with minerals to form insoluble salt crystals, thus reducing the bioavailability and absorption of calcium and iron in diets. Calcium oxalate crystals may deposit in the kidneys of certain people as a common form of kidney stone. Horticultural traits, adaptation, and yield are also important. The most economical means of disease and insect 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 pesticides and cultural practices to control diseases and insects. The use of resistant cultivars may reduce expenses for chemicals, energy, and labor associated with pesticide applications and minimize potential adverse effects of pesticide use. In this study, a wide range of genetic variation and sources of resistance to Stemphylium leaf spot and leafminers as well as low oxalic acid content were found in the USDA spinach genebank. The results suggest that improvements for genetic resistance and nutritional quality seem feasible in spinach. Indeed, research is currently in progress to incorporate the resistance and quality traits identified in this study into elite cultivars in our spinach breeding program. We are also screening spinach genebank varieties for resistance to downy mildew and Verticillium wilt diseases. Impatiens Necrotic Spot Virus (INSV) was detected on several commercial spinach cultivars in an experimental field in Salinas in October 2008. Numerous lettuce fields in the Salinas Valley tested positive for INSV in recent years, but to our knowledge this is the first report of natural occurrence of INSV on spinach plants in California. Growers should be vigilant as it may pose a new threat to the California spinach production.

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Objective 1. Screening for Resistance to Stemphylium Leaf Spot Diseases of Spinach (with Steve Koike and Lindsey du Toit).

Procedures. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, Calif. 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*), 4 accessions of *S. turkestanica*, and 2 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 x 10 cm x 10 cm) filled with Sunshine Mix #1 (Sun Gro Horticulture Canada Ltd., Seba Beach, Canada) and seedlings were thinned to 5 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 agar under a 12 h/12 h light/dark regime. A conidial suspension (approximately 10^5 conidia per ml) was prepared in water and applied using a hand-held mister onto leaves of each genotype five 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^{o}C (night/day cycle). Three weeks after inoculation, the plants in each pot were evaluated for incidence (% of plants with symptoms), and severity (% of leaf area with symptoms) of Stemphylium leaf spot symptoms per plant. Re-isolations 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 8 susceptible accessions and 22 commercial cultivars included in Test 1, were planted for a second inoculation trial (Test 2), which was carried out as described above 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.

Disease severity values 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 $P = 0.05$.

Results and Discussion. 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 Test 1, disease incidence ranged from 10 to 100% and averaged 53.4 ± 7.3% (mean ± standard error), while 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 Test 2, the disease incidence ranged from 5 to 100% with an average of 52.3 ± 7.2%, while disease severity ranged from 0.3 to 12.8% and averaged 5.0 ± 0.3% (Appendix Table 1). *S. 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 semi-flat leaf surface, while 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. (2001) 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.

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 reduce expenses for chemicals, energy, and labor associated with pesticide applications. In this study, a wide range of genetic variability in response to Stemphylium 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.

Objective 2. Studying Leafminer Resistance in Spinach.

Procedures. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, Calif. We screened spinach collections from the USDA (seeds were provided by the North Central Regional Plant Introduction Station, Iowa State University, Ames, Iowa) for leafminer resistance in a preliminary study. The collection includes 332 accessions of cultivated spinach, 4 accessions of *Spinacia turkestanica* (Ames 23666, PI 494751, PI 604792, and PI 608713), and 2 accessions of *S. tetrandra* (Ames 23664 and PI 608712). Sixteen seeds from each accession were planted in a plastic pot (10 x 10 x 10-cm) with 2 sand : 1 soil (by volume) in greenhouse, and seedlings were thinned to 10 plants per pot. Plants were moved into an outdoor insect cage (2 m high by 4 m wide by 8 m deep) made of polypropylene shade cloth for resistance screening five weeks after planting. Lettuce leaves with leafminer mines were collected from newly harvested fields around Salinas and hung in shade to allow leafminer larvae to emerge from the leaves and pupate. Pupae were collected and put in plastic containers to allow adult flies to emerge. About 3500 flies were then released in the outdoor cage to feed on the spinach plants. After 10 days, number of stings per unit area was counted on the leaf with most leafminer stings on each plant, using an optical glass binocular magnifier (OptiVisor, Donegan Optical Co., Lenexa, Kans., U.S.A.), and the number of plants with mines for each accession was recorded.

From the preliminary screening, 22 accessions with the fewest stings and 10 accessions with the most stings per unit leaf area were selected for further testing. These genotypes plus six commercial hybrid cultivars and two Chinese local varieties were planted in plastic pots (10 x 10 x 10-cm) with soil and were thinned to five plants per pot. Plants were placed in the outdoor insect cage six weeks after planting and were arranged in a randomized complete block with a single pot as the experimental unit and eight replications. About 4300 leafminer flies were released in the cage. Stings per unit area were counted on the leaf with the highest sting density on each plant 10 days after the introduction of flies in the cage. Mines per plant and the fresh weight of the plant excluding roots were also recorded. The same 40 genotypes were planted in the field in a randomized complete block design with 8 replications for two years. Each plot consisted of 5 plants of a genotype, with 30 cm between plants and 35 cm between rows on double-row beds of 1-m center. Leafminer stings were counted in a 20-cm^2 leaf area with the

highest sting density on each plant five weeks after planting. Mines per plant and plant weight excluding roots were also recorded.

Per plant values were averaged and analysis was conducted on the basis of pot or plot means. Data were analyzed by analysis of variance (ANOVA) using the general linear model procedure of JMP v. 5 (SAS Institute, Cary, N.C.). Genotype was considered the fixed effect, and replication was considered the random effect. For comparisons between genotypes, least significant differences (LSD) were calculated with an error rate of $P = 0.05$. A correlation matrix for genotype means was calculated for all variables using the multivariate platform of JMP. To test differences in rank order among the genotypes grown in different environments, Spearman's rank correlations were calculated (Steel and Torrie, 1980).

Results and Discussion. None of the genotypes tested was immune to leafminers, as all genotypes had at least a few stings or mines. Significant genotypic differences were found for leafminer stings per unit leaf area, mines per plant, mines per 100 g plant weight, and plant weight both in the outdoor insect cage and in the field (Appendix Table 2). Leafminer stings per cm^2 leaf area ranged from 1.8 to 7.1 in the cage, from 10.5 to 30.8 in Year 1 and from 9.9 to 23.8 in Year 2 in the field for the genotypes tested. Mines per plant varied from 0.4 to 3.1 in the cage, from 6.3 to 38.4 in Year 1 and from 14.8 to 79.5 in Year 2 in the field. Because plant sizes differed among plants, we divided mines per plant by plant weight to standardize the result. Mines per 100 g fresh weight ranged from 5.6 to 85.1 in insect cage and from 3.5 to 48.1 in Year 1 and from 9.5 to 55.5 in Year 2 in the field. These results suggest that there is substantial genetic variation among spinach genotypes in their suitability as a leafminer host.

None of the four accessions of *Spinacia turkestanica* and two accessions of *S. tetrandra* had low leafminer sting density in the preliminary screening and they were not included in further cage and field tests. PI 274065 (cultivar name 'Wisemona') had the lowest sting density (10.5 and 9.9 sting/cm^2 in Year 1 and Year 2) in the field where the six commercial cultivars averaged 20.4 and 17.3 stings per cm^2 leaf area in Year 1 and year 2, respectively. Two accessions from Turkey showed the fewest mines per 100 g fresh weight (3.5 and 9.5 for PI 174385 and 4.4 and 10.5 for PI 169673 in Year 1 and Year 2, respectively) among genotypes in the field, as compared to 15.3 and 26.5 for the six commercial cultivars in Year 1 and year 2, respectively. These results showed a great potential to improve the level of leafminer resistance in the current spinach cultivars.

Rank order of stings per cm^2 did not significantly change for the genotypes in cage, Year 1 and Year 2 field tests, as indicated by the high Spearman's rank correlation. The sting results from different tests were also highly correlated. The consistency in performance demonstrated that differences in sting density were stable and a cage test can be used to screen germplasm for fewer leafminer stings. It also suggests that sting per unit leaf area is a reliable trait for the selection of leafminer feeding non-preference. Similar results were found in a study of leafminer resistance in lettuce (Mou and Liu, 2003).

Although the rank correlation for mines per plant or per 100 g plant weight was highly significant between the two field tests, the correlation was only moderate or non-significant between cage and field experiments. This may be partly due to the fact that the level of mine

damage in the cage was low (about one mine per plant on average, Table 2). These results suggest that mines per plant or per unit plant weight are also relatively stable over different years in the field, but a cage test may not be a good method of germplasm screening for resistance to mines.

The majority of stings are caused by feeding activities of leafminer adult. It is interesting to know whether leafminer flies tend to lay fewer eggs on a spinach plant if they do not like to feed on that plant. Leafminer eggs are difficult to count because they are tiny and are laid within leaf tissue. We had to use number of mines as an indirect measure of eggs laid in the plant. Stings per unit leaf area were not correlated with mines per plant or per 100 g plant weight either in the insect cage or in the field. This suggests that feeding non-preference does not necessarily mean oviposition-nonpreference for a spinach genotype, and these two traits can be improved independently.

In these choice tests, fewer stings or mines suggest host nonpreference (antixenotic resistance). Resistance based on antixenosis would be desirable because even the losses in photosynthetic capacity and appearance caused by adult feeding and oviposition would be reduced (Trumble et al., 1985). This is especially important for spinach as more than 95% of the value of spinach crop in the United States is from fresh market use (National Agricultural Statistics Service, 2007), and quality standards for fresh market spinach are extremely high. Antixenosis could prompt leafminer movements to weeds or crops tolerant to insect damage. For example, broccoli and cauliflower (both important crops in central California) with six or more leaves are rarely damaged by leafminers, regardless of population numbers (University of California, 1992).

Sting per cm^2 and mine per 100 g plant weight had little correlation with plant weight in cage and field tests. This suggests that leafminer sting and mine densities are not associated with plant biomass and it is possible to improve and combine leafminer resistance and yield traits in a spinach cultivar.

Chemical control of leafminers usually lasts only a short period of time, and adult control with contact insecticides is especially unsatisfactory because flies can easily move around, and the treated field is subject to reinfestation from adjacent untreated crops and weeds (LeStrange et al., 1999). Many studies have shown that leafminers can develop a high degree of resistance to a broad range of insecticides (Keil and Parrella, 1990; Mason et al., 1987; Parrella and Trumble, 1989). In California, chemical control is often not an option for spinach. Fresh market "baby leaves" are harvested as short as 24 days after planting (at the four- to five-leaf stage), with "junior leaves" harvested four days later. Many systemic insecticides for larval control have a requirement of 14-day preharvest spray interval (the period with no chemical sprays before harvest). This means that fields have to be sprayed about 10 days after planting or earlier, when plants are still small and most of the sprays hits the ground and is wasted. Consequently some growers try to avoid the leafminers by planting spinach in fields where the insect pressure is low, but growers may not have that luxury and leafminer infestation is often unpredictable. As a result, spinach is often tainted with the stipples of adult feeding or tunnels (mines) from larva feeding, and has reduced quality, appearance, and value. Therefore, it is essential to develop alternative management strategies for leafminers.

Resistant varieties remain the most economical means of insect control. Their use may reduce the costs of chemicals, energy, and labor associated with pesticide spray and minimize potential adverse effects of pesticide use. However, commercial spinach cultivars with high levels of resistance to leafminers are not currently available. In our study, a wide range of genetic variation in traits related to leafminer resistance was found in spinach germplasm. Some genotypes had much lower levels of leafminer stings and mines than commercial cultivars. From these findings, genetic improvement of spinach for leafminer resistance seems feasible.

Objective 3. Evaluation of Oxalate Concentration in the U.S. Spinach Germplasm Collection.

Procedures. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, Calif. (lat. 36°40'N, long. 121°36'W). During the course of these studies, the entire USDA spinach collection from NCRPIS plus 11 commercial cultivars were screened for oxalate concentration. The USDA collection includes 332 accessions of cultivated spinach, 4 accessions of *S. turkestanica*, and 2 accessions of *S. tetrandra*. Eight seeds from each genotype were planted in a plastic pot (10 x 10 x 10-cm) filled with soil in a greenhouse. The experimental design was a randomized complete block with two replications.

After plant emergence, each pot was fertilized weekly with 50 ml of a soluble fertilizer as a combination of ammonium phosphate, potassium nitrate, and urea (20N-8.8P-16.6K, Nortrace, Ltd., Greeley, CO) at a concentration of 0.8 $g.L^{-1}$. The nitrogen component consists of 2.9% ammoniac N, 5.0% nitrate N, and 12.1% urea N. The air temperature varied between 8-27°C night/day and the day length changed from 10 h 10 min to 11 h 22 min during the experiment. Four weeks after planting, chlorophyll content of five randomly selected mature leaves in each pot was measured by using a chlorophyll meter (Minolta Chlorophyll Meter SPAD-502, Spectrum Technologies, Plainfield, IL) and the readings were averaged for each pot. Five weeks after planting, all leaves in a pot were harvested without petioles in the morning and fresh weight of the leaves was determined. Harvested leaves were dried at 60°C for 24 h before being weighed for dry weight.

The leaves were re-dried at 60°C overnight, broken into small pieces by hand, and mixed. A 0.01 g leaf sample was homogenized in 5 ml de-ionized water for 6 min with a homogenizer (Ultra-turrax T25, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) at 24,000 rpm. The sample was diluted with 5 ml EDTA (10 mM, pH 7.6) and centrifuged at 1,500 rcf for 5 min. The oxalate concentration in the supernatant was determined using an oxalate kit (Procedure No. 591, Trinity Biotech, St. Louis, MO). The oxalate concentrations were calculated on both fresh and dry weight bases.

From the initial screening, 40 putative accessions with low oxalate concentration were identified for further testing. These genotypes, plus 13 accessions with high oxalate concentration and the 11 commercial cultivars, were planted for another test using methods described above. The experimental design was a randomized complete block with five replications. The air temperature was 12-27°C night/day in the greenhouse.

Fresh leaf weight from each pot was divided by the number of plants in the pot to derive leaf weight per plant. Data were analyzed by analysis of variance (ANOVA) using the general linear model procedure of JMP v. 5 (SAS Institute, Cary, N.C.). Genotype was considered the fixed effect, and replication was considered the random effect. For comparisons between genotypes, least significant differences (LSD) were calculated with an error rate of $P = 0.05$. For correlation analysis, leaf types of all genotypes in the 2006 experiment were rated on a 1-4 scale (1 = flat, 2 = semi-flat, 3 = semi-savoy, and 4 = savoy). A correlation matrix for genotype means was calculated for all variables using the multivariate platform of JMP.

Results and Discussion. There were significant differences in leaf weight per plant, chlorophyll content, moisture %, and oxalate concentration expressed on fresh and dry weight basis among the genotypes evaluated. Among the 349 germplasm accessions and cultivars screened, oxalate concentration ranged from 647.2 to 1286.9 mg/100 g fresh weight and 53.4 to 116.2 $mg.g^{-1}$ dry weight (Figure 1). This is in line with the results of Kitchen et al. (1964) who found the amount of anhydrous oxalic acid ranging from 5.4 to 9.8% on a dry weight bases among 39 spinach breeding lines, hybrids, and F_2 populations. From the initial screening, 64 genotypes were further evaluated. Oxalate content of these genotypes averaged 913.0 mg/100 g and 1024.3 mg/100 g on a fresh weight basis, and 75.1 $mg.g^{-1}$ and 83.2 $mg.g^{-1}$ on a dry weight basis in the two tests. This shows that besides genotypic effect, environment also has a great influence on oxalate content in spinach.

When expressed on a fresh weight basis, oxalate concentration may be affected by the moisture content of the plant. The oxalate concentration of NSL 4658 was higher than average on a dry weight basis, but was lower than average on a fresh weight basis as the concentration was diluted by its high water content (89.3%). 'Bordeaux' had low oxalate concentration on a dry weight basis, but showed only average concentration on a fresh weight basis due to its low water content (85.0%). Similarly, the effect of moisture content on carotenoid concentration was reported in lettuce (Mou, 2005). Nevertheless, oxalate concentration on a fresh weight basis was highly correlated with oxalate concentration on a dry weight basis ($r = 0.781$, $P < 0.01$).

Whether on a fresh weight or on a dry weight basis, two accessions from Syria, PI 445782 (cultivar name Shami) and PI 445784 (cultivar name Baladi), consistently had low oxalate concentrations. Many genotypes had lower oxalate concentration than 'Low Acid', a variety claimed to have "very little oxalic acid" (Anonymous, 2008b). The low-oxalate genotypes identified in our experiments are all *S. oleracea*. None of the two *S. tetrandra* and four *S. turkestanica* accessions screened contained low levels of oxalate in the initial (2006) screening and they were excluded from further testing.

Water content was correlated with oxalate concentration on a dry weight basis ($r = 0.547$, $P < 0.01$) but not on a fresh weight basis. On the other hand, chlorophyll content was correlated with oxalate content on a fresh weight basis ($r = 0.291$, $P < 0.01$) but not on a dry weight basis. This again shows that when oxalic acid is studied in spinach, it is important to express oxalate concentration on both fresh weight and dry weight bases.

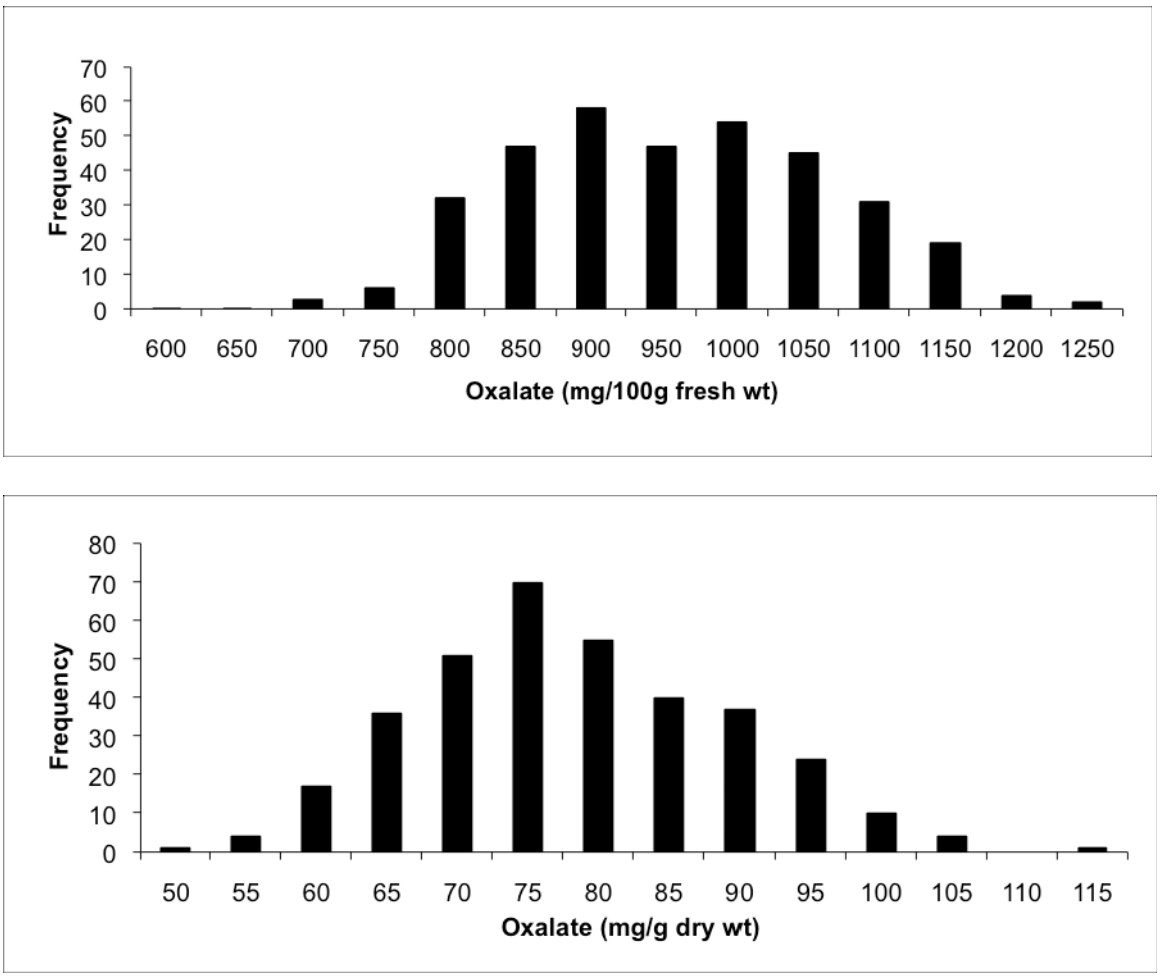

Figure 1. Frequency distribution of oxalate concentration of 349 spinach accessions screened, expressed on a fresh and dry weight basis respectively.

Leaf type had a significant but weak correlation with oxalate concentration on a fresh weight basis (r = -0.109, $P < 0.05$) but showed no association with oxalate concentration on a dry weight basis. Kitchen et al. (1964) found that savoy types had lower amount of oxalates on a dry weight basis than smooth or semi-savoy forms in 39 spinach breeding lines, hybrids, and F_2 populations. Our study had a total of 349 genotypes from all over the world and contained 46 flat, 191 semi-flat, 84 semi-savoy, and 28 savoy leaf types.

Leaf type was correlated with chlorophyll content (r = 0.309, $P < 0.01$), suggesting that more savoy types of spinach tend to have higher chlorophyll content. That is conceivable as savoy types generally have thicker and darker green leaves than flat types. Leaf weight per plant showed a weak correlation with oxalate content on a dry weight basis (r = 0.119, $P < 0.05$) but no such correlation with oxalate concentration on a fresh weight basis. This suggests that leaf weight per plant and oxalate concentration can be improved independently, and it is possible to combine high leaf yield and low oxalate content in a cultivar. F_1 hybrid cultivars grew faster than other open-pollinated accessions but generally had average oxalate

concentrations. Kaminishi and Kita (2006) found that fast-growing spinach cultivars contained lower oxalate concentrations. They took plant samples of different cultivars for oxalate analysis at different times, i.e. when a cultivar reached harvest maturity. The fast-growing cultivars probably had less time to accumulate oxalate. They also harvested both leaf blades and petioles for oxalate analysis. Savoy leaf types tend to grow slower and have shorter petioles, while flat leaf types generally grow faster and have longer petioles which have lower oxalate concentration than leaf blades. However, the leaf types of the cultivars were not described in their report. We harvested only leaf blades from all spinach genotypes at the same time (five weeks after planting).

Because of the health benefits of consuming fruits and vegetables, there is great interest in reducing the level of anti-nutritional oxalate in spinach. Our study demonstrated that a wide range of genetic variability in oxalate content exists in spinach germplasm. Two accessions from Syria, PI 445782 ('Shami') and PI 445784 ('Baladi'), may be used as sources of low oxalate concentration in a spinach breeding program. Oxalate concentration had little correlation with leaf type and leaf weight per plant, suggesting that it is possible to combine low oxalate and high yield in different types of spinach. These results suggest that genetic improvement of spinach for a low level of oxalate is feasible.

Other Research Projects:

Downy Mildew (with Steve Koike) We continued to collect downy mildew isolates for germplasm screening. We tried and modified different methods of pathogen inoculation. Several rounds of inoculum increase were performed on susceptible cultivars to maintain and produce sufficient inoculums for germplasm screening. Races of field-collected isolates were identified by using a set of ten differential cultivars.

Verticillium Wilt (with Krishna Subbarao, Karunakaran Maruthachalam) Twenty seeds from each of the 390 accessions of the entire USDA spinach germplasm collection and 5 commercial cultivars were plated on NP-10 medium and 21 accessions were found positive for *Verticillium dahliae*. We are working with the USDA spinach germplasm curator in Ames, IA to identify the source of the pathogen. Based on the seed test, 60 USDA accessions and 9 commercial cultivars were planted in 3 inoculated reps and 2 uninoculated reps in greenhouse to screen for resistance.

Impatiens Necrotic Spot Virus (INSV, with Hsing-Yeh Liu) INSV was detected on several commercial spinach cultivars in an experimental field in Salinas in October 2008. Infected plants exhibited severe stunting, interveinal yellowing, thickening, and deformation (Figure 2). Symptomatic plants were positive for INSV and negative for TSWV, CMV, and TMV with immunostrips (Agdia, Figure 3). The INSV-positive spinach was sap transmitted to spinach and the inoculated plants showed the same symptoms (Figure 3) and were positive for INSV with immunostrips. RT-PCR was used to amplify INSV coat protein gene from both field infected and mechanically inoculated spinach plants. The amplified products were sequenced and showed 99% nucleotide identity with INSV coat protein sequence in GenBank. Numerous lettuce fields in the Salinas Valley tested positive for INSV in recent years, but to our knowledge this is the first report of natural occurrence of INSV on spinach plants in California.

Figure 2. Healthy 'Lazio' plants (left) and INSV infected 'Lazio' plants (right) in an experimental field in Salinas, CA in October, 2008.

Figure 3. Left: Symptomatic plants tested positive for INSV and negative for TSWV, CMV, and TMV with immunostrips (Agdia). Right: The INSV-positive spinach was sap transmitted to healthy spinach and the inoculated plants showed the INSV symptoms.

Publications relevant to this project in 2008-09:

Mou, B. 2008. Leafminer resistance in spinach. HortScience 43: 1716-1719.

Mou, B., Koike, S., and du Toit, L. 2008. Screening for resistance to leaf spot diseases of spinach. HortScience 43: 1706-1710.

Mou, B. 2008. Evaluation of oxalate concentration in the U.S. spinach germplasm collection. HortScience 43: 1690-1693.

Appendix.

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

			Incidence (%)		Severity (%)	
Genotype	Source[z]	Leaf type	Test 1	Test 2	Test 1	Test 2
PI 249920	USDA (Spain)	Semi-savoy	90.0	83.8	2.5	12.8
Seven R	Seminis	Semi-savoy	80.0	81.7	4.7	11.1
PI 179590	USDA (Belgium)	Semi-flat	100.0	71.7	7.3	10.5
Bossanova	Seminis	Semi-flat	75.0	66.7	1.8	9.5
NSL 4657	USDA (U. S.)	Semi-savoy	100.0	80.0	3.6	9.4
PI 419004	USDA (China)	Semi-flat	90.0	78.8	4.3	9.4
Whale	Rijk Zwaan	Semi-flat	80.0	50.0	2.9	9.0
PI 169673	USDA (Turkey)	Semi-flat	22.5	61.3	7.0	8.0
PI 171865	USDA (Turkey)	Semi-flat	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.)	Semi-flat	80.0	88.8	2.0	7.3
PI 169671	USDA (Turkey)	Semi-savoy	30.0	70.0	2.1	7.3
Springfield	Gowan Seed	Semi-flat	70.0	55.8	1.2	7.2
PI 164965	USDA (Turkey)	Semi-flat	43.3	19.6	0.5	6.8
Indian Summer	JSS	Semi-savoy	40.0	45.0	2.4	6.7
Cheetah	Gowan Seed	Semi-flat	54.2	70.4	1.5	6.7
PI 274057	USDA (unknown)	Semi-flat	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)	Semi-flat	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)	Semi-flat	100.0	65.0	6.4	5.5
Bolero	Seminis	Semi-flat	75.0	81.3	7.0	5.3
PI 222749	USDA (Iran)	Semi-flat	80.0	56.3	2.2	5.2
ASR6710362	Seminis	Semi-flat	77.5	53.8	4.0	5.0
PI 171864	USDA (Turkey)	Flat	25.0	47.9	0.2	4.9
Eagle	Rijk Zwaan	Semi-flat	62.5	35.0	4.9	4.8
Symphonie	Gautier Graines	Semi-flat	10.0	62.5	2.5	4.5
PI 176775	USDA (Turkey)	Semi-flat	90.0	83.8	2.8	4.5
Unipack 144	Seminis	Semi-flat	83.3	100.0	0.4	4.1
Alrite	American Takii	Flat	70.0	56.3	0.8	4.1
Hellcat	Seminis	Semi-flat	37.5	32.5	0.8	4.1
PI 165012	USDA (Turkey)	Semi-flat	30.0	50.0	0.4	4.0
Polka	Seminis	Semi-flat	63.3	63.3	3.8	3.9
Unipack 12	Seminis	Semi-flat	50.0	36.3	3.7	3.8
PI 183246	USDA (Egypt)	Semi-flat	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.)	Semi-savoy	36.7	14.6	0.3	3.1
PI 103063	USDA (China)	Flat	40.0	52.1	0.7	3.0

Table 1. Continued.

Genotype	Source	Leaf type	Incidence(%)		Severity (%)	
			Test 1	Test 2	Test 1	Test 2
Melody	Seminis	Savoy	100.0	41.7	0.9	3.0
Unipack 277	Seminis	Semi-flat	62.5	28.3	6.9	3.0
Space	Gowan Seed	Semi-flat	70.0	48.8	3.4	2.8
PI 361127	USDA (England)	Savoy	16.7	45.8	0.1	2.6
PI 174385	USDA (Turkey)	Semi-flat	30.0	27.5	0.1	2.6
NSL 6782	USDA (Holland)	Semi-savoy	100.0	81.3	4.0	2.6
PV 0063	Gowan Seed	Semi-flat	67.5	67.5	5.3	2.5
NSL 4659	USDA (U.S.)	Semi-savoy	20.0	26.3	0.3	2.3
Nordic IV	Gowan Seed	Semi-flat	63.3	30.0	0.6	2.2
NSL 22149	USDA (U.S.)	Semi-savoy	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	Semi-flat	70.0	59.6	2.8	1.3
Tyee	JSS	Semi-savoy	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)	Semi-flat	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. Means and least significant differences (LSD) at $P \leq 0.05$ for leafminer resistance traits of 40 spinach genotypes tested in an outdoor insect cage and in the field in Salinas, Calif. for two years.

	Plant wt (g)			Sting/cm^2 leaf area			Mine/plant			Mine/100 g plant wt		
Genotype[z]	Cage	Year 1	Year 2	Cage	Year 1	Year 2	Cage	Year 1	Year 2	Cage	Year 1	Year2
PI 169673	6.3	167.7	141.4	5.7	25.2	17.3	0.8	7.8	14.8	15.9	4.4	10.5
PI 171862	7.3	261.5	294.5	3.2	19.7	19.0	1.0	19.1	44.6	14.2	10.1	15.9
PI 174385	3.7	186.8	195.9	4.3	30.5	22.2	0.5	6.3	18.1	14.3	3.5	9.5
PI 175312	4.6	207.3	212.2	6.2	30.8	23.8	0.7	10.9	28.9	17.5	7.4	15.9
PI 181808	5.7	420.8	418.9	4.9	17.9	18.4	0.9	19.5	52.3	13.6	7.2	11.7
PI 212921	4.5	312.5	116.2	7.1	29.1	21.2	0.9	17.3	24.8	20.3	6.6	23.7
PI 220121	4.3	235.8	241.2	2.4	14.6	11.8	0.8	22.9	47.5	16.3	10.4	21.8
PI 223536	4.3	370.8	302.2	5.5	24.8	20.0	0.5	30.9	61.6	11.2	11.8	20.2
PI 261787	8.9	204.4	312.2	2.6	13.1	12.7	1.5	16.2	39.4	16.0	17.0	12.9
PI 274058	5.8	133.7	216.3	3.5	17.8	13.5	1.3	24.3	51.7	15.8	19.9	26.9
PI 274059	7.8	246.3	311.5	3.7	14.8	11.5	1.5	16.5	53.0	21.7	9.4	19.2
PI 274065	5.0	188.7	317.0	3.0	10.5	9.9	1.4	22.9	54.6	29.6	23.5	16.9
PI 339545	6.5	134.2	113.7	3.8	17.1	14.2	0.8	10.5	25.6	12.7	9.7	22.1
PI 358248	5.5	224.7	274.7	1.9	13.7	11.4	0.5	18.3	37.8	10.9	8.9	13.2
PI 358253	4.2	301.6	321.9	2.2	13.6	11.4	1.1	21.4	54.4	24.9	7.5	20.9
PI 370602	3.8	156.5	177.1	4.6	17.9	20.4	1.3	16.6	41.8	30.9	11.3	22.6
PI 433208	4.5	157.5	201.0	4.0	28.3	17.3	1.8	22.0	69.3	39.9	16.3	38.4
PI 445783	5.5	453.5	439.3	3.7	15.8	11.7	0.8	19.5	79.5	15.3	5.7	18.6
PI 449353	4.8	206.9	343.9	3.1	13.6	12.3	1.4	19.9	52.4	32.4	11.9	15.9
PI 527332	6.3	161.5	207.9	2.4	17.8	12.8	1.6	24.9	60.0	27.9	16.0	32.0
PI 531449	6.0	256.9	288.1	2.5	13.8	11.8	0.9	18.9	47.6	15.5	8.3	18.4
PI 531454	5.1	156.5	179.0	3.1	18.7	10.6	0.4	14.9	30.2	9.9	9.1	19.5
PI 531457	4.2	227.1	300.3	3.6	12.8	15.1	0.8	13.6	29.1	20.8	9.0	11.1
PI 604778	4.8	272.7	272.2	4.2	20.3	14.8	1.5	25.3	50.9	32.4	9.1	19.7
PI 604783	6.4	180.9	153.4	2.3	13.6	13.8	0.5	12.0	24.7	11.2	8.1	20.4
PI 604787	3.9	184.1	197.0	3.7	15.4	13.6	0.8	12.9	30.8	21.7	7.2	16.9
PI 604789	3.4	182.1	138.3	3.2	14.5	14.0	1.1	23.4	28.6	32.7	16.2	20.9
PI 604791	4.3	156.0	149.2	1.8	16.5	12.0	0.6	20.4	35.3	16.1	17.4	28.5

Table 2. Continued.

Genotype	Plant wt (g)			Sting/cm^2 leaf area			Mine/plant			Mine/100 g plant wt		
	Cage	Year 1	Year 2	Cage	Year 1	Year 2	Cage	Year 1	Year 2	Cage	Year 1	Year 2
PI 648957	3.5	260.4	392.5	2.3	13.0	10.0	0.9	25.6	63.4	26.5	10.0	18.7
NSL 4683	6.1	120.4	152.8	3.3	13.3	13.7	1.2	12.4	17.4	20.9	11.4	12.7
NSL 6085	4.1	265.8	302.1	2.9	15.5	12.4	0.7	21.9	56.0	19.0	12.0	19.8
NSL 6093	4.7	184.4	199.3	3.0	12.3	10.8	0.5	24.2	33.6	13.8	15.6	17.8
Jianye Bocai	10.5	84.7	286.4	2.0	12.3	15.3	3.1	14.8	62.4	29.2	26.3	24.7
Shuangcheng Jianye	3.4	58.1	122.1	4.3	20.1	17.5	2.9	21.9	64.1	85.1	48.1	55.5
Alrite	6.8	288.0	459.0	4.2	21.8	20.2	0.9	25.6	78.5	12.4	10.6	17.5
Hellcat	8.1	197.7	197.4	3.8	19.1	14.1	0.6	33.6	57.8	5.6	19.6	30.8
Lion	5.7	223.6	198.2	4.0	19.4	17.1	1.5	38.4	67.4	28.7	19.4	38.1
Melody	4.5	121.5	245.6	4.0	21.7	18.8	1.9	13.6	45.7	42.2	16.0	18.9
Nordic IV	4.8	241.3	279.8	3.6	21.9	15.0	1.0	35.0	75.6	23.6	14.2	28.1
Spring Field	5.0	258.6	248.8	2.9	18.3	18.7	0.8	31.1	62.1	14.6	12.1	25.5
Mean	5.4	216.3	248.0	3.6	18.0	15.0	1.1	20.2	46.8	22.1	13.0	21.3
$LSD_{0.05}$	2.1	110.0	98.2	1.5	5.6	2.6	1.0	10.3	21.4	20.0	11.6	10.6

[z] The last six genotypes are commercial cultivars.