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. 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 Race 10 of downy mildew pathogen and Verticillium wilt disease were found in the USDA spinach genebank. The results suggest that improvements for genetic resistance to these diseases seem feasible in spinach. Indeed, research is currently in progress to incorporate the resistance traits identified in this study into elite cultivars in our spinach breeding program. We are also breeding spinach for resistance to leafminers. Impatiens Necrotic Spot Virus (INSV) was detected on several commercial spinach cultivars in an experimental field in Salinas in October 2008. INSV was again found in a spinach experimental field in Salinas in October 2009. This shows that INSV is here to stay in spinach. The INSV from lettuce can be sap transmitted to spinach. Numerous lettuce fields in the Salinas Valley tested positive for INSV in recent years, this suggests that the INSV in spinach came from lettuce. We also found significant differences in resistance to beet necrotic yellow vein virus (BNYVV, normally a sugarbeet pathogen) among spinach cultivars. Vein-clearing symptoms of BNYVV showed up in spinach as early as 28 days after planting (4 - 6 true leaf stage). Sugarbeet was widely grown in California 10 years ago. BNYVV and its vector (*Polymyxa betae*) can persist in soil for more than 20 years. The increasing acreage of spinach host may increase BNYVV in soil. Diseased spinach plants were found in a grower's field in Ventura County last year and were tested positive for an aggressive (resistance-breaking) strain of BNYVV. The results suggest that BNYVV is a new threat to spinach production in California.

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Objective 1. Screening for Resistance to Downy Mildew Race 10 in the USDA Spinach Germplasm Collection.

Procedures.

Plant materials. Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, CA. The 135 accessions from the USDA spinach collection (seeds provided by the North Central Regional Plant Introduction Station, Iowa State University, Ames, IA) and 7 commercial cultivars were screened for resistance to downy mildew in a greenhouse test. The USDA collection includes accessions of cultivated spinach (*Spinacia oleracea*), and wild species *S. turkestanica* and *S. tetrandra*. Cultivar 'Viroflay' with no resistant gene was the susceptible control and 'Lazio' served as the resistant control. The experimental design was a randomized complete block with two replications. In each replication, 8 seeds of each accession was planted in Sunshine Plug 5 Growing Mix (Sun Gro Horticulture, Inc., Bellevue, Wash.) in plastic transplanting trays (128 cells, 3 x 3 x 5 cm in length x width x height) in a greenhouse.

Inoculations. Downy mildew Race 10 inoculums was increased on cultivar 'Tarpy' that has resistance to Race 1-7. The inoculums was also tested against a set of downy mildew race differentials. Only fresh inoculums were used to screen germplasm. Leaves with pathogen

spores were put into a flask with refrigerated distilled water and shaken vigorously. The spore suspension was poured through two layers of cheesecloth to remove debris and adjusted to 3.0 x 10^5 spores/ml. Spores were sprayed onto three-week-old spinach seedlings of different accessions with a hand-held mister until runoff. Plants were incubated in a dew chamber at 18^{o}C and 100% relative humidity for 48 hours, and then in a growth room for 5 days. Plants were then returned to the dew chamber maintained at 18^{o}C and 100% relative humidity for 24 hours to induce sporulation.

Disease Evaluations. After 24 hours in a dew chamber to induce sporulation, cotyledons and true leaves of the plants were rated qualitatively and quantitatively with the aid of a Opti Visor magnifier. Cotyledons were evaluated for the presence or absence of sporulation. True leaves were rated for the percentage of leaf area with symptoms and sporulating lesions. Disease incidence (% diseased cotyledons and true leaves) and severity (% diseased leaf area) of each genotype were calculated.

Results and Discussion.

There were significant differences in both incidence and severity of the disease among the genotypes tested. Of the 142 genotypes evaluated, disease incidence on cotyledons ranged from 0 to 55% (Fig.1). Similarly, disease incidence on true leaves ranged from 0 to 100% (Fig. 2). Disease severity on true leaves had a range of 0 to 100% (Fig. 3). These results show that there is large genetic variation in resistance to downy mildew Race 10 in the USDA spinach germplasm collection we screened.

Eight accessions, Ames 20169, Ames 26366, NSL 22149, NSL 4652, NSL 6083, NSL 6094, NSL 92009, and PI 169683, had no disease incidence and severity on cotyledons and true leaves, just like the resistant control 'Lazio' (Table 1). These genotypes may be potential source of resistance against the downy mildew Race 10.

It is interesting that six accessions, NSL 184398, NSL 42771, NSL 4683, NSL 6098, NSL 6782, and PI 169674, had varying disease incidence on cotyledons, but showed no disease incidence on true leaves (Table 2). This could suggest that these genotypes might have quantitative resistance against Race 10. Quantitative resistance (controlled by many genes) is considered more durable than the qualitative resistance (conferred by a few major genes), because it may be harder for the pathogen to mutate and overcome many genes.

However, these results are preliminary and the experiments need to be repeated to confirm the findings. We will test the putative resistant accessions again with more replications and controls in future experiments.

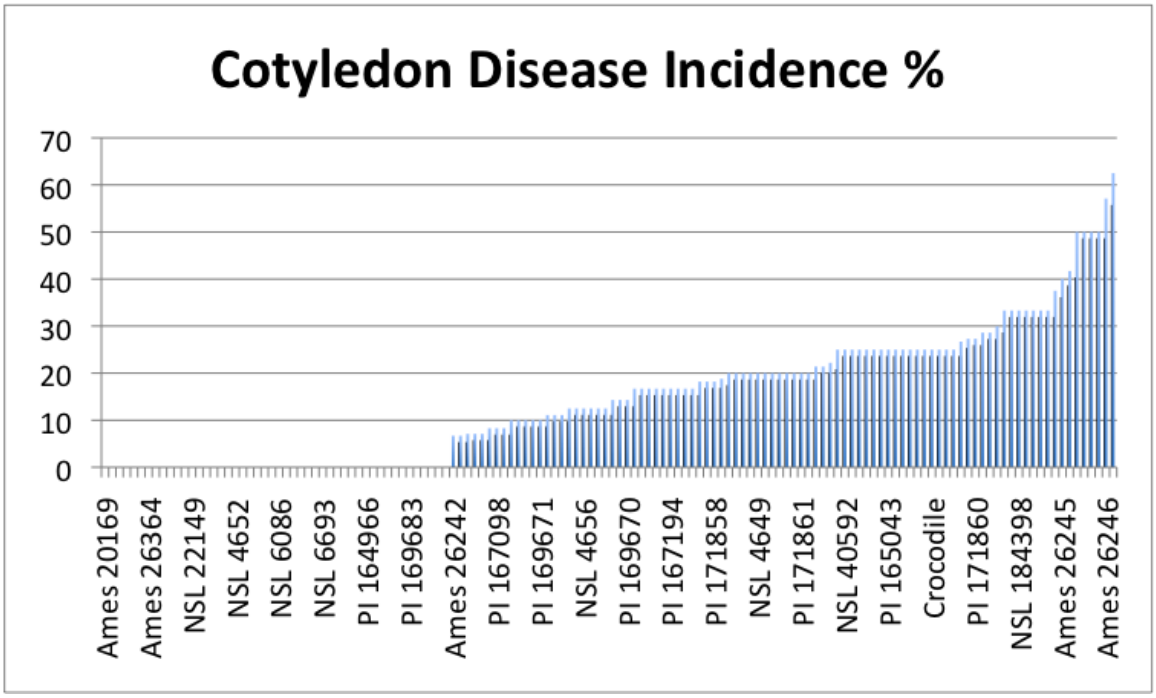

Fig. 1. Downy mildew Race 10 disease incidence % on cotyledons of the USDA spinach germplasm collection.

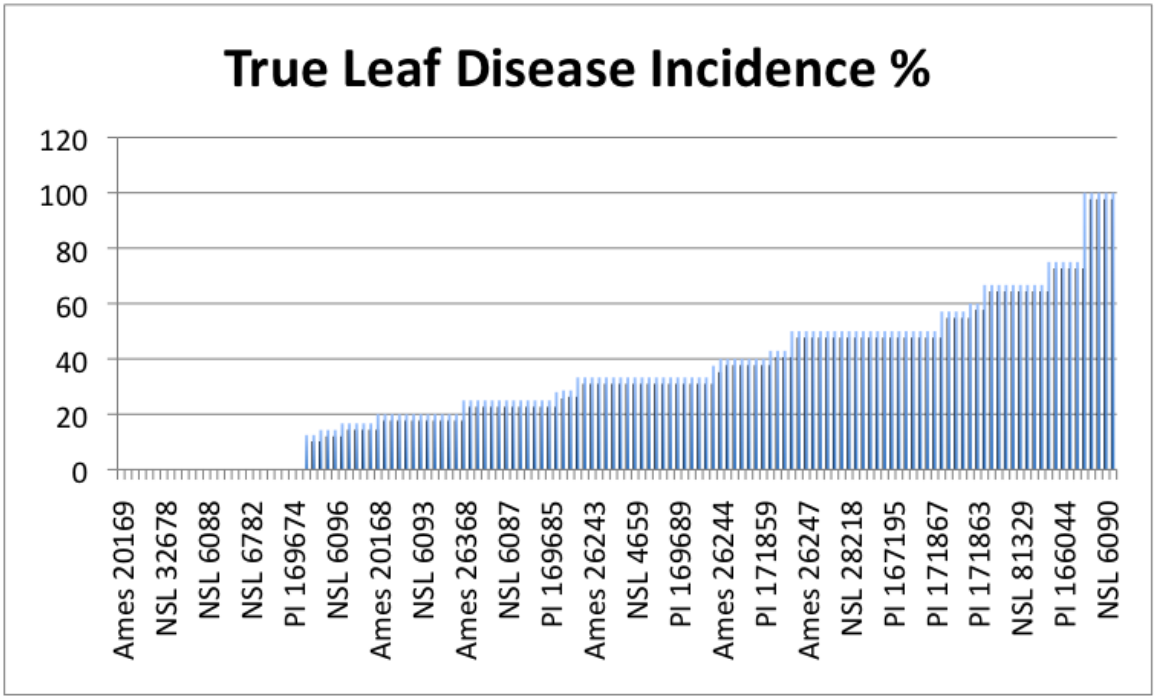

Fig. 2. Downy mildew Race 10 disease incidence % on true leaves of the USDA spinach germplasm collection.

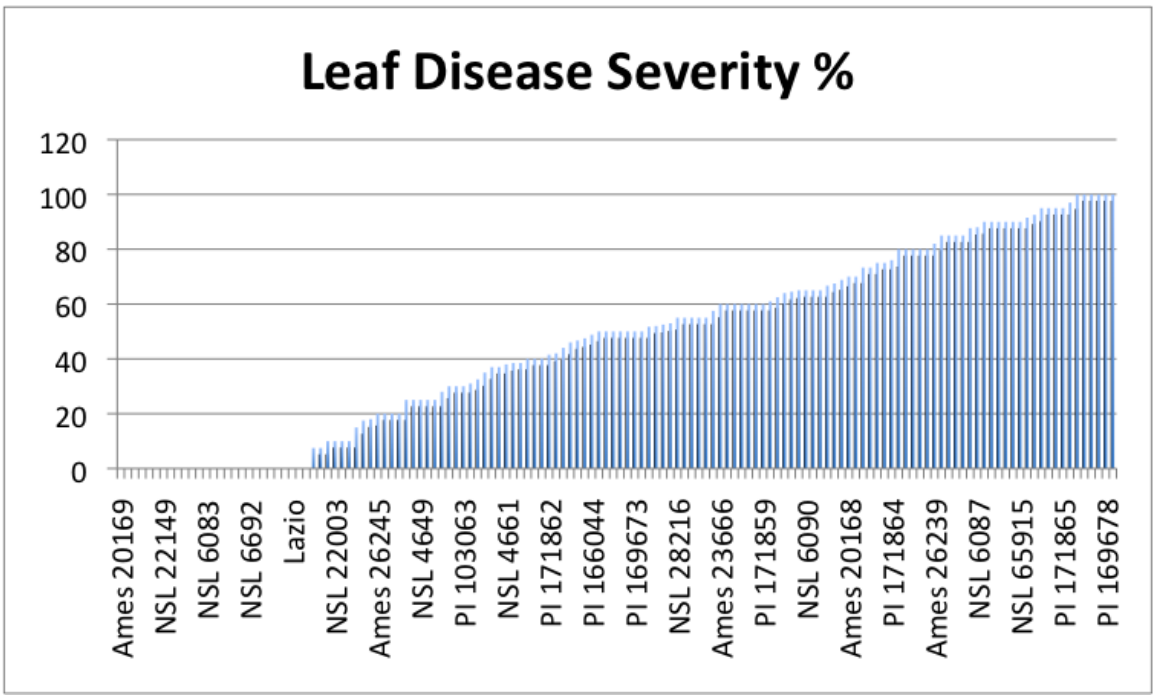

Fig. 3. Downy mildew Race 10 disease severity % on true leaves of the USDA spinach germplasm collection.

Table 1. Downy mildew Race 10 disease incidence and severity on cotyledons and true leaves of selected commercial cultivars and accessions of the USDA spinach germplasm collection.

	Incidence %		Leaf
Genotype	Cotyledon	True leaf	severity %
Dolphin	16.7	33.3	25.0
Lion	0.0	100.0	80.0
Crocodile	25.0	33.3	17.5
Lazio	0.0	0.0	0.0
Ames 20169	0.0	0.0	0.0
Ames 26366	0.0	0.0	0.0
NSL 22149	0.0	0.0	0.0
NSL 4652	0.0	0.0	0.0
NSL 6083	0.0	0.0	0.0
NSL 6094	0.0	0.0	0.0
NSL 92009	0.0	0.0	0.0
PI 169683	0.0	0.0	0.0

Table 2. Downy mildew Race 10 disease incidence on cotyledons and true leaves of selected accessions of the USDA spinach germplasm collection.

	Incidence %	
Genotype	Cotyledon	True leaf
NSL 184398	33.3	0.0
NSL 42771	20.0	0.0
NSL 4683	20.0	0.0
NSL 6098	50.0	0.0
NSL 6782	16.7	0.0
PI 169674	33.3	0.0

Objective 2. Screening for Resistance to Verticillium wilt in the USDA Spinach Germplasm Collection (with Krishna Subbarao, Steve Klosterman and Karunakaran Maruthachalam).

Procedures.

Plant materials. Sixty accessions from the USDA spinach collection that have not been screened plus 9 commercial cultivars were screened for resistance to Verticillium wilt in a greenhouse test. The experimental design was a randomized complete block with five replications. In each replication, 8 seeds of each accession were planted in Sunshine Plug 5 Growing Mix in plastic transplanting trays (128 cells, 3 x 3 x 5 cm in length x width x height) in a greenhouse in winter to control day length.

Inoculations. Three replications were inoculated with a Race 1 isolate So923 from spinach while the other two replications were used as uninoculated checks. Seedlings were inoculated at 2, 3, and 4 weeks after sowing by saturating the soil in each plug tray well with a 3-ml suspension containing 10^6 conidia/ml in sterile, distilled water. Seedlings were incubated for another week after last inoculation and then transplanted into 0.5-liter (16 oz) foam-insulated cups filled with a pasteurized sand:potting soil mixture (3:1, vol/vol). Two weeks after transplanting, day length was extended to 18 hr/day by supplemental lighting to promote bolting, as symptoms of Verticillium wilt on spinach mainly develop after bolting stage.

Evaluations. Starting from three weeks after last inoculation, severity of symptoms were rated weekly using a scale of 0 to 4: 0 = no symptoms, 1 = lower leaves with patches of yellow areas, 2 = middle leaves with patches of yellow areas, 3 = upper leaves with patches of yellow areas, and 4 = all leaves died. After final rating, roots were cleaned of sand and cut longitudinally to evaluate disease severity as the % brown discoloration of vascular tissue in the roots, crown, and lower stem, characteristic of Verticillium wilt. The growth period of the inoculated plants was compared with the uninoculated control. To confirm the presence of the pathogen, *V. dahliae* was re-isolated from diseased tissue. Roots, crown, and lower stems were placed on NP-10 medium and examined microscopically for development of conidiophores and/or microsclerotia of *V. dahliae*.

Results and Discussion.

The Verticillium disease incidence varied greatly among the 69 genotypes, ranging from 0 to 100% (Fig. 4). Thee were also large variation in disease severity among different accessions and cultivars, which ranged from 0 to 3 (Fig. 5). These results suggest that there are significant genetic differences in Verticillium disease resistance among the genotypes tested.

Four USDA spinach germplasm accessions, NSL 6097, NSL 6092, Ames 26364, and PI 167194, had low or no disease incidence, severity, root discoloration, and reduction of growth period (Table 3). The nine commercial cultivars al had higher disease ratings than these accessions (Table 3). These spinach genotypes could potentially serve as source of resistance to Verticillium wilt disease. Again, these are preliminary experiment results and need to be confirmed in further testing. We are also testing the resistance of these accessions against Race 2 of Verticillium wilt disease. Nevertheless, these results are encouraging and suggest that the development of Verticillium-resistant spinach cultivars is feasible.

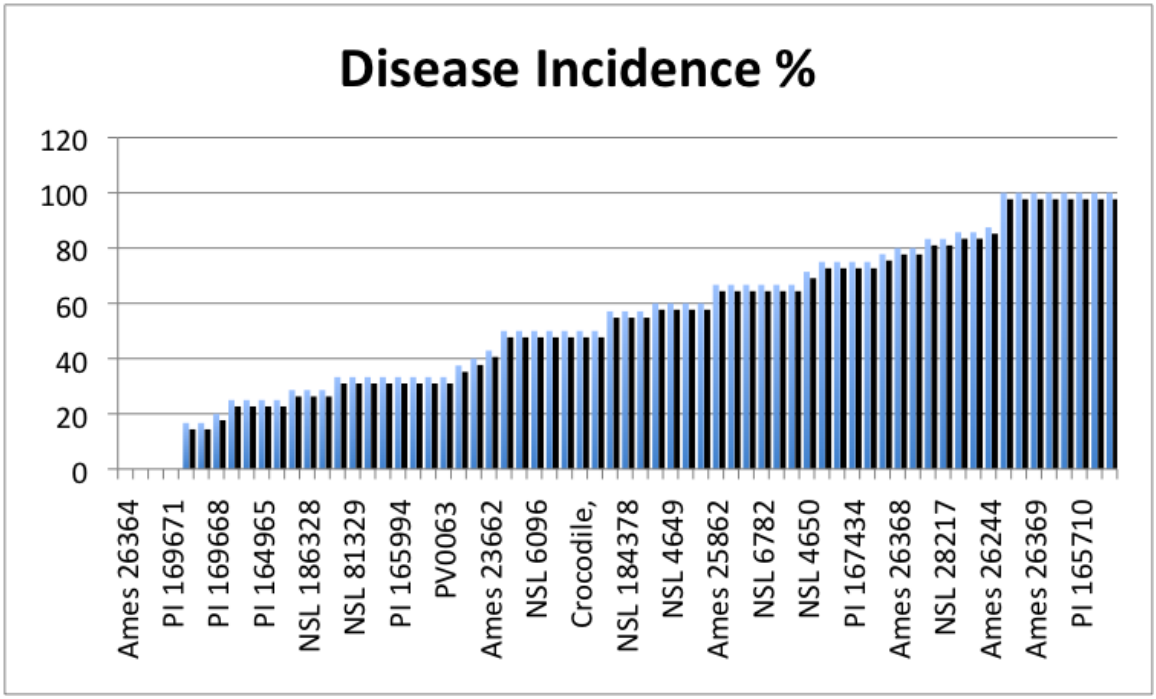

Fig. 4. Verticillium wilt disease incidence of 60 accessions from the USDA spinach germplasm collection and 9 commercial cultivars.

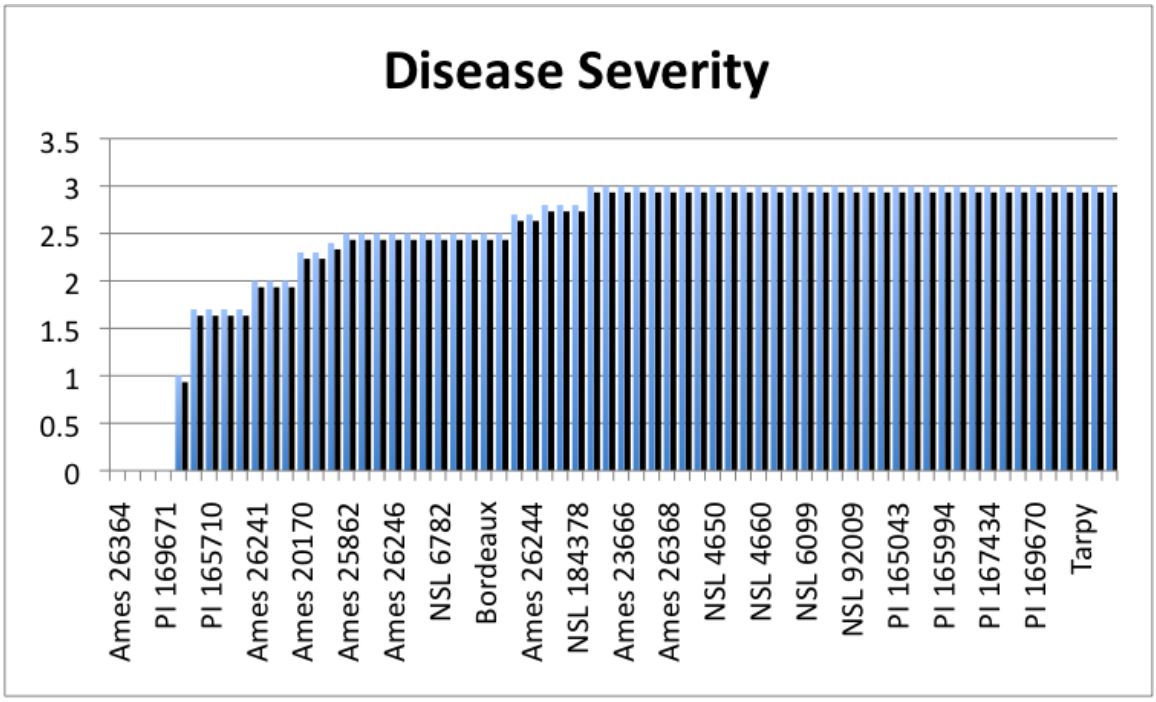

Fig. 5. Verticillium wilt disease severity of 60 accessions from the USDA spinach germplasm collection and 9 commercial cultivars.

Table 3. Verticillium disease incidence, severity, root discoloration, and reduced growth period as compared to uninoculated control of selected commercial cultivars and accessions of the USDA spinach germplasm collection.

Genotype	Incidence %	Severity 0 – 3	% Root discoloration	Reduced growth, day
Hector	83.3 A	2.3 ABC	100.0 A	4.3 BC
Bordeaux	75.6 AB	2.2 ABC	100.0 A	6.1 BC
Tarpy	61.1 ABC	3.0 A	80.0 AB	16.8 A
Avenger	61.1 ABC	3.0 A	100.0 A	14.3 AB
Space	55.5 ABC	2.7 AB	61.7 AB	7.5 ABC
Polar Bear	47.2 BCD	2.6 AB	97.8 A	9.8 ABC
Tyee	33.3 CDE	1.3 BCDE	36.7 BC	0.7 C
Crocodile	27.8 CDE	2.6 AB	100.0 A	9.6 ABC
Lazio(PV63)	27.8 CDE	1.7 ABCD	66.7 AB	12.7 AB
NSL 6097	16.7 DE	0.3 DE	0.0 C	0.0 C
NSL 6092	8.3 E	1.0 CDE	33.3 BC	0.0 C
Ames 26364	0.0 E	0.0 E	0.0 C	4.2 BC
PI 167194	0.0 E	0.0 E	0.0 C	5.3 BC

Other Research Projects:

Leafminer A recurrent selection method was used to increase the level of resistance to leafminers in 10 populations of different leaf types. Plants with fewer leafminer stings or mines were selected and transplanted into isolators to produce seeds for further rounds of evaluation and selection.

Beet Necrotic Yellow Vein Virus (BNYVV, with Hsing-Yeh Liu, Kelley Richardson, Sharon Benzen, and Steve Koike) To investigate whether BNYVV can cause disease in spinach, 8 commercial spinach cultivars were planted in two fields with BNYVV activity and two control fields. Many plants on the BNYVV fields showed disease symptoms of yellow-green or light-green vein clearing, mottling or yellow-green chlorotic lesions on younger leaves. Leaves may also become stiff, more crinkled, and necrotic. There is an increase of lateral roots compared to healthy plants. Infected plants often become stunted, deformed, wilted, or dead. Symptomatic leaves and roots from plants with or without leaf symptoms all tested positive for BNYVV by ELISA. There were significant differences in disease incidence among cultivars, ranging from 8%(Unipack 277) to 44%(Polar Bear). Vein-clearing symptoms showed up as early as 28 days after planting (4 - 6 true leaf stage). Sugarbeet was widely grown in California 10 years ago. BNYVV and its vector (*Polymyxa betae*) can persist in soil for more than 20 years. The increasing acreage of spinach host may increase BNYVV in soil. Diseased spinach plants were found in a grower's field in Ventura County last year and were tested positive for the aggressive (resistance-breaking) strain of BNYVV (Liu, et al., 2010). The results suggest that BNYVV is a new threat to spinach production in California.

Impatiens Necrotic Spot Virus (INSV, with Hsing-Yeh Liu) We reported at last CLGRP annual meeting that INSV was detected on several commercial spinach cultivars in an experimental field in Salinas in October 2008. INSV was again found in a spinach experimental field in Salinas in October 2009. Infected spinach plants exhibited severe stunting, interveinal yellowing, thickening, deformation, wilting, and death. Symptomatic plants were positive for INSV and negative for TSWV, CMV, and TMV with immunostrips (Agdia). This shows that INSV is here to stay in spinach. The INSV from lettuce can be sap transmitted to spinach. Numerous lettuce fields in the Salinas Valley tested positive for INSV in recent years, this suggests that the INSV in spinach came from lettuce.

Publications relevant to this project in 2009-10:

Liu, H. Y., B. Mou, K. Richardson, and S.T. Koike. 2010. First report of *Beet necrotic yellow vein virus* infecting spinach in California. Plant Disease 94(5): 640.

Liu, H. Y., J. L. Sears, and B. Mou. 2009. Spinach (*Spinacia oleracea*) is a new natural host of *Impatiens necrotic spot virus* in California. Plant Disease 93(6): 673.