Screening and breeding for resistance to leafminer (*Liriomyza langei*) in lettuce and spinach

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Abstract: Leafminer (*Liriomyza langei* Frick) is a major pest that causes considerable damage to a wide variety of vegetable crops including lettuce and spinach. Forty-eight lettuce cultivars and introduction lines and 338 spinach accessions were screened in an insect cage for leafminer resistance. Significant genetic variation for leafminer stings per unit leaf area was observed among genotypes tested. Resistant lines with fewer leafminer stings were found in cultivated lettuce and spinach, *Lactuca saligna, L. serriola, and L. virosa*. The resistance in lettuce was confirmed in a field experiment. Crosses were made to combine leafminer resistance and superior horticultural traits in crisphead, green leaf, red leaf, romaine, and butterhead lettuces. Leaf miner resistant plants were selected in F_2 progenies of such crosses, and were backcrossed to restore horticultural type. A phenotypic recurrent selection method was used to increase the level of leafminer resistance in spinach.

Keywords: *Lactuca sativa, L. serriola, L. saligna, L. virosa*, *Spinacia oleracea*, *S. tetrandra, S. turkestanica,* insect resistance

Introduction

Leafminers are major insect pests of many important agricultural crops including lettuce and spinach (Parrella, 1987). Adult sting and larval mining of leaves reduce photosynthetic capacity, render leaves unmarketable, and provide an entrance for disease organisms (University of California, 1992; LeStrange et al., 1999). The principal leafminer species affecting vegetables include *Liriomyza brassicae, L. sativae, L. trifolii, L. huidobrensis*, and *L. langei.* Scheffer and co-workers (2001) identified the leafminers in central California as *L. langei* by using polymerase chain reaction (PCR) amplification of mitochondrial DNA.

Few studies on leafminer resistance in vegetables have been reported. Larval (*L. trifolii*) antibiosis was found in four interspecific hybrids of *Lycopersicon pennellii, L. cheesmanii, and L. hirsutum*, and adult antibiosis and antixenosis for feeding was partially a result of the tomato plant's trichome exudates (Erb et al., 1993). No resistance to leafminers (*L. trifolii*) has been observed in cultivated celery, but an accession from a wild species, *Apium prostratum,* was found to be practically immune (Trumble and Quiros, 1988). No feeding or oviposition was observed in this species. It has been used in a backcross program in an attempt to develop leafminer-resistant celery lines (Quiros, 1993). In addition, an accession from another wild species, *A. nodiflorum,* demonstrated substantial insect toxicity; few mines were observed and no larvae survived to the pupal stage (Trumble et al., 1990). In lettuce, significant differences among four romaine cultivars were found in the number of pupae produced and in the total numbers of stipples when exposed to leafminers (*L. trifolii*), but these differences resulted from differences in adult survival. Female leafminers survived significantly longer and produced more pupae on the cultivar Tall Guzmaine than on three other cultivars (Nuessly and Nagata, 1994; Nagata et al., 1998).

Lettuce and spinach cultivars highly resistant to leafminers are not presently available. The genetic variation of leafminer resistance in the germplasm of lettuce and spinach, including the wild species, has not been fully explored. The purposes of the present study

were to evaluate differences in leafminer resistance among lettuce and spinach genotypes and to incorporate the resistance into elite cultivars.

Material and methods

Lettuce

The experiments were conducted at the Agricultural Research Station of the U.S. Department of Agriculture, Salinas, California in 2002. Forty-eight genotypes from the lettuce germplasm collection maintained at the station were studied in an insect cage. They include cultivated lettuce (*Lactuca sativa*), and the wild species *L. serriola, L. saligna, and L. virosa* from different geographic areas of the world.

Three weeks after planting, 8 plants of each genotype were transplanted individually into plastic pots (10 x 10 x 10-cm) with soil. Plants were placed in a field cage (2 m high by 4 m wide by 8 m deep) made of polypropylene shade cloth and were arranged in a randomized complete block with a single plant as the experimental unit and 8 replications. Lettuce leaves with leafminer mines were collected from newly harvested fields around Salinas and leafminer larvae were allowed to emerge from the leaves and pupate. Pupae were collected and put in plastic containers to allow the adult flies to emerge. About 2,800 flies were released in the field cage. The leaf with the most leafminer stings on each plant was counted for the number of stings per 20-cm^2 leaf area with the aid of an optical glass binocular magnifier (OptiVisor, Donegan Optical Co., Lenexa, Kansas, USA) 7 days after the introduction of leafminer flies in the cage.

Fifty-four genotypes were also transplanted in a field on the station in summer. Each plot consisted of eight plants, with 30 cm between plants and 35 cm between rows on double-row beds with 1-m centers. The experimental design was a randomized complete block with 8 replications. The leaf with the most leafminer stings on each plant was counted for the number of stings per 20-cm^2 leaf area when plants reached maturity.

Data were analyzed statistically, using Analysis of Variance (ANOVA) with Microsoft Excel (Office 2001, Microsoft Co., Redmond, Washington, USA). The per plant values were averaged and the analysis was conducted on the basis of plot means for the field experiment. Means of different lettuce genotypes were compared with *t* tests (Petersen, 1985).

Spinach

We screened the spinach accessions from the USDA collection (maintained at Iowa State University, Ames, Iowa, USA) for leafminer resistance at our research station in 2001 in a preliminary study. The collection includes 332 accessions of cultivated spinach (*Spinacia oleracea*), 4 accessions of *S. turkestanica*, and 2 accessions of *S. tetrandra*. About 16 seeds from each accession were planted in a pot (10 x 10 x 10-cm) with sand: soil 2: 1 in the greenhouse, and thinned to 10 plants per pot. Plants were moved into a field cage (as described above) for screening three weeks after planting. Lettuce leaves with mines were collected from fields near Salinas and larvae were allowed to pupate and become adults. The adult (about 3,500) flies were then released in the cage to feed on the spinach plants. After 10 days, the 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), and the number of plants with mines for each accession was recorded.

Results and discussion

Significant differences among lettuce genotypes were found for the number of stings per unit leaf area, and the nine genotypes with fewest stings from the cage experiment are shown in

Fig. 1. Five *L. saligna* lines (PI 509525, PI 509519, PI 491159, PI 491207, and PI 261653), two *L. serriola* lines (PI 491251 and PI 281876), and a *L. virosa* line (PI 273597) had the least leafminer stings among genotypes tested. Among the cultivated lettuces (*L. sativa),* PI 187238 showed fewer leafminer stings per unit leaf area than other lines. 'Iceberg' registered the most sting damage among the genotypes tested. These results demonstrate the existence of large genetic variability in leafminer resistance in different lettuce species. However, all genotypes had at least a few stings, indicating that none of the genotypes tested was immune to leafminers.

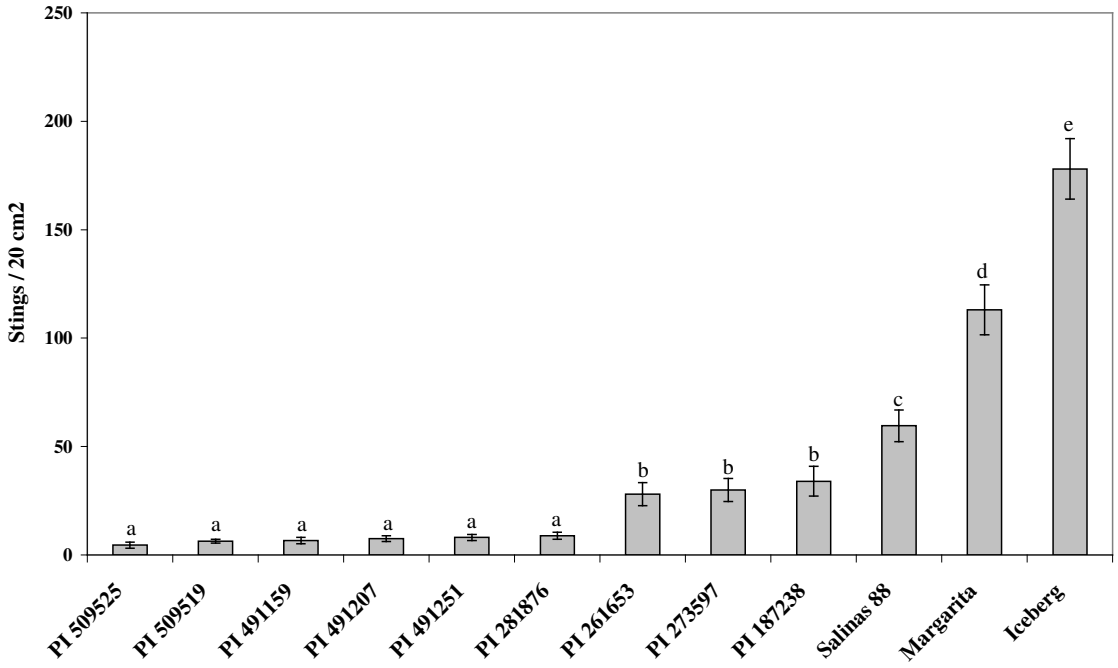

Figure 1. Mean and standard errors of leafminer stings per 20-cm^2 leaf area of selected lettuce genotypes tested in an insect cage. Means with same letters are not significantly different at *P* = 0.05 level.

The results from the cage test were confirmed in the field experiment (Fig. 2). Although the plants in the cage experiment and in the field trial were at different ages and were subjected to different environments and leafminer pressures, genotypes with fewer stings per unit leaf area in the insect cage also had fewer stings in the field. These similarities in performance suggest that differences in resistance were stable and a cage test can be used to screen lettuce plants for leafminer resistance.

We are attempting to incorporate the leafminer resistance into elite lettuce cultivars in a breeding program. Resistant sources were crossed to cultivars of crisphead, green leaf, red leaf, romaine, and butterhead type of lettuces, and the progenies were selected by the pedigree method. Since some resistant sources are from wild species, it may be necessary to backcross to the adapted cultivars a few times to restore the horticultural traits. The progenies of the crosses can also be used to study the inheritance of leafminer resistance. Crosses were also made among the resistant sources, and their progenies are being selected to elevate the level of resistance.

The 20 spinach accessions with the least leafminer stings and the 4 lines with the most stipples are listed in Table 1. There is much genetic variation in mean leafminer stings per cm^2 leaf area, ranging from 1.1 for PI 220121 to 13.4 for PI 212921. Percent of plants with

mines varied from 0 to 100 for different accessions. These results demonstrated that partial resistance to leafminer exists in spinach germplasm, which may serve as the foundation for a breeding program to increase the level of resistance and to transfer the resistance into elite spinach varieties.

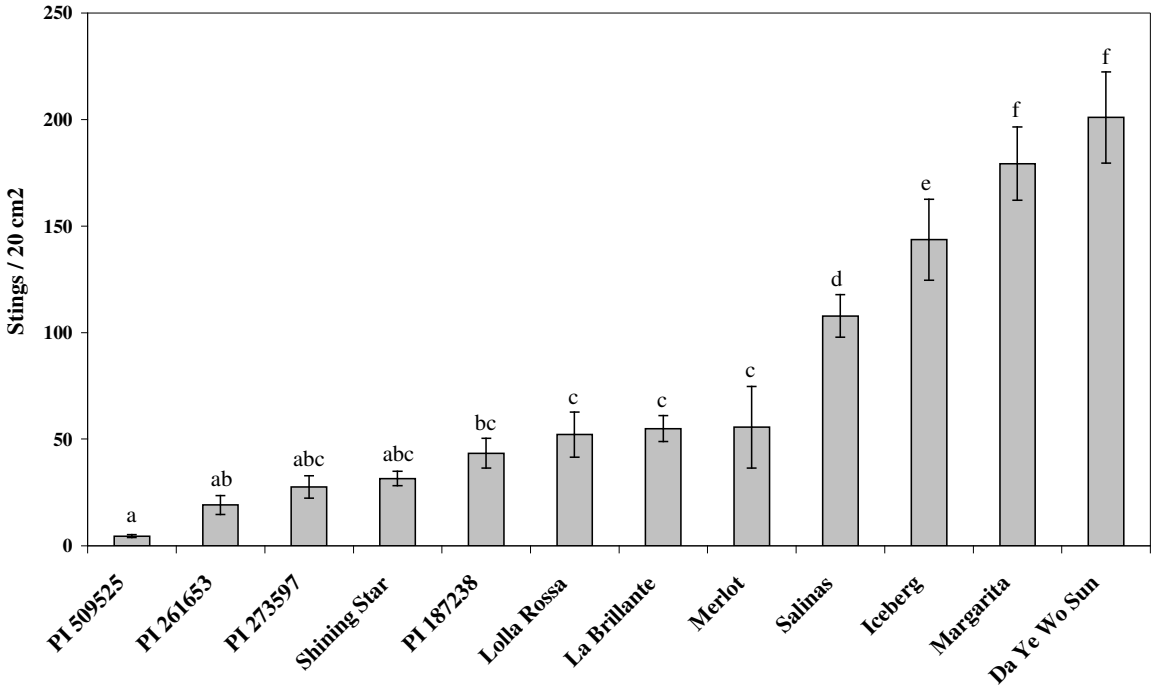

Figure 2. Mean and standard errors of leafminer stings per 20-cm^2 leaf area of selected lettuce genotypes tested in the field. Means with same letters are not significantly different at P = 0.05 level.

Table 1. Mean number of stings per cm^2 leaf area and percent of plants with mines for selected spinach accessions from the USDA collection at Iowa State University.

Accession	Mean stings / cm^2	% Plants with mines	Accession	Mean stings / cm^2	% Plants with mines
PI220121	1.1	14.3	PI358253	2.7	0.0
PI445783	1.6	0.0	PI531449	2.7	57.1
PI358248	1.9	0.0	PI531457	2.7	0.0
PI531454	1.9	12.5	PI261787	2.8	12.5
PI274059	2.0	0.0	PI274065	2.8	22.2
PI449353	2.0	25.0	NSL4683	2.8	10.0
PI604789	2.4	9.1	NSL6093	2.9	14.3
PI604791	2.4	45.5	PI604787	2.9	36.4
PI604783	2.5	33.3	PI171862	10.8	100.0
PI339545	2.6	33.3	PI223536	11.2	0.0
PI527332	2.6	33.3	PI175312	11.7	0.0
PI274058	2.7	20.0	PI212921	13.4	66.7

We are using a phenotypic recurrent selection method to increase the level of leafminer resistance in spinach. Plants with fewer leafminer stings and/or mines were selected from

resistant accessions and placed in glass isolators on the station to open-pollinate with each other. Seed harvested from these plants were planted in the field next season. Leafminer-resistant plants were again selected for another cycle of hybridization. This cycle will continue until a satisfactory level of resistance is reached. The method has been successfully used at the University of Arkansas to breed for white rust disease resistance in spinach (Goode et al., 1988). Starting with 27 yellow clover aphid-resistant plants selected from a wide diversity of germplasm, Gorz and co-workers (1979) used phenotypic recurrent selection to improve resistance to aphids in red clover. Through five cycles of testing and selection for yellow clover aphid resistance and three such cycles for pea aphid resistance, a synthetic variety ('N-2') was developed that had a high level of resistance to both aphids.

In summary, a wide range of genetic variation of leafminer resistance was found in different types and species of lettuce and spinach. Coupled with the stability of the trait, genetic improvement of both crops for leafminer resistance seems feasible. We are currently incorporating the resistance sources found in this study into elite cultivars in a breeding program.

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