Breeding for resistance to new and emerging lettuce diseases in California

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Abstract: Preventing crop loss due to diseases has historically been the primary focus of public lettuce (*Lactuca sativa*) breeding efforts in the United States. Recent years have seen a shift in the industry, with increasing percentages of romaine and mixed lettuces being grown under intensive production systems. Possibly related to this change, several diseases have recently been reported for the first time or have increased in incidence. Two of these, lettuce dieback and crown rot, affect primarily romaine lettuce, whereas a third, Fusarium wilt, threatens all types. Lettuce dieback is caused by soilborne viruses of the family Tombusviridae. This disease may be identical to 'brown blight', which was widespread in the 1940s but vanished when resistant crisphead cultivars were developed. Fusarium wilt of lettuce was initially observed in California in 1990, and first caused significant crop losses in both California and Arizona in 2001. Crown rot of romaine, now known as Phoma basal rot, was first observed in the Salinas Valley of California in 2001. The cause of this disease was recently identified as *Phoma exigua*. Progress and results of breeding for genetic resistance to these diseases will be discussed.

Keywords: *Lactuca sativa*, crown rot, *Phoma exigua*, basal rot, tomato bushy stunt virus, lettuce necrotic stunt virus, lettuce dieback, *Fusarium oxysporum* f. sp. *lactucae,* host resistance.

Introduction

Lettuce dieback was first observed in California in the mid-1980s, and reports of the disease have increased over the last 10 years. Complete crop losses have occurred in fields of romaine lettuce, and no commercial romaine cultivar has been shown to be resistant to the disease. In the U.S., romaine is a rapidly growing market segment, having increased 68% over the last five years (USDA, 2002). The disease has occurred in commercial fields of some leaf lettuce cultivars; however, symptoms have never been observed on any modern crisphead (iceberg) cultivars. Lettuce dieback is caused by several related tombusviruses including tomato bushy stunt virus (TBSV) and lettuce necrotic stunt virus (LNSV) (Liu et al., 1999; Obermeier et al., 2001). These are soilborne, highly stable, and mechanically transmitted, and have no known vector. The conditions affecting symptom development remain poorly understood. The disease is frequently observed in low-lying areas of fields with a prior history of flooding, suggesting that the virus may be carried in river water and/or that disease symptoms may be associated with increased root stresses such as those presented by excess moisture. No effective cultural or chemical control methods have yet been identified.

Fusarium wilt was first recognized on lettuce around 1955 in Japan and was attributed to *Fusarium oxysporum* f. sp. *lactucae* n. f. (Matuo and Motohashi, 1967). In 1995, a second race that was virulent on cultivars resistant to race 1 was found simultaneously in two areas of Japan. Resistance to both races has been reported in different cultivars (Fujinaga et al., 2001), but its inheritance is not known. Fusarium wilt was first observed in the U.S. in 1990 near Huron, California and was attributed to *Fusarium oxysporum* f. sp. *lactucum* (Hubbard and Gerik, 1993), but did not become a serious problem until 2001 in Huron and Yuma, Arizona. Most recently, *Fusarium* wilt was described in a California lettuce coastal district and in Italy (S. T. Koike, unpublished; Garibaldi et al., 2002). California isolates showed similar pathogenicity as Japanese race 1 and it has been proposed that f. sp. *lactucum* is identical to f. sp. *lactucae* (M. Fujinaga, personal communication).

In 2000, an unidentified crown rot disease was observed in a field of romaine lettuce in Watsonville, California. The disease occurred throughout the Salinas Valley in 2001, and was observed in both Santa Maria and Salinas Valleys in 2002. Infected plants initially had wilted outer leaves, and a characteristic dry, black, sunken lesion located on one side of the crown, which often extended deep into the center of the root. Infected plants often grew asymmetrically and were stunted on the side of the plant with the lesion. The disease was initially referred to as ‘crown rot of romaine’. The causative agent of the disease was recently identified as *Phoma exigua* (S. Koike, unpublished results), which has caused Phoma basal rot in greenhouse-grown lettuce in the U.K. (Anonymous, 1991). As a result, this name is being used to describe the crown rot disease caused by *P. exigua* in the field. Preliminary studies were conducted to determine the feasibility of controlling the disease chemically or culturally (Koike, unpublished results). Because Phoma basal rot has been observed almost exclusively in romaine fields, we hypothesized that genetic resistance exists in other lettuce types.

To survey the existing genetic variability in susceptibility to each of these diseases, we evaluated an array of lettuce germplasm including modern cultivars of all major types, including heirloom cultivars and plant introduction (PI) accessions. Our initial objectives were to identify sources of genetic resistance and to incorporate resistance into lettuce types where resistance is not available. Long term goals include determining the inheritance and mechanisms of identified resistance gene(s).

Materials and methods

Lettuce dieback resistance evaluation

Lettuce genotypes were direct seeded in commercial growers’ fields with prior histories of lettuce dieback. The heirloom crisphead cv. Iceberg and the modern crisphead cv. Salinas were used as susceptible and resistant controls, respectively. In each experiment, plants were monitored regularly for symptoms, and symptomatic susceptible control tissue was sampled to confirm the presence of the virus. Virus was detected by mechanical inoculation onto indicator plant species, by reverse transcriptase-polymerase chain reaction (RT-PCR), or both, using the methods described by Obermeier et al. (2001).

Fusarium resistance evaluation

Based on its pathogenicity on crisphead cvs. Empire and Vanguard in a preliminary test, isolate 121 collected from lettuce in Yuma, AZ (K. V. Subbarao, Univ. of California, Davis) was used. Three U.S. crisphead cultivars and the Japanese cv. River Green, which was reported to be resistant to race 1 (Zen Takamiya, Sakata Seed America, Inc., personal communication) were evaluated. The greenhouse testing protocol was established based on earlier work at Salinas (Hubbard and Gerik, 1993). Lettuce seeds were germinated in sand. Roots of seedlings at the first true leaf stage (ca. 14 days old) were soaked in a spore suspension (5 x 106 spores/ml) for 5-10 min and then individually transplanted into sand in Styrofoam cups and placed in a greenhouse. Symptoms were visible 10 days post inoculation (dpi) and plants were evaluated ca. 28 dpi. Plants were rated on a 1-4 scale: 1, no apparent disease or stunting; 2, slight to moderate stunting; 3, severe stunting and yellowing; 4, senesced. For each cultivar, 20 plants were inoculated, five plants were transplanted with no treatment, and five were mock-inoculated with distilled water. Unless otherwise specified, Student’s T test was used to compare treatment means.

Phoma basal rot resistance evaluation

Lettuce genotypes were direct-seeded in Spring, 2002 in a field where previous crops had exhibited high incidence of *Phoma* basal rot. Crisphead and leaf cultivars were included as resistant controls, based on extremely low incidence of basal rot in commercial fields of these types. Commercial romaine cultivars that had shown symptoms in 2000 and 2001 (e.g. Green Towers) were used as susceptible controls. Each genotype was planted in two replicates, each of which comprised a 25’ double-row bed. Disease incidence was assessed visually for 20 plants per genotype per replicate, by cutting each plant at the crown and determining presence or absence of the characteristic dry black lesion. Several samples were taken from plants located throughout the field to confirm the identity of the pathogen.

Table 1. Reaction of lettuce germplasm to field infections of lettuce dieback.

Type	Resistant		Susceptible			
Romaine	PI 491214		Athena	Green Towers	Gladiator	Coastal Star
	PI 491224		Caesar	Heart’s Delight	Lobjoits	Parris Island
			Clemente	Vert Maraichere	Red Cos	King Henry
			Darkland	Queen of Hearts	Red Eye	Signal
			Romance	Romaine du Prat	Verde	Valmaine
			Valcos	Romana Larga Verde	Majestic	
Leaf	Flame		Big Red	Red Oak Leaf	Merlot	Aragon Red
	Grand Rapids		Impulse	Red Salad Bowl	Rodan	Royal Red
	Pybas Green		Red Rage	Bronze Beauty	Rolina	Rubens Red
	Waldmann’s Green		Cavalry	Rossi di Trente	Redina	Selma Lolla
			Carnival	Red Grenobloise	Valeria	Lolla Rossa
			Deep Red	Black-Seed Simpson	Vulcan	Glossy Green
			Ferrari	Bronze Arrowhead	Prizehead	Green Vision
Crisphead	Imperial	Vanguard	Iceberg	New York		
	Salinas	Sharp Shooter				
	Glacier					
Butter	Dark Green Boston		Pavane	Cabernet Red	Kordaat	Ben Shemen
	Margarita		Saffier	Brune d’Hiver		
Primitive/ Stem	PI 171666	Balady Banha	Celtuce	Balady Aswan	Balady Cairo	
	PI 273589	PI 178924b				

Results and discussion

Lettuce dieback - germplasm evaluation and breeding

Over 100 cultivars and PI accessions of lettuce were evaluated in lettuce dieback-infested fields. Potentially resistant genotypes were tested in additional fields. Genotypes were classified as resistant if they were asymptomatic in at least two experiments in which susceptible controls were infected, and susceptible if they showed symptoms in one or more experiments (Table 1). All 21 romaine cultivars were susceptible, which is consistent with anecdotal evidence from commercial plantings. In contrast, none of the modern crisphead cultivars were susceptible, and no symptoms have been reported in commercial plantings of any crisphead variety. Heirloom crisphead cultivars, however, including Iceberg and New York, were susceptible. The disease ‘brown blight’ was common in the 1930s but disappeared when the susceptible ‘New York’ type cultivars were replaced by resistant ‘Imperial’ cultivars, and the causal agent was never identified (Jagger, 1940). The susceptibility of ‘New York’ and resistance of ‘Imperial’ and modern crisphead cultivars to lettuce dieback, in conjunction with similar symptoms and epidemiology, suggests that brown blight and dieback are the same disease. If true, this suggests that the resistance in crisphead cultivars has been effective and durable for decades despite widespread cultivation.

Although most leaf and butterhead cultivars tested were susceptible, four leaf and two butterhead cultivars were resistant, suggesting that there is variability within the commercial varieties of these types. There was also variability among the PI accessions evaluated. Several primitive or stem lettuces were resistant, but many, including several not reported in Table 1, were susceptible (data not shown). Two PI accessions with romaine-type horticultural characteristics, PI 491214 and PI 491224, were resistant. Of these, PI 491224 had superior type and was chosen for further development. PI 491224 was free of lettuce dieback symptoms in eight trials conducted over three years in six commercial growers' fields in CA. Over this period, selections from PI 491224 with improved uniformity and reduced incidence of tipburn were generated and released as dark green romaine breeding lines with open growth habits (01-778M, 01-781M, and 01-789M) (Grube and Ryder, submitted). Crosses were made between PI 491224 and several romaine cultivars to develop widely adapted cultivars with resistance to dieback and other diseases. Evaluation of these lines and segregating populations for inheritance studies is ongoing.

Fusarium

For 'Vanguard', 'Empire', and 'River Green', the mean disease ratings of inoculated plants were significantly higher than uninoculated controls (Table 2). Disease ratings of inoculated 'Salinas 88' plants were not significantly different from those of control plants. 'Vanguard' had a significantly higher mean disease rating and more severely infected plants than 'Empire'. This was consistent with observations of infected commercial crops in which 'Empire' was more resistant than 'Vanguard'-type cultivars. Both 'River Green' and 'Salinas 88' were resistant compared with 'Vanguard' and 'Empire'. The mean disease rating of 'Salinas 88' was significantly lower than that of 'River Green' at a = 0.05 (but not at a = 0.01), and 18/20 'Salinas 88' plants had the lowest disease rating, compared with 13/20 of 'River Green'. Examination of inoculated roots revealed that 'Salinas 88' had fewer discolored (7/20) roots than 'River Green' (16/20). For comparison, all 'Vanguard' plants and 16/20 'Empire' plants had discolored roots. Although evidence of root discoloration in 'Salinas 88' suggests that this genotype is not likely to be immune to the pathogen, our results suggest that 'Salinas 88' may have a higher level of resistance than 'River Green'. If this is confirmed, this will hasten the process of developing crisphead types adapted to the western U.S. production areas. Responses of these cultivars and additional candidate sources of resistance (M. Tsukada, personal communication) will be verified in field and additional greenhouse tests.

Table 2. Disease ratings of four lettuce cultivars inoculated with *Fusarium oxysporum*, 28 dpi.

	Mean disease rating		Disease rating			
Cultivar	Control	Inoculated	1	2	3	4
Empire	1.0	2.0**[x]	8[y]	5	6	1
River Green	1.0	1.5*	13	5	2	0
Salinas 88	1.0	1.1	18	2	0	0
Vanguard	1.0	3.2**	0	5	6	9
$LSD_{0.05}$	*ns*	*0.4*				

[x] control and inoculated are significantly different at the 0.05 (*) and 0.01 (**) level.

[y] number of plants (out of 16) with each disease rating.

Phoma basal rot

Romaine, crisphead and leaf lettuce cultivars and romaine and stem-type PI accessions were evaluated for incidence of Phoma basal rot in a commercial field plot in Spring, 2002. Over 80% of the plants of the most severely affected cultivar, ‘Green Towers’, showed symptoms of Phoma basal rot. For 6/7 romaine cultivars evaluated, disease incidence was over 50%. The two crisphead and 5/6 leaf cultivars tested had significantly fewer diseased plants than ‘Green Towers’. All cultivars had at least 10% disease incidence. These data are consistent with reports of crop losses in commercial fields, where the highest proportion of plants with symptoms were observed in romaine fields. Although crisphead and leaf lettuces were generally less severely affected than romaine cultivars, these types were not immune, suggesting that yield losses may occur in these types when disease pressure is severe. In fact, moderate (8%, 13%) incidences of Phoma basal rot were reported for the first time in two commercial crisphead fields in 2002.

Our results suggest that, within a given type, cultivars vary in response to Phoma basal rot. For example, although most leaf cultivars tested were significantly more resistant than ‘Green Towers’, ‘Waldmann’s Green’ had over 60% infected plants. In contrast, the red romaine cultivar ‘Majestic’ had significantly lower disease incidence (30%) than ‘Green Towers’. Genetic variability was also detected among PI accessions. Out of 15 PI accessions evaluated, six were significantly more resistant than ‘Green Towers’; however, most exhibited undesirable traits such as woody stems, early bolting, and excessive lateral branching. Confirmation of these results and additional germplasm evaluation is ongoing, and will be used as a foundation for breeding efforts and to guide growers’ choice of cultivars. Future directions include transfer of resistance into more widely adapted romaine cultivars. A better understanding of the conditions that favor disease development will facilitate breeding and evaluation efforts, since laborious field screening is currently required to evaluate resistance. Additional control methods have been investigated, and preliminary results of our coworkers suggest that fungicide applications may also help reduce disease incidence in susceptible cultivars (S. Koike, unpublished results).

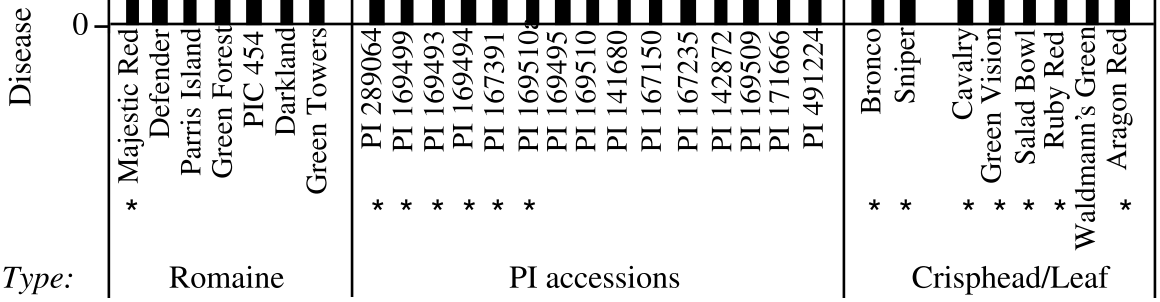

Figure 1. Incidence of Phoma basal rot in lettuce germplasm. Mean disease incidence plus standard deviation is given. * = significantly less than ‘Green Towers’ (Hsu’s MCB, $\alpha = 0.05$).

Acknowledgements

We thank R. Aburomia, A. Anchieta, C. Barr, P. Fashing, K. Kammeijer, D. Kile, C. Obermeier, E. Saavedra, J. Sears, B. Shepherd, and G. Wisler for technical assistance and discussions. We also thank commercial lettuce growers and the California Lettuce Research Board for generously providing field space for trials, information, and financial support for this work.

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