Variation for Resistance to Verticillium Wilt in Lettuce (*Lactuca sativa* L.)

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ABSTRACT

Hayes, R. J., Vallad, G. E., Qin, Q.-M., Grube, R. C., and Subbarao, K. V. 2007. Variation for resistance to Verticillium wilt in lettuce (*Lactuca sativa* L.). Plant Dis. 91:439-445.

Host resistance offers the most cost-effective method of Verticillium wilt control in lettuce (*Lactuca sativa*). In 2004 and 2005, 107 and 22 lettuce cultivars, respectively, were screened for resistance in a field infested with *Verticillium dahliae*, and disease progress on resistant and susceptible cultivars was determined. Greenhouse experiments were conducted to evaluate 16 cultivars for resistance to a race 1 and a race 2 isolate. Significant differences for resistance were observed within cultivated lettuce. In susceptible cultivars, disease levels increased through the season, whereas disease in resistant cultivars remained constant. Resistance in greenhouse tests was dependant upon the race used. Seven cultivars were resistant to race 1, whereas all were susceptible to race 2. Cultivar reactions to race 1 in greenhouse and field experiments were correlated, indicating the utility of greenhouse evaluations. The identification of resistance in diverse lettuce types is beneficial to the breeding process. However, because of the existence of resistance-breaking race 2 isolates, this resistance may not be durable. Alternatively, targeted releases of race-1-resistant cultivars to fields with only race 1 pathogen genotypes may extend the life of these cultivars.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a serious disease of lettuce (*Lactuca sativa* L.) that first was identified in the central coast of California in 1995 (20). Although the disease impacts a broad range of plants, it is especially devastating on lettuce, because plants often remain symptomless until they near vegetative or market maturity, at which time the symptoms develop quickly. Discoloration of the root vascular tissue is first evident at midheading, followed by foliar symptoms of angular chlorosis, necrosis, and wilting of basal leaves that progress acropetally and eventually lead to death as the plant reaches vegetative or market maturity (21). Verticillium wilt is most damaging in iceberg lettuce cultivars, where the basal "wrapper leaves" completely cover the outer part of the head as the disease progresses leading to early collapse and death. Lettuce heads with symptoms of Verticillium wilt are not marketable, and the disease threatens the agroeconomic sustainability of coastal lettuce production in California, which is a $1 billion industry (2). Furthermore, the pathogen is seed transmitted, which raises even greater concerns of potential spread to other lettuce production areas (23).

Cultural control methods for Verticillium wilt are expensive or of limited feasibility. Crop rotation is limited, because isolates of *V. dahliae* from lettuce are cross-pathogenic on many crops grown in the region (4,16). Soil fumigation is currently the only control method available for Verticillium wilt in lettuce. However, fumigation is cost prohibitive for lettuce; therefore, growers often rotate to strawberry production where the practice is standard. Host resistance to Verticillium wilt represents the best long-term control method for this disease in lettuce.

Substantial diversity exists within cultivated lettuce for resistance to biotic and abiotic stresses and for horticultural traits (18). Within cultivated lettuce, crisphead, romaine, butterhead, Latin, and red or green leaf can be recognized as distinct types with unique morphology (18). Within crisphead are the subtypes Batavia and iceberg, which are closely related and share many horticultural characteristics (5). Although modern iceberg and romaine cultivars are the most widely grown types in the United States, understanding the variation within each lettuce type for traits of economic significance is important. Lettuce may be derived from multiple gene pools (10), and each lettuce type represents an important and unique resource for the identification of new genes.

Resistance to Verticillium wilt has been identified in the Batavia cv. La Brillante and the Latin cv. Little Gem using disease screens conducted in infested field sites (R. J. Hayes, G. E. Vallad, R. Grube, and K. V. Subbarao, *unpublished data*). Although field testing has proven useful for screening lettuce germplasm and breeding resistance for Verticillium wilt, it has limitations because of time and space requirements and heterogeneous disease pressure due to variation in the environment and the pathogen distribution. In addition, consistent access to fields with a history of Verticillium wilt has been a problem, because growers often choose to fumigate these fields and grow strawberry in an attempt to limit the spread of the disease and continue lettuce production afterward. A greenhouse testing method that is correlated with field results is needed for further germplasm screening and future genetic studies. In greenhouse experiments, Vallad et al. (24) used a root dip inoculation method to test La Brillante, Little Gem, and the susceptible iceberg cvs. Salinas and Sniper against 30 isolates of *V. dahliae* and 2 isolates of *V. albo-atrum*. None of the *V. albo-atrum* or *V. dahliae* isolates from cruciferous hosts were pathogenic on the cultivars tested. However, the remaining *V. dahliae* isolates from various noncruciferous hosts, including lettuce, could be categorized further into two races based on a differential response of the resistant cultivars. Isolates designated as race 1 were pathogenic only on Salinas and Sniper, whereas isolates pathogenic on Salinas, Sniper, La Brillante, and Little Gem were designated as race 2. Furthermore, these races are genetically distinct based on the sequence of the intergenic spacer region from the nuclear ribosomal RNA gene complex (16). Resistance to race 1 lettuce isolates in modern cultivars, particularly the widely grown romaine and iceberg types, has not been reported, nor has resistance to race 2 isolates been identified in any *Lactuca* germplasm. The objectives of this research were to (i) determine the genetic variation for resistance to race 1 and race 2 isolates of *V. dahliae* causing Verticillium wilt in

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Accepted for publication 26 October 2006.

doi:10.1094/PDIS-91-4-0439

cultivated lettuce, (ii) determine the effectiveness of greenhouse testing for resistance to Verticillium wilt, and (iii) identify resistant parents for use in a lettuce-breeding program.

MATERIALS AND METHODS

Field experiments. Experiments were conducted in 2004 and 2005 in a *V. dahliae*-infested field located near Watsonville, CA. The field has a history of Verticillium wilt on lettuce, with an average microsclerotia count of over 60 microsclerotia g^{-1} of soil (G. E. Vallad and K. V. Subbarao, *unpublished data*). In all, 107 cultivars of Batavia, iceberg, romaine, butterhead, Latin, red leaf, and green leaf lettuce types were direct seeded on 7 May 2004 as a randomized complete block design with four replications. Each plot was 6 m long and consisted of two seed lines on 1-m-wide beds standard for lettuce production in coastal California. Plant spacing was approximately 30 cm between seed lines and 30 cm between plants within a seed line. All trials were maintained using standard cultural practices for coastal California lettuce production. Cultivars were evaluated for disease at market maturity, which varies widely between each lettuce type. Butterhead cultivars were evaluated 56 days after planting (DAP); Batavia, Latin, romaine, green leaf, and red leaf cultivars were evaluated 60 DAP; and iceberg cultivars were evaluated 68 DAP. The disease incidence in each plot was assessed by uprooting 10 plants, cutting each root longitudinally, and recording the number of plants exhibiting any amount of discoloration of root vascular tissues typical of Verticillium wilt. In 2005, 22 cultivars were reevaluated based on prior variation for Verticillium wilt incidence. These cultivars were sown on 26 August in the same field used for the 2004 experiments as a randomized complete block design with three replications. Rather than timing disease evaluations based on the market maturity of specific lettuce types, all plots were evaluated 83 DAP. Plant spacing, cultural practices, and disease assessment were identical to those in 2004.

Disease progress was evaluated in 2004 and 2005 using a subset of resistant and susceptible cultivars identified in previous field experiments. Plots (12 m long) of each cultivar were planted in a randomized complete block and disease incidence was determined on 10 plants per plot at three time periods through the season. In 2004, the resistant cvs. La Brillante, Little Gem, Pavane, and Merlot and the susceptible cvs. Salinas and Pacific were planted with four replications and evaluated 70, 75, and 84 DAP. In 2005, the resistant cvs. La Brillante, Little Gem, Pavane, and Merlot and the susceptible cvs. Salinas 88, Pacific, and Sniper were planted with three replications and evaluated 66, 73, and 83 DAP. Within-plot spacing, cultural management, and disease assessment were the same as in previous field experiments.

Greenhouse evaluations. Verticillium wilt resistance was evaluated in greenhouse experiments using 16 cultivars inoculated with *V. dahliae* isolates VdLs16 (race 1) and VdLs17 (race 2) that were prepared and maintained according to Vallad et al. (24). Seeds of each cultivar were sown in 200-well plug trays. Triplicate sets of plug trays were started, one tray for each isolate and the noninoculated control to insure no cross contamination during the experiment. Seed were first germinated at 10ºC in the dark for 48 h in a growth chamber, and grown at 20ºC with a 16-h photoperiod. Seedlings were inoculated at 2, 3, and 4 weeks after sowing by saturat-

Table 1. Verticillium wilt disease incidence of 107 Batavia, butterhead, green leaf, iceberg, Latin, red leaf, and romaine lettuce cultivars in a *Verticillium dahlia*-infested field in Watsonville, CA, in 2004

	Disease incidence (% symptomatic plants)				
			Relative marginal effect[a]		
				95% Confidence interval	
Cultivar	Median	Maximum	Estimate	Lower	Upper
Red Leaf					
Merlot	0	0	0.12	0.08	0.18
Battalion	5	10	0.20	0.13	0.31
Regiment	5	20	0.22	0.13	0.35
Sentry	5	30	0.23	0.13	0.38
Guardian	20	50	0.33	0.20	0.51
Red Grenoble	40	50	0.39	0.24	0.57
Aragon Red	45	100	0.56	0.33	0.77
Red Tide	55	70	0.59	0.51	0.66
Prizehead	70	90	0.62	0.48	0.74
Deep Red	80	100	0.74	0.54	0.86
Red Rage	95	100	0.80	0.68	0.88
New Red	95	100	0.82	0.71	0.88
Big Red	100	100	0.89	0.84	0.93
Green Leaf					
Plymouth	5	30	0.17	0.07	0.42
Two Star	5	30	0.17	0.07	0.42
Franklin	10	20	0.17	0.10	0.31
Deer's Tongue	15	30	0.27	0.17	0.41
Green Vision	20	30	0.34	0.26	0.44
Grand Rapids	20	70	0.42	0.18	0.71
Pybas Green	35	40	0.43	0.28	0.60
Alpine	40	50	0.50	0.30	0.71
Tehama	45	50	0.61	0.48	0.73
Waldmann's Green	50	60	0.68	0.50	0.81
Shining Star	50	60	0.75	0.67	0.82
Genecorps Green	60	80	0.78	0.59	0.89
Western Green	90	100	0.96	0.92	0.96
Romaine					
Defender	5	20	0.19	0.08	0.41
Infantry	5	20	0.19	0.08	0.41
Annapolis	10	20	0.22	0.11	0.40
Avalanche	10	20	0.22	0.11	0.40
Eruption	10	20	0.22	0.11	0.40
Heart's Delight	15	20	0.28	0.14	0.48
Braveheart	10	44	0.35	0.14	0.64
EXP1752	15	30	0.36	0.20	0.57
Caesar	15	50	0.38	0.14	0.71
Klamath	15	50	0.38	0.14	0.71
USDA03-243-3	15	60	0.40	0.14	0.75
Clemente	20	30	0.42	0.27	0.59
Triple Threat	20	30	0.42	0.27	0.59
King Henry	20	50	0.44	0.19	0.73
Green Towers	20	30	0.48	0.37	0.58
Heavy Heart	30	40	0.49	0.22	0.76
Conquistador	25	60	0.51	0.21	0.80
Parris Island Cos	25	50	0.52	0.28	0.75
VJO3R	25	50	0.52	0.28	0.75
USDA03-246-1	30	30	0.58	0.46	0.68
Camino Real	35	50	0.65	0.47	0.80
Darkland EL	35	50	0.65	0.47	0.80
PIC714	40	60	0.67	0.37	0.87
Fresh Heart	35	60	0.67	0.46	0.83
Passport	47	80	0.75	0.48	0.90
Sunbelt	40	90	0.77	0.56	0.89

(*continued on next page*)

[a] The relative marginal effect and 95% confidence intervals were calculated from analysis of the rank values of the disease incidence data.

ing the soil in each plug tray well with a 3-ml suspension containing 2 × 10^6 conidia/ml in sterile, distilled water. Seedlings were incubated for another 1 to 2 weeks after the third inoculation and transplanted into 0.5-liter foam-insulated cups filled with a pasteurized sand:potting soil mixture (3:1, vol/vol). Each cultivar–isolate treatment was arranged in a randomized complete block design with three replications of up to five plants per block. A nonreplicated block of noninoculated plants of each cultivar also was included. Plants were maintained in the greenhouse for approximately 8 to 10 weeks after transplanting until foliar symptoms were evident on the susceptible cultivars, and then evaluated for disease severity. Plants were uprooted and roots were cleaned of soil and cut longitudinally to evaluate disease severity by rating root discoloration on a 0-to-5 scale, where 0 = no vascular discoloration, 1 = 1 to 25% of the vascular tissue exhibiting discoloration; 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% discoloration in the absence of foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt. Plants with less than 100% root discoloration and foliar symptoms were not observed because the disease progresses acropetally (25). Root and crown tissues of inoculated and noninoculated plants were sampled periodically and placed on a potato dextrose agar medium to confirm the presence or absence of the pathogen. The first experiment was seeded on 10 August 2005 and rated 79 DAP, and repeated on 19 October 2005 and rated 96 DAP.

Table 1. (*continued from preceding page*)

	Disease incidence (% symptomatic plants)				
			Relative marginal effect[a]		
				95% Confidence interval	
Cultivar	Median	Maximum	Estimate	Lower	Upper
Coastal Star	50	50	0.81	0.73	0.87
Triton	45	90	0.82	0.67	0.90
Lobjoits	90	100	0.97	0.94	0.98
Iceberg					
Salinas 88	15	30	0.24	0.10	0.48
Sun Devil	20	20	0.25	0.17	0.35
Cannery Row	20	30	0.28	0.14	0.49
Vanguard	20	30	0.30	0.18	0.47
Laguna Fresca	15	40	0.31	0.13	0.60
Monument	15	50	0.32	0.10	0.69
Sure Shot	20	40	0.33	0.13	0.61
Pacific	25	30	0.33	0.16	0.56
El Dorado	20	40	0.34	0.13	0.66
Salinas	25	30	0.35	0.21	0.54
Tiber	20	40	0.39	0.22	0.59
Big Ben	25	50	0.41	0.16	0.72
Corona	20	50	0.42	0.20	0.68
Grand Slam	25	50	0.42	0.14	0.77
Trojan	30	30	0.44	0.33	0.56
Icon	30	40	0.45	0.25	0.67
Legend	30	50	0.47	0.20	0.75
Telluride	25	70	0.47	0.19	0.77
Bayview	30	50	0.48	0.21	0.77
Durango	30	50	0.49	0.25	0.74
Hallmark W	30	70	0.49	0.17	0.82
Empire	30	80	0.52	0.23	0.80
Home Run	35	50	0.54	0.27	0.78
Silverado	30	60	0.60	0.39	0.77
Liberty	35	50	0.63	0.46	0.77
Tribute	40	90	0.66	0.36	0.87
Great Lakes 54	40	50	0.67	0.53	0.79
Early Bird	40	50	0.72	0.63	0.80
Tiber-USDA	50	80	0.73	0.42	0.90
Sniper	70	100	0.73	0.22	0.95
Calmar	50	50	0.74	0.55	0.87
Vandenberg	45	60	0.78	0.65	0.87
Venus	45	70	0.79	0.64	0.88
Sharp Shooter	60	90	0.91	0.82	0.95
Butterhead					
Ostinata	0	20	0.29	0.14	0.55
Tania	0	30	0.33	0.14	0.66
Grappa	5	30	0.40	0.19	0.68
Allegiance	15	20	0.48	0.28	0.68
Martin	15	30	0.52	0.29	0.74
Bennett	20	50	0.60	0.29	0.83
Boston	20	30	0.63	0.42	0.80
Margarita	27	60	0.75	0.46	0.89
Latin					
Gallega	5	10	0.43	0.24	0.65
Little Gem	5	10	0.43	0.24	0.65
Pavane	5	10	0.43	0.24	0.65
Barnwood Gem	5	20	0.48	0.25	0.73
Sucrine	10	30	0.57	0.25	0.82
Brigade	15	30	0.67	0.35	0.85
Batavia					
La Brillante	5	10	0.39	0.28	0.54
Sierra	5	30	0.44	0.24	0.69
Batavia Reine des Glaces	20	60	0.64	0.34	0.83
Carnival	25	40	0.64	0.34	0.83

Statistical analysis. Disease severity data from greenhouse and disease incidence data from field experiments were analyzed using analysis of variance type statistics of ranked data using the PROC Mixed procedure in SAS (2004, version 9.1), and the LD_CI macro to generate relative marginal effects (RME) for each treatment and 95% confidence intervals for detection of statistical differences between treatments (6,19). The disease incidence data from the field experiments were calculated as the percentage of symptomatic plants and were analyzed as factorial experiments, with cultivars as fixed effects and blocks as random effects. The median and maximum disease incidence also was calculated for each cultivar. In 2004, the analysis was conducted separately for each lettuce type. Disease progress data was analyzed using cultivars, DAP, and cultivar–DAP as fixed effects and blocks as random effects. For both greenhouse experiments, disease severity values were averaged across the plants within each block prior to statistical analysis. The results from the two greenhouse experiments were consistent and, therefore, were pooled together into one factorial analysis having experiments and blocks as random effects and isolates and cultivars as fixed effects. Median and maximum disease severity was calculated for each cultivar. The noninoculated controls in the greenhouse experiments remained free of Verticillium wilt and were not included in the analysis. Spearman rank correlation was used to compare cultivar performance between the 2004 and 2005 field results and the race 1 and race 2 inoculations of each greenhouse experiment. The correlation was calculated using the percentage of symptomatic plants from each experiment. In the greenhouse experiments, the percentage of symptomatic plants was calculated using the number of plants with severity scores greater than 1 as the number of symptomatic plants.

RESULTS

Variation for disease incidence was observed within lettuce cultivars from seven diverse lettuce types in the 2004 field experiment. Significant differences for the RME of disease incidence were observed within red leaf, green leaf, romaine, and iceberg lettuce cultivars (Table 1). The red leaf cultivars were the most variable, with the median disease incidence ranging from 0 to 100% (Table 1). Significant differences within butterhead, Latin, and Batavia cultivars were not detected, with the lower variation explained by a higher frequency of cultivars with low disease incidence. For example, 53% of the butterhead, Latin, and Batavia cultivars had a median disease incidence ≤5%, despite the fact that these lettuce types represented only 17% of the cultivars in this trial. The Latin cultivars were the least variable for disease incidence, and exhibited a median disease incidence ≤15% and a maximum disease incidence ≤30% (Table 1). Ostinata, Tania, and Merlot were the only cultivars with a median disease incidence of 0; only Merlot was completely free of Verticillium wilt symptoms in this experiment (Table 1).

Several candidate resistant and susceptible cultivars were retested in 2005, and variation for disease incidence was observed. Furthermore, results from the 2005 experiment were consistent with those from 2004, with a significant correlation of 0.70 ($P = 0.0005$). In all, 13 romaine, Latin, red leaf, green leaf, and Batavia cultivars were free of Verticillium wilt symptoms, with a median and maximum disease incidence of 0, and with RMEs (0.28) significantly better than the 7 most susceptible cultivars (RME ≥ 0.68) (Table 2). Two Star (RME = 0.49) and Red Grenoble (RME = 0.58) exhibited intermediate disease incidence, and did not differ significantly from any other cultivar (Table 2). Iceberg cultivars were the most susceptible, with three of the four cultivars exhibiting a median disease incidence of 40% or higher (Table 2). Pacific, with a median disease incidence of 10% and a maximum disease incidence of 40%, had the least amount of disease of the iceberg cultivars. Salinas 88 had the most disease, with a median disease incidence of 70% (Table 2). Because Salinas 88 was the most resistant iceberg tested in 2004 (Table 1), there is a high likelihood that no useable levels of resistance are available within the iceberg cultivars tested.

Analysis of disease progress in 2004 and 2005 indicate that disease incidence over time in resistant cultivars remained constant whereas, in susceptible cultivars, disease incidence increased or remained at a consistently high level (Fig. 1). The cultivar–time interaction was significant in 2004 ($P = 0.02$) and in 2005 ($P = 0.03$), further indicating that this set of cultivars had different disease progress curves. When the first disease assessments were made 70 DAP in 2004, the RME of four of the six cultivars tested were not significantly different (Fig. 1). By 84 DAP, Little Gem, Pavane, Merlot, and La Brillante were significantly different from Pacific and Salinas (Fig. 1). Merlot and Pacific were the only resistant and susceptible cultivars that were not significantly different by the end of the experiment. A similar trend was observed in 2005. No significant differences for RME were present at 66 DAP. By 73 DAP, all resistant cultivars were significantly different from the susceptible cultivars, except for Little Gem and Pacific (Fig. 1). At 83 DAP, the resistant genotypes Merlot, Little Gem, La Brillante, and Pavane had no disease, and had significantly lower RME than Sniper, Pacific, and Salinas 88 (Fig. 1).

Variation for resistance to a race 1 isolate of *V. dahliae* was observed in greenhouse experiments (Table 3). Resistant, susceptible, and intermediate groups of cultivars were evident based on the significance groupings of RME. The cultivars resistant to the race 1 isolate were Annapolis, Defender, Eruption, Infantry (romaines), La Brillante (Batavia), Merlot (red leaf), and Pavane (Latin). These cultivars were free of disease symptoms (0 median and maximum disease severity) and had RMEs significantly lower than the six most susceptible cultivars (Table 3). The most susceptible cultivars were Heart's Delight, Franklin, Salinas 88, Salinas, Avalanche, and Two Star; these had median disease severities ≥1.1 and maximum disease severities of ≥3 (Table 3). Sentry, Regiment, and Plymouth formed a group of intermediate cultivars that had observable disease, but RME estimates not significantly different from the resistant cultivars (Table 3). These cultivars cannot be considered equivalent to the resistant cultivars because some symptomatic plants were identified, but could be distinguished from susceptible cultivars in that most plants had no disease (median disease severity values of 0) (Table 3). This indicates that, although symptomatic plants were present, most plants either were free or exhibited only weak symptoms of Verticillium wilt. The results from

Table 2. Verticillium wilt disease incidence for 22 lettuce cultivars in a *Verticillium dahliae*-infested field in Watsonville, CA, in 2005

		Disease incidence (% symptomatic plants)				
				Relative marginal effect[a]		
					95% Confidence interval	
Cultivar	**Lettuce type**	**Median**	**Maximum**	**Estimate**	**Lower**	**Upper**
Annapolis	Romaine	0	0	0.28	0.27	0.30
Barnwood Gem	Latin	0	0	0.28	0.27	0.30
Battalion	Red Leaf	0	0	0.28	0.27	0.30
Defender	Romaine	0	0	0.28	0.27	0.30
Eruption	Romaine	0	0	0.28	0.27	0.30
Infantry	Romaine	0	0	0.28	0.27	0.30
La Brillante	Batavia	0	0	0.28	0.27	0.30
Little Gem	Latin	0	0	0.28	0.27	0.30
Merlot	Red Leaf	0	0	0.28	0.27	0.30
Pavane	Latin	0	0	0.28	0.27	0.30
Plymouth	Green Leaf	0	0	0.28	0.27	0.30
Regiment	Red Leaf	0	0	0.28	0.27	0.30
Sentry	Red Leaf	0	0	0.28	0.27	0.30
Two Star	Green Leaf	10	20	0.49	0.17	0.82
Red Grenoble	Red Leaf	20	30	0.58	0.30	0.82
Pacific	Iceberg	10	40	0.68	0.53	0.79
Heart's Delight	Romaine	10	60	0.70	0.50	0.84
Lobjoits	Romaine	20	60	0.73	0.55	0.85
Avalanche	Romaine	20	58	0.75	0.63	0.83
Sniper	Iceberg	40	80	0.82	0.70	0.89
Salinas	Iceberg	60	80	0.86	0.77	0.91
Salinas 88	Iceberg	70	100	0.92	0.77	0.97

[a] The relative marginal effect and 95% confidence intervals were calculated from analysis of the rank values of the disease incidence data.

the two greenhouse experiments that evaluated cultivars against the race 1 isolate correlated not only with each other (r = 0.78, P = 0.0005) but also with results from the field experiments in 2004 (r = 0.66, $P < 0.0105$; and r = 0.51, P = 0.0455) and 2005 (r = 0.81, P = 0.0004 and r = 0.75, P = 0.0012), further substantiating the validity of the results.

Symptoms of Verticillium wilt were observed across all cultivars inoculated with the race 2 isolate of *V. dahliae* (Table 3). All of the cultivars resistant to race 1 were susceptible to race 2, and a significant genotype–isolate interaction was detected (P = 0.0004). Resistance to race 2, if any, was not expressed in the same manner as resistance to race 1 in this population of cultivars. The median disease severity values ranged from 1.5 to 4.3; no median disease severity values of 0 were observed as in race 1 inoculations (Table 3). Significant differences were detected; namely, that Salinas and Two Star were significantly more susceptible than 12 other cultivars. The cultivars Annapolis, Defender, Regiment, and Heart's Delight had the least disease; each cultivar exhibited a median disease severity of less than 2, but a maximum disease severity value greater than 2.6, indicating the potential for high disease (Table 3). Cultivar performance against the race 2 isolate in the two greenhouse experiments was not significantly correlated with either the 2004 field results (r = 0.15 and 0.05) or the 2005 field results (r = 0.36 and 0.01), or with those of the race 1 inoculations (r = 0.45, 0.45, 0.36, and 0.08). Furthermore, the two independent experiments using inoculations with the race 2 isolate were not significantly correlated (r = –0.07), and suggest that further testing is needed to fully characterize this population's variation for susceptibility to race 2 of *V. dahliae*.

DISCUSSION

Resistance to Verticillium wilt was identified in cultivated lettuce in field and greenhouse experiments. In greenhouse experiments, resistance was dependant upon the race of *V. dahliae* used in the inoculation. This finding supports a previous report on the existence of two races of *V. dahliae* among isolates pathogenic on lettuce (24). The correlation data between the field experiments and the race 1 greenhouse experiments suggest that a race 1 isolate or isolates mostly contributed to the disease symptoms observed in the field experiments. Furthermore, greenhouse tests were a useful alternative to field testing to determine resistance in lettuce to Verticillium wilt. Based on these tests, resistance to race 1 was identified in seven cultivars from four diverse lettuce types that are immediately useful in a breeding program as parents. No resistance was identified to the race 2 isolate.

Substantial diversity exists among those cultivars identified as resistant to the race 1 isolate. The horticultural characteristics of these genotypes vary considerably and, consequently, their use in the lettuce industry is diverse. Cv. La Brillante is an heirloom Batavia cultivar that is not used in commercial lettuce production. However, Batavia and modern iceberg cultivars both are subtypes of crisphead lettuce and share many horticultural characteristics. Because of this, La Brillante is an important parent for developing crisphead cultivars with resistance to Verticillium wilt. Among the resistant romaines identified, Annapolis, Defender, Eruption, and Infantry are predominantly used for baby leaf production, but not whole head production. Additionally, Annapolis, Eruption, and Infantry are red romaines, while Defender is a green romaine. Merlot is a dark red leaf cultivar also used for baby leaf production. Other cultivars, such as the Latin cvs. Pavane and Little Gem, have small acreage for whole head and baby leaf production. Resistance was not identified in any modern cultivar of any lettuce type that is grown exclusively for whole head production. How or if traits desirable for baby leaf production relate to Verticillium wilt resistance is not known and should be further investigated. Still, common ancestry may exist among these resistant cultivars. For example, Defender is a descendant of Pavane, and likely could have inherited its resistance from this source. Because pedigree information is limited, how the remaining resistant cultivars are related to each other is not exactly known. The Latin, Batavia, and butterhead-type cultivars were rich sources of resistance to Verticillium wilt. The butterheads were not pursued as sources of resistance because they are not widely grown in the United States. Consequently, considerable genetic variation for Verticillium wilt resistance may still exist in this group. The existence of resistance in cultivars from diverse lettuce types is beneficial to the breeding process. This diversity eliminates the need for between-type crosses (i.e., iceberg × romaine) to develop new cultivars for each market type, an approach that is more challenging than within-type crosses. Furthermore, the use

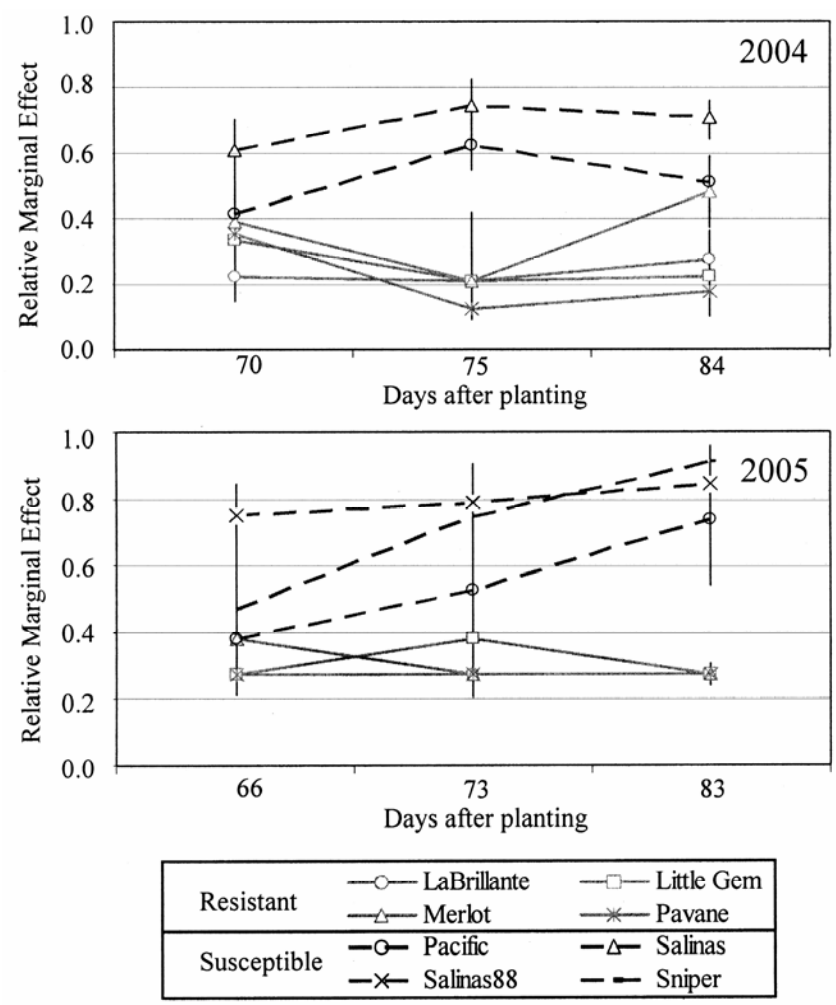

Fig. 1. Relative marginal effect of Verticillium wilt disease incidence over three evaluation dates in 2004 and 2005 *Verticillium dahliae*-infested field experiments. The relative marginal effect and 95% confidence intervals were calculated from analysis of the rank values of the disease incidence data. Error bars indicate 95% confidence intervals.

of diverse parents in breeding increases genetic variation and, thereby, can increase breeding efficiency for the many traits needed in lettuce cultivars.

On the basis of the disease progress data, disease incidence in susceptible cultivars increased throughout the season while disease remained constant in resistant cultivars. Consequently, evaluating disease too early in the season, even at market maturity, may lead to the misclassification of susceptible cultivars as resistant. For example, market maturity for iceberg cultivars in 2005 was 70 to 75 DAP. A clear difference between Little Gem (resistant) and Pacific (susceptible) was not evident until 83 DAP, more than a week past market maturity for Pacific and 2 weeks past market maturity for Little Gem. Disease development in susceptible lettuce genotypes may be related to plant development, as has been reported in other species (25). The resistant genotypes La Brillante, Pavane, Little Gem, and Merlot generally maintained minimal or no disease through the season despite progressing through an apparently normal plant development. Although we do not know the mechanism of resistance operating in these diverse cultivars, it suggests that some lettuce genotypes offer a high level of resistance in greenhouse and field environments that may be independent of plant development or protect the plants later into reproductive development. High levels of Verticillium wilt resistance that protect plants through flowering have been reported in other *Compositae* species (11,15). Sentry, Regiment, and Plymouth demonstrated resistance equivalent to La Brillante in field trials. However, a small number of plants infected with the race 1 isolate were identified in greenhouse trials. The green leaf cv. Two Star also had low levels of infected plants in both field trials, but it was among the most susceptible cultivars in greenhouse experiments. These results suggest that Sentry, Regiment, Plymouth, and Two Star differ from La Brillante and other similar sources of resistance. Further studies are needed to characterize Sentry, Regiment, Plymouth, and Two Star.

Table 3. Disease severity for 16 greenhouse-grown lettuce cultivars inoculated with race 1 or race 2 isolates of *Verticillium dahliae* in two independent greenhouse experiments

	Disease severity[a]				
			Relative marginal effect[b]		
				95% Confidence interval	
Cultivar	**Median**	**Maximum**	**Estimate**	**Lower**	**Upper**
Race 1					
Annapolis	0.0	0.0	0.17	0.15	0.18
Defender	0.0	0.0	0.17	0.15	0.18
Eruption	0.0	0.0	0.17	0.15	0.18
Infantry	0.0	0.0	0.17	0.15	0.18
La Brillante	0.0	0.0	0.17	0.15	0.18
Merlot	0.0	0.0	0.17	0.15	0.18
Pavane	0.0	0.0	0.17	0.15	0.18
Sentry	0.0	0.8	0.20	0.14	0.27
Regiment	0.0	3.0	0.28	0.15	0.47
Plymouth	0.0	2.0	0.27	0.16	0.42
Heart's Delight	1.1	4.8	0.28	0.22	0.69
Franklin	2.8	5.0	0.61	0.37	0.80
Salinas 88	3.0	3.0	0.68	0.49	0.82
Salinas	3.1	5.0	0.62	0.50	0.74
Avalanche	3.4	4.0	0.72	0.61	0.81
Two Star	4.5	5.0	0.88	0.76	0.94
Race 2					
Annapolis	1.5	2.6	0.44	0.32	0.57
Defender	1.7	4.7	0.53	0.33	0.72
Eruption	2.8	5.0	0.62	0.35	0.83
Infantry	2.7	4.8	0.65	0.47	0.79
La Brillante	2.5	3.8	0.62	0.51	0.71
Merlot	2.6	4.4	0.61	0.48	0.73
Pavane	2.7	4.5	0.61	0.38	0.79
Sentry	3.1	3.6	0.65	0.52	0.76
Regiment	1.8	4.6	0.54	0.38	0.70
Plymouth	4.3	5.0	0.76	0.39	0.94
Heart's Delight	1.5	3.6	0.46	0.31	0.62
Franklin	3.1	3.8	0.66	0.54	0.76
Salinas 88	4.0	4.8	0.59	0.46	0.71
Salinas	2.5	3.8	0.88	0.81	0.93
Avalanche	2.9	3.8	0.64	0.54	0.74
Two Star	4.3	5.0	0.89	0.83	0.94

[a] Disease severity scored on a 0-to-5 scale, where 0 = no discoloration to 5 = 100% discoloration and the presence of foliar symptoms.

[b] The relative marginal effect and 95% confidence intervals are calculated from analysis of the rank values of the disease severity data.

All of the genotypes tested in the greenhouse assay were susceptible to the race 2 isolate of *V. dahliae*. Although the cultivars tested differed in their susceptibility, none exhibited a level of resistance that would be useful. Lettuce and tomato are the only crop species reported to have well-defined races of *V. dahliae* that differ in virulence. In tomato, the closely linked Ve_1 and Ve_2 genes at the *Ve* locus condition high levels of resistance to race 1 isolates (7,9; Schiable et al. 1951). Race 2 isolates that overcome *Ve* resistance were identified quickly and now predominate in many tomato-production regions (1,14). Consequently, the usefulness of *Ve*-mediated resistance has been limited. La Brillante and Little Gem previously were reported as resistant to race 1, whereas Salinas and Sniper were susceptible (24). All four genotypes were susceptible to race 2. Similarly, in this research, seven genotypes were resistant to race 1 but susceptible to race 2. The race 1 and 2 lettuce isolates used in this study were collected from fields in Watsonville, CA, where the initial disease outbreak occurred in 1995 (K. V. Subbarao, *unpublished data*). The existence of race 2 isolates in lettuce production fields raises serious doubts regarding the durability of the race 1 resistance, and some infected resistant cultivars were observed in the 2004 field experiment. A race 2 isolate may have been responsible for symptoms on these resistant cultivars, and experiments are in progress to test several isolates collected. Why this was not observed in 2005 is not known. It is possible that race 1 isolates may be more fit than race 2 isolates and, thus, would become more prevalent in the field with subsequent lettuce crops (strawberry was the crop grown immediately prior to the 2004 experiment). Grogan et al. (8) found differences in virulence between race 1 and race 2 tomato isolates of *V. dahliae* on susceptible cultivars, with race 1 being the more virulent. However, such differences in virulence were not apparent between the race 1 and race 2 lettuce isolates used in the experiments described here (24). Regardless of the relative distribution of race 1 and 2 isolates in affected fields and the existence of resistance in lettuce to race 2 isolates, testing of individual fields prior to planting for the resident race and planting lettuce cultivars resistant to race 1 in fields that carry only race 1 pathogen genotypes is a useful way to extend the life of lettuce cultivars resistant to race 1.

In tomato, germplasm resistant to race 2 was identified, although its resistance was less effective than that conferred by the *Ve* gene to race 1 (3,12) and was conditioned by multiple genes with a lower heritability (13). Transgene-based resistance to race 2 also has been described (17,22). Further screening of *Lactuca* germplasm for resistance to race 2 of *V. dahliae* is needed. Although there is no guarantee which race will predominate in the future, it appears that a race 1 isolate or isolates contributed mostly to the disease we observed in our field locations. This indicates that the greenhouse-based evaluation methods developed here will be crucial for the continued screening of lettuce germplasm for resistance to race 2.

ACKNOWLEDGMENTS

This research was supported by the California Lettuce Research Board and the California Department of Food and Agriculture under the "Buy California Initiative." We thank M. Orozco, S. Martin, F. Hernandez, R. Margarito, B. Robinson, A. Pepper, A. Folck, and M. Renteria for their help throughout this project; and L. Knerr for pedigree information.

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