Delayed Foliar Symptoms Caused by *Verticillium dahliae* as an Alternative Resistance Trait in Iceberg Lettuce

Germán V. Sandoya

Horticultural Sciences Department, University of Florida—IFAS, 3200 E. Palm Beach Road, Belle Glade, FL 33430

Krishna Subbarao

Department of Plant Pathology, University of California, Davis, 1636 E. Alisal Street, Salinas, CA 93905

Ryan Hayes^{1,2}

U.S. Department of Agriculture, Agricultural Research Station, 1636 E. Alisal Street, Salinas, CA 93905

Additional index words. *Verticillium dahliae*, lettuce, verticillium wilt, *Verticillium dahliae* race 1, *Verticillium dahliae* race 2, breeding, genetics

Abstract. Verticillium wilt caused by *Verticillium dahliae* Kleb. is an economically damaging disease of iceberg lettuce on the Central Coast of California. Foliar wilting symptoms that manifest near or at peak market maturity (MM) lead to collapse of the head, making it unmarketable. Complete resistance to race 1 of the pathogen is known, but adequate levels of resistance are not available against race 2. Additional mechanisms or traits that reduce foliar symptoms (FS) are needed to lessen economic losses from this disease. Since the disease affects leaves, the harvested product, identification of iceberg cultivars that delay the onset of FS past peak MM could reduce yield loss from the disease. The goal of this research was to identify iceberg lettuce germplasm with delayed onset of FS. Diverse iceberg cultivars were evaluated in replicated field experiments for MM, FS severity, and adaptation. A few winter-adapted cultivars showed fewer FS past MM and seem to be promising candidates for breeding. These cultivars are not adapted to the California Central Coast where the disease currently predominates. Further studies will determine the usefulness of this trait for breeding improved cultivars for use in *V. dahliae*-infested fields. Developing new cultivars that combine currently available sources of partial resistance against race 2 with delayed onset of FS could lead to reduced crop losses should race 2 of *V. dahliae* become widespread.

Verticillium wilt is a destructive disease of lettuce (*Lactuca sativa* L.) in the Salinas Valley of California, a region that accounts for 50% of the U.S. lettuce production (Monterey County Crop Report, 2012). The disease is caused by the soilborne fungus *V. dahliae* and is a threat to crops grown in California such as strawberry, artichoke, tomato, and lettuce. The pathogen attacking lettuce exists as two races (Gurung

et al., 2014). Complete resistance to race 1 was identified in several heirloom lettuce cultivars (cvs.) (Hayes et al., 2007) and resistant iceberg germplasm was bred using ‘La Brillante’ as a parent (Hayes et al., 2011a, 2015). It is expected that widespread production of race 1 resistant cvs. will cause race 2 strains to increase in frequency and developing resistance to this strain is a priority for the lettuce industry. Although race 2 isolates are largely limited to the Pajaro Valley, a few have been found in the Salinas Valley (Gurung et al., 2014). Adequate levels of resistance to race 2 are not known (Hayes et al., 2011b) and alternative approaches to breed crops that tolerate the disease or escape economic damage could be useful to complement existing resistance.

Verticillium dahliae penetrates the secondary roots of lettuce and moves into the taproot through the vascular system (Vallad and Subbarao, 2008). In susceptible plants, the fungus moves into the foliage of the plant causing wilting, chlorosis, and necrosis, and finally death. These symptoms progress acropetally, from base to apex (Fradin and Thomma, 2006). In most crops, reduced yields of fruit, seeds, or tubers from verticillium wilt occurs indirectly, through reduced photosynthesis as

a result of leaf wilting and death. Economic damage in lettuce occurs immediately whenever chlorosis is expressed on leaves, a characteristic unique to leafy vegetable and ornamental crops.

Cultivated lettuce contains a diversity of market types that exhibit unique leaf characteristics and plant architectures. The popularity of each type is usually geographically dependent; in the United States, romaine and iceberg types are predominant. Although *V. dahliae* causes wilt in all types of lettuce, the economic damage to iceberg lettuce is the most severe due to its unique architecture and development (Vallad et al., 2006). Iceberg cvs. form a solid, spherical head. The heading process begins when outer leaves cup to form a sphere, an event that is genetically and environmentally dependent (Still, 2007). The head becomes solid as new leaves grow and fill the inside of the head. When plants are infected with verticillium wilt, the older and outer leaves of the iceberg head are the first to show symptoms. As the iceberg plant reaches maturity, these outer leaves that wrap around the lettuce head become the most severely wilted, leading to plant collapse and death. Healthy looking crops can often turn diseased in as little as a week. In some situations, growers may harvest iceberg crops before peak maturity but before severe symptoms occur. Conversely, iceberg cvs. that delay the onset of symptoms past peak harvest maturity could be useful to reduce economic damage from verticillium wilt. Similar characteristics are known in other lettuce diseases, but have not been pursued for verticillium wilt (Simko et al., 2014).

The majority of U.S. iceberg lettuce is produced year round in the southwestern United States. In Coastal California, lettuce is harvested from April to October. Later in the year (October to November), the production moves to the San Joaquin Valley and then the winter production is concentrated in the low desert of California and Arizona near the border with Mexico. Proper heading is a major determinant of iceberg cv. adaptation, and is a process influenced by genotype × environment interactions (Simko et al., 2014). Because of this, iceberg lettuce cvs. are generally bred to be narrowly adapted to specific locations and production times. Production of cvs. outside of the environment for which they were bred often results in poor heading and low yields (Simko et al., 2014).

The objectives of this research were to determine: 1) the genetic diversity for onset of FS in iceberg lettuce and 2) the adaptation of cvs. with delayed onset of symptoms to Coastal California production environments.

Materials and Methods

Lettuce germplasm evaluated. Thirty-four iceberg lettuce cvs. were chosen for this study (Table 1). This population included cvs. adapted to Coastal California, the low desert (southern California and Arizona), and other U.S. and international locations. We are unaware of any reports of these cvs. being tested for verticillium wilt resistance. The controls

Received for publication 22 Sept. 2016. Accepted for publication 9 Dec. 2016.

We would like to acknowledge funding from USDA-NIFA-SCRI Grant 2010-51181-21631 and USDA-NIFA-AFRI Grant 2013-01846 that made this research possible. We are also grateful to the California Leafy Green Research Board (CLGRB) for additional funding and Tom Bengard of Bengard Ranch for hosting outside experiments on company ranches. We also acknowledge the research assistance of Jose Orozco, USDA-ARS and Rosa Marchebout, University of California, Davis.

¹Current address: National Forage Seed Production Research Center, U.S. Department of Agriculture, 3450 SW Campus Way, Corvallis, OR 97331

²Corresponding author. E-mail: ryan.hayes@ars.usda.gov.

used in these experiments were the susceptible cv. Salinas and the resistant breeding line RH11-1798 (Hayes et al., 2015).

Disease and head maturity assessment. Ten plants per plot were evaluated in each experiment. FS were rated on a scale of 0 to 5 where 0 = no foliar wilting, 1 = 1% to 25% of the lettuce head showing wilting, 2 = 26% to 50% of the lettuce head showing wilting, 3 = 51% to 75% of lettuce head with wilting, 4 = 76% to 99% with wilting, and 5 = plants completely wilted. MM was rated on a 1 to 5 scale where 1 = very soft head, 2 = semisoft head, 3 = harvestable head that compresses slightly under pressure, 4 = very firm head, and 5 = head splitting open. Root discoloration (RD) was evaluated using the same plants evaluated for MM using a 0 to 5 scale in which 0 = roots with no vascular discolor-

ation, 1 = 1% to 25% of the taproot with vascular discoloration, 2 = 26% to 50% of taproot with vascular discoloration, 3 = 51% to 75% of taproot with vascular discoloration, 4 = 76% to 100% of taproot with vascular discoloration and no FS, and 5 = 100% of taproot with discoloration and leaves with wilting symptoms. Root discoloration data were used to calculate the root discoloration incidence (RDI, percentage of plants with visible RD) and the root discoloration severity (RDS, plot average of RD) (Hayes et al., 2007).

USDA, Salinas, CA, experiments. Three experiments were planted in a field at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Research Station in Salinas, CA (Table 2). This site was previously artificially infested with the race

1 isolate VdLs16 of *V. dahliae* (Hayes et al., 2011c). All the experiments were grown using typical lettuce production practices for the Central Coast of California (Ryder, 1999). The lettuce cvs. were arranged in a randomized complete block design (RCBD) with three replicates, hand-planted in a single seed line 12-m long, then thinned to a distance of 0.30 m between plants.

The first experiment (VERT2012-1) was planted on 16 May 2012 using 33 iceberg cvs. Each plot was evaluated at three time points for MM, RDI, and RDS corresponding to before, at, and after peak harvest maturity of cv. Salinas. The frequency between time points was about 1 week (Table 2). No assessment of FS was made in VERT2012-1. In 2013, a second experiment (VERT2013-1) with 34 iceberg cvs. was planted on 18 May to assess MM, RDI, RDS, and FS at three time points as done in 2012. This experiment was repeated in 2014 (VERT2014-1) with a 13 May planting. Two cvs. planted in the 2014 experiment were not assessed due to poor germination and seed contamination. Additionally, the 2014 experiment was evaluated at two time points coinciding with peak MM and past peak maturity of cv. Salinas.

Commercial field experiments. A subset of nine cvs. was chosen for two experiments in commercial fields with a history of verticillium wilt (Table 2). The first experiment (VERT2013-2) was planted on 1 June 2013 near Davis Road in Salinas, CA, as a RCBD with four replicates. This planting also included the cv. Tiber, the recurrent parent of RH11-1798. The experiment used single seed line plots that were 8-m long. Plants were thinned to a spacing of 0.30 m. A second experiment (VERT2014-2) with 10 cvs. was planted in a different commercial field near Somavia Road, Chualar, CA, on 13 July 2014. The experiment was conducted with the same specifications described above but did not include cv. Tiber. Both commercial field experiments were evaluated for RDI, RDS, FS, and MM. In 2013, FS, MM, RDI, and RDS were taken at just one time point (past peak maturity). In 2014, the FS and MM were evaluated at three time points (before, during, and past peak maturity) but RDI and RDS were only evaluated at the last time point. The horticultural characters head weight (grams), head height (centimeter), and core or stem length (centimeter) were assessed on 10 heads per plot. Heads were harvested by cutting the plant at the crown to separate the head from the roots. Each head was weighed and then split in half longitudinally to expose the core for measurement.

Table 1. Thirty-four iceberg lettuce cultivars and their origin.

Cultivar	Reported adaptation ²
Alaska	Alaska, United States
Annie	San Joaquin Valley and low desert ³
Anuenue	Low land Hawaii, United States
Batavia Reine des Glaces (BRG)	France, Europe
Bubba	San Joaquin Valley and low desert
Cibola	Low desert
Climax	Low desert
Coyote	San Joaquin Valley and low desert
Del Rio	San Joaquin Valley and low desert
Desert Storm	Unknown
Dominguez 67	Unknown
El Dorado	Coastal California
Emperor	San Joaquin Valley and low desert
Floriscrisp 1265	Florida, United States
Gabilan	Coastal California, San Joaquin Valley, and low desert
Green Lake	Wisconsin, United States
Green Lightning	San Joaquin Valley and low desert
Grizzly	San Joaquin Valley and low desert
Head Master	San Joaquin Valley and low desert
Honcho II	San Joaquin Valley and low desert
Ithaca	Northeast, United States
Kikugawa	Japan
Oswego	Northeast
Premiere	San Juan Bautista, CA
Salad Crisp	Northeast, United States
Shinano Hope	Japan
Star Ray	Unknown
Sun Devil	San Joaquin Valley
Valley Green	Unknown
Valverde	Lower Rio Grande Valley, TX
Vanguard 75	Low desert
Yuma	San Joaquin Valley and low desert
Controls	
Salinas (susceptible)	Coastal California
RH11-1798 (resistant) ^x	Coastal California Tiber × (La Brillante × Pacific)

²Adaptation data summarized from the vegetable cultivar list (<http://cuke.hort.ncsu.edu/cucurbit/wehner/vegcult/vgclintro.html>) and plant variety protection certificates (<https://apps.ams.usda.gov/CMS/default.aspx>).

³Low desert is the Imperial Valley, CA, and Yuma Valley, AZ. The San Joaquin Valley is in California. Coastal California is the Salinas Valley, Pajaro Valley, and Santa Maria area in California.

^xBreeding line released as RH12-3197 (Hayes et al., 2015).

Table 2. Experiments conducted in this research.

Expt.	Location	Planting	Evaluation	Entries	Experimental design	Variables
VERT2012-1	USDA, Salinas	16 May 2012	Three time points	33	RCBD	RDI, ² RDS, MM
VERT2013-1	USDA, Salinas	18 May 2013	Three time points	34	RCBD	RDI, RDS, FS, MM
VERT2014-1	USDA, Salinas	13 May 2014	Two time points	34	RCBD	RDI, RDS, FS, MM
VERT2013-2	Davis Road, Salinas, CA	1 June 2013	One time point	9	RCBD	RDI, RDS, FS, MM, Horticultural
VERT2014-2	Somavia Road, Chualar, CA	13 July 2014	Three time points	10	RCBD	RDI, RDS, FS, MM, Horticultural

²RDI = root discoloration incidence; RDS = root discoloration severity; FS = foliar symptoms; MM = market maturity; Horticultural = head weight, head height, core height, and tipburn incidence; RCBD = randomized complete block design.

Every harvested head was evaluated for the physiological disorder tipburn and the data from each plot were expressed as the proportion of plants with the disorder.

Statistical analysis. Foliar severity, RDI, RDS, and MM from VERT2012-1, VERT2013-1, and VERT2014-1 were analyzed as a repeated measure experiment using the nonparametric approach (Shah and Madden, 2004) in SAS (version 9.3; SAS Institute, Cary, NC). The data from each experiment were analyzed separately. The analysis of variance (ANOVA) F was calculated using PROC MIXED with an unstructured covariance model. Cultivars, times, and the interaction of cultivar $\times$ time were considered as fixed factors. The LD_CI and F1_LD_F1 macros were used to calculate the relative marginal effects (RMEs) and their 95% confidence intervals (Brunner et al., 2002). Orthogonal contrasts were calculated for FS, RDI, and RDS for comparisons between the resistant and susceptible checks and other cvs. within an assessment time point. Additionally, a ratio of the median severity of FS over median maturity was calculated for each time point in experiments VERT2013-1 and VERT2014-1 and analyzed using the same repeated measures model described above.

Foliar severity, RDI, RDS, and MM from VERT2013-2 and VERT2014-2 were analyzed as an RCBD using the nonparametric analysis of ordinal data taken at the past MM time point using the nonparametric statistic described by Shah and Madden (2004) in SAS. The LD_CI macro was used to generate the RME and their 95% confidence intervals (Brunner et al., 2002; Shah and Madden, 2004).

Head weight and height, core length, and tipburn incidence from VERT2013-2 and VERT2014-2 were analyzed as an RCBD as well, using PROC MIXED of SAS; cvs. were considered fixed effects and replicates as random effects. The least square means were compared using the Tukey–Kramer test at 95% probability. The disease (FS, RDI, and RDS) and maturity (MM) data as well as yield data described in the last two paragraphs were analyzed for experiments conducted in commercial field experiments in Davis Road, Salinas, CA, and Somavia Road, Chualar, CA. Mean separation in this trait was calculated using the macro pdmix800 that converts mean separation into letter grouping in PROC MIXED (Saxton, 1998).

Results

Genetic variation for RDI and RDS. The median RDI for the susceptible cv. Salinas

was 80% in all 3 years of the study at the USDA-ARS field site (experiments VERT12012-1, 13-1, and 14-1), which demonstrates that the environment was conducive for verticillium wilt (Table 3). All cvs. except for breeding line RH11-1798 had some level of RD, though the incidence of RD varied among cvs. indicating differences in resistance. The 34 iceberg lettuce cvs. showed significant differences for RDI and RDS in 2012, 2013, and 2014 ($P < 0.0001$). Time was a significant factor for RDI and RDS in 2012 ($P < 0.0001$) and 2013 ($P < 0.0001$), but nonsignificant in 2014 ($P > 0.05$). Regardless, the amount of disease increased over time in all field experiments (data not shown). The cultivar $\times$ time interaction was not significant for RDI and RDS in any year ($P > 0.05$). The experiment median RDI was 70 in all experiments, indicating that overall cv. disease levels were similar in all years (Table 3). The cvs. with the fewest diseased plants and lowest RDIs were ‘Anuenue’, ‘BRG’, and ‘Kikugawa’. In 2012, cvs. Anuenue (20%) and BRG (40%) had significantly lower RDI compared with ‘Salinas’ (80%); followed by ‘Green Lakes’ (40%) and ‘Honcho II’ (40%). In 2013, ‘Anuenue’ (10%), ‘BRG’ (20%), and ‘Kikugawa’ (40%) were significantly lower than ‘Salinas’ (80%). The only

Table 3. Median root discoloration incidence (RDI), relative marginal effect (RME) and their 95% confidence interval (CI) for 34 iceberg cultivars evaluated past market maturity in 2012, 2013, and 2014 replicated experiments grown in a race-1 *Verticillium dahliae* infested field in Salinas, CA.

	2012 RDI			2013 RDI			2014 RDI			Multi-yr avg RDI
	Median	RME	95% CI	Median	RME	95% CI	Median	RME	95% CI	
Control										
Salinas	80	0.78	0.56–0.90	80	0.79	0.69–0.86	80	0.67	0.36–0.87	80
RH11-1798	0	0.02	0.02–0.04	0	0.02	0.01–0.02	0	0.01	0.0–0.03	0
Cultivars										
Anuenue	20	0.11	0.04–0.27	10	0.11	0.05–0.24	60	0.41	0.33–0.51	30
Honcho II	40	0.25	0.19–0.33	80	0.81	0.70–0.88	70	0.60	0.46–0.73	63
BRG	40	0.31	0.18–0.48	20	0.17	0.13–0.21	60	0.44	0.10–0.86	40
Green Lakes	40	0.32	0.24–0.41	60	0.56	0.30–0.79	90	0.64	0.22–0.91	63
Valverde	50	0.50	0.19–0.81	70	0.67	0.56–0.77	80	0.62	0.28–0.87	67
Yuma	60	0.47	0.40–0.55	80	0.66	0.18–0.94	70	0.47	0.25–0.71	70
Kikugawa	60	0.51	0.08–0.92	40	0.32	0.20–0.47	40	0.16	0.09–0.26	47
Salad Crisp	60	0.52	0.15–0.87	70	0.69	0.50–0.83	60	0.46	0.21–0.72	63
Green Lightning	60	0.68	0.39–0.87	80	0.83	0.68–0.92	80	0.65	0.31–0.88	73
Vanguard 75 ²	—	—	—	60	0.57	0.07–0.96	90	0.74	0.63–0.82	75
Desert Storm	70	0.58	0.18–0.89	60	0.58	0.19–0.89	60	0.39	0.27–0.52	63
Cibola	70	0.57	0.36–0.76	60	0.47	0.27–0.67	70	0.56	0.38–0.72	67
Emperor	70	0.53	0.33–0.72	80	0.68	0.20–0.94	40	0.36	0.05–0.86	63
Floricrisp 1265	70	0.61	0.10–0.96	60	0.62	0.52–0.72	60	0.31	0.17–0.49	63
Oswego	70	0.65	0.58–0.72	70	0.61	0.22–0.90	50	0.32	0.13–0.60	63
Shinano Hope	70	0.60	0.42–0.76	70	0.53	0.15–0.87	30	0.16	0.05–0.43	57
Grizzly	70	0.62	0.45–0.77	60	0.62	0.38–0.82	90	0.66	0.22–0.91	73
Bubba	80	0.72	0.40–0.91	80	0.74	0.38–0.93	100	0.90	0.78–0.95	87
Ithaca	80	0.61	0.21–0.90	80	0.89	0.78–0.95	40	0.31	0.09–0.69	67
Star Ray ³	80	0.56	0.21–0.86	70	0.70	0.63–0.76	—	—	—	75
Sun Devil	80	0.69	0.56–0.81	60	0.71	0.41–0.89	70	0.52	0.22–0.81	70
Valley Green	80	0.69	0.56–0.81	80	0.79	0.62–0.89	80	0.63	0.45–0.78	80
Annie	90	0.81	0.56–0.93	60	0.58	0.37–0.76	70	0.58	0.41–0.73	73
Del Rio	90	0.77	0.45–0.93	80	0.77	0.52–0.91	90	0.84	0.69–0.92	87
Dominguez 67	90	0.72	0.33–0.93	70	0.72	0.43–0.89	60	0.41	0.19–0.67	73
Climax ³	90	0.75	0.38–0.93	60	0.67	0.47–0.82	—	—	—	75
Head Master	90	0.75	0.53–0.88	100	0.84	0.43–0.97	80	0.63	0.30–0.87	90
El Dorado	100	0.92	0.84–0.96	70	0.69	0.47–0.85	60	0.36	0.27–0.45	77
Coyote	100	0.92	0.85–0.95	80	0.70	0.28–0.93	90	0.63	0.22–0.91	90
Gabilan	100	0.90	0.76–0.96	90	0.95	0.90–0.97	100	0.84	0.58–0.95	97
Premiere	100	0.82	0.47–0.96	90	0.74	0.25–0.96	80	0.71	0.44–0.88	90
Experiment total	70			70			70			68

²Cultivar Vanguard 75 was not planted in 2012.

³Cultivars Star Ray and Climax were discarded in 2014 because of poor germination and seed contamination.

cv. that showed significant differences compared with ‘Salinas’ (80%) in 2014 was ‘Kikugawa’ (40%). The same patterns were observed for RDS (data not shown).

Genetic variation for foliar severity. Cultivars exhibited differences in FS. In 2013 and in 2014, significant differences ($P < 0.0001$) were detected among the tested cvs. for FS. A significant cultivar $\times$ time interaction was detected in 2013 ($P < 0.0001$) but not in 2014 ($P = 0.2476$). The lack of significance in 2014 is likely because this year only had two assessment time points. The significant cultivar $\times$ time interaction indicates that the advancement or worsening

of FS over time was not the same for each cv. Breeding line RH11-1798 had the lowest FS of all tested lines or cvs. (Fig. 1). Cultivars Climax, Desert Storm, Anuenue, BRG, and Bubba showed the least FS of the remaining cvs. as indicated by lower RME values. According to the orthogonal contrasts, these cvs. had significantly lower FS compared with cv. Salinas ($P = 0.0005$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). Additionally, ‘Vanguard 75’, ‘Yuma’, ‘Honcho II’, and ‘Cibola’ also showed less FS compared with ‘Salinas’ ($P = 0.0170$, $P = 0.0056$, $P = 0.0226$, and $P = 0.0257$, respectively). The FS in 2014 were higher

compared with 2013 but FS in 2013 was correlated with FS in 2014 ($r = 0.50$; $P < 0.0001$). Some cvs. that showed delayed symptoms in 2013 again expressed lower FS in 2014 (‘Cibola’, ‘Desert Storm’, and ‘Yuma’), though these cvs. were not significantly different from cv. Salinas for FS past MM in 2014 ($P = 0.2142$, $P = 0.2138$, and $P = 0.1719$, respectively) (Fig. 2). However, in an earlier assessment time point ‘Cibola’ ($P = 0.0402$), ‘Desert Storm’ ($P = 0.0004$), and ‘Yuma’ ($P = 0.0002$) showed significant differences compared with cv. Salinas.

Genetic variation for MM. MM showed significant differences among cvs. ($P < 0.0001$)

Fig. 1. Relative marginal effects (RMEs) for foliar severity (FS, black dots) and market maturity (MM, gray bars) of 34 iceberg cultivars assessed after peak MM in a Salinas, CA, replicated field experiment in 2013. Median MM and FS are shown for each cultivar in the in-set box. The error bars in the FS data represent confidence intervals (CIs) at 95% probability. CI for MM are omitted in the figure.

Fig. 2. Relative marginal effects (RMEs) for foliar severity (FS, black dots) and market maturity (MM, gray bars) of 32 iceberg cultivars assessed after peak MM in a Salinas, CA, replicated field experiment in 2014. Median MM and FS are shown for each cultivar in the in-set box. The error bars in the FS data represent confidence intervals (CIs) at 95% probability. CI for MM are omitted in the figure.

in years 2012, 2013, and 2014. In the ANOVA F, time was also significant ($P < 0.0001$) in all years as well as the interaction of cultivar $\times$ time in 2012 ($P < 0.0001$) and 2013 ($P = 0.0445$) but not in 2014 ($P = 0.3629$). This indicates that the advancement of maturity over time was cv. dependent. Most cvs. reached a median MM above 3.0 in 2012 (data not shown) and 2013 (Fig. 1), which is considered the minimum marketable maturity for iceberg lettuce. Exceptions included 'Vanguard 75' (2.4), 'Climax' (2.7), and 'Bubba' (2.9) (Fig. 1). Evaluations during 2014 were made on more mature plants compared with previous years. The median MM of cv. Salinas was 4.0 at the last time point, whereas the same cv. at the last time point in 2013 had a median MM of 3.0. Because of the later evaluation in 2014, 15 cvs. were over mature (MM > 4) and only cvs. Bubba and Yuma failed to reach a median MM of at least three by the end of the experiment (Fig. 2).

Comparison of FS adjusted for MM. FS severity was dependent on MM in some years. Correlations between the traits ranged from 0.15 ($P = 0.1423$) to 0.47 ($P < 0.0001$) and cvs. that had high RDI (i.e., susceptible to *V. dahliae*) but exhibited slow advancement of FS were generally cvs. that were also slow to mature (Figs. 1 and 2).

The ratio between FS and MM expresses the severity of FS relative to MM across three time points in 2013 and two time points in 2014 (Table 4). Increases in the ratio indicate a worsening of FS. In 2013, these ratios increased through time in all cvs. The race 1 resistant breeding line RH11-1798 was the only treatment that did not experience an increase. 'Climax' demonstrated a slight increase, whereas cvs. Anuenue, Desert Storm, BRG, and Honcho II showed lower than average increases. Values from the 2014 experiment were higher for all treatments at the second evaluation time point, including the resistant control RH11-1798. Despite this, cvs. Anuenue, Desert Storm, Bubba, and Yuma again had among the lowest ratios at time point one in the 2014 experiment (Table 4). Other cvs. with low ratios in 2014 include 'Cibola' and 'Grizzly'. Cultivars Desert Storm, Bubba, Yuma, Cibola, Grizzly, and Honcho II are notable for all being low-desert winter-production-adapted cvs.

Resistance and horticultural traits of iceberg cultivars in Coastal California commercial fields. Cultivars tested in commercial fields exhibited genetic variation for FS, RDI, RDS, and MM (RDI and RDS data not shown). Evaluation of these experiments was conducted when cv. Salinas and breeding line RH11-1798 were at peak maturity; most of the remaining cvs. were mature or slightly under mature at both sites at this evaluation time point (Fig. 3A and B). The cv. Yuma was the least mature at both sites indicating a lack of adaptation to Coastal California growing conditions. Overall, the Davis Road trial had higher RDI and RDS compared with Somavia Road (data not shown). The RDI for 'Salinas' and RH11-1798 was 75% and 0%, respectively, at the Davis Road location but

Table 4. The ratio of the foliar symptom severity over market maturity of 34 iceberg cultivars evaluated at three time points in replicated field experiments in Salinas, CA, in 2013 and 2014.

Cultivars	2013 field expt.			2014 field expt.	
	Evaluation time points ^z			Evaluation time points	
	1	2	3	1	2
RH11-1798	0.03	0.07	0.04	0.00	0.61
Salinas	0.19	0.71	0.83	0.42	0.81
Climax ^y	0.02	0.14	0.12	—	—
Anuenue	0.01	0.13	0.22	0.17	0.57
Desert Storm	0.15	0.28	0.25	0.06	0.81
BRG	0.05	0.14	0.30	0.30	1.27
Honcho II	0.08	0.44	0.31	0.19	1.06
Bubba	0.09	0.31	0.41	0.12	1.25
Yuma	0.25	0.50	0.51	0.06	0.77
Alaska	0.09	0.40	0.54	0.13	0.84
Ithaca	0.05	0.32	0.54	0.58	0.90
Emperor	0.13	0.60	0.54	0.54	1.00
Green Lakes	0.07	0.37	0.55	0.56	0.93
Cibola	0.19	0.47	0.56	0.07	0.84
Coyote	0.14	0.52	0.59	0.22	0.91
Vanguard 75	0.28	0.69	0.60	0.18	0.91
Domingo	0.24	0.77	0.60	0.25	0.91
Floricrisp	0.07	0.34	0.61	0.69	1.01
Kikugawa	0.26	0.51	0.62	0.29	0.91
Valverde	0.06	0.52	0.62	0.80	0.91
Grizzly	0.09	0.46	0.65	0.12	0.88
Oswego	0.05	0.40	0.66	0.40	0.79
Premiere	0.19	0.67	0.69	0.42	0.88
Sun Devil	0.04	0.71	0.69	0.49	0.87
Valley Green	0.28	0.82	0.71	0.71	1.01
Star Ray ^y	0.15	0.58	0.72	—	—
Shinano Hope	0.20	0.56	0.73	0.17	0.65
El Dorado	0.18	0.76	0.73	0.47	0.86
Del Rio	0.30	0.60	0.78	0.37	1.07
Annie	0.26	0.84	0.83	0.72	0.95
Salad Crisp	0.07	0.44	0.85	0.43	0.87
Head Master	0.20	0.76	0.85	0.48	1.02
Green Lightning	0.15	0.60	0.87	0.36	1.08
Gabilan	0.15	0.70	0.97	0.67	1.06

^zEvaluation time points correspond to before, at, and after peak market maturity in 2013 and at and after peak market maturity in 2014.

^yCultivars Star Ray and Climax were discarded in 2014 because of poor germination and seed contamination.

25% and 0% at Somavia Road. The least diseased cvs. were 'Anuenue' and 'BRG' with the first having significantly less disease compared with cv. Salinas ($P = 0.0493$). At the Davis Road experiment, 'Yuma' had the least FS followed by 'Anuenue', 'BRG', 'Coyote', and 'Honcho II' (Fig. 3A), as indicated by their significant orthogonal contrasts compared with cv. Salinas with P values of 0.0016, 0.0035, 0.0104, 0.0009, and 0.0092, respectively. FS were lower in the Somavia Road experiment (Fig. 3B). In this experiment, only cv. Yuma had FS significantly lower than cv. Salinas ($P = 0.0333$).

Highly significant differences ($P < 0.001$) between cvs. were detected for the horticultural traits evaluated at both locations. The heaviest heads were found in cv. Coyote, which was statistically similar to RH11-1798 and cvs. Tiber and Gabilan in the Davis Road experiment (Table 5). In the Somavia Road experiment, cv. Coyote again had the highest head weight followed by RH11-1798 and cvs. Climax, Yuma, Honcho II, and Salinas. This group of cvs. was significantly better than the rest of cvs. in the Somavia Road trial (Table 5).

Core lengths in the Davis Road experiment in 2013 were generally within an

acceptable length (< 5 cm), ranging from 3.20 cm (cv. Green Lakes) to 6.29 cm (cv. BRG). However, cores were bigger in the Somavia Road experiment in 2014 as a consequence of the trial being more mature (Table 5). In fact, all cvs. had cores > 8 cm, except for cvs. Gabilan, Green Lakes, and the breeding line RH11-1798. Differences between cvs. were detected for head height in each experiment (Table 5).

The physiological defect known as tipburn occurred at the Davis and Somavia Road experiments. In both locations, the cvs. with lowest incidence of tipburn were cvs. Yuma, Anuenue, and Gabilan. Cultivar Tiber, which was bred for a low occurrence of tipburn, had zero tipburn at the Davis Road experiment but was not tested at Somavia Road. These cvs. were significantly different (data not shown) from the cvs. with the highest tipburn incidence such as 'Coyote' and 'Honcho II' in both sites and from 'Climax' at the Somavia Road site (Table 5).

Discussion

In this study, we identified iceberg cvs. that exhibit less FS at peak maturity and past peak MM. This research is the first attempt

to elucidate if this can be considered a resistance characteristic for lettuce. We initially focused on identifying iceberg lettuce cvs. that exhibit low RD. Outside of the USDA breeding line (RH11-1798), only cvs. Anuenue and BRG demonstrated low RDI and severity in some experiments. Among the remaining susceptible cultivars (i.e., those with high RDI and RDS), ‘Yuma’,

‘Climax’, and ‘Desert Storm’, and possibly a few others showed less foliar wilting. In some experiments, cvs. or breeding lines with low or zero RD exhibited FS indicating that FS may be caused by stresses other than *V. dahliae*. Regardless, these findings indicate that some cvs. may possess genes that reduce FS severity without influencing resistance to pathogen colonization.

Race-specific resistant cvs. of tomato and lettuce block systemic spread of the fungus (Fradin and Thomma, 2006; Maruthachalam et al., 2010; Vallad and Subbarao, 2008). Little is known about the subcellular or molecular events in lettuce root defense against *V. dahliae* infection in resistant and susceptible interactions. In tomato, fungal infection of resistant cvs. upregulated genes in the roots related to the phenylpropanoid pathway (Gayoso et al., 2010; Tan et al., 2015). Even less is known about the defense response in different plant organs. Different *phenylalanine ammonia-lyase* genes were also expressed in tomato hypocotyls, epicotyls, cotyledons, leaves, and flowers as a consequence of *V. dahliae* colonization (Gayoso et al., 2010). This finding implies that low expression of FS could be based on genes that are different from those involved with reduced RD-based resistance.

We do not know at this point if the ability of these cvs. to exhibit fewer FS past MM can be considered as tolerance to *V. dahliae* infection. Tolerance to verticillium wilt has been described. In tomato, a susceptible cv. was reported to develop little or no symptoms to a nontomato isolate of *V. dahliae*, even though the fungus systemically colonized plants (Chen et al., 2004). In other reports using pathogenic isolates on tomato (Blackhurst and Wood, 1963; Robb et al., 2007) and *Arabidopsis* (Veronese et al., 2003), plants colonized by *V. dahliae* did not show the typical foliar wilting symptoms and appeared as disease free. However, the concept of tolerance is not well defined for soilborne pathosystems such as verticillium wilt. Although there are many concepts of tolerance of plants against biotic stresses, crops able to produce expected yield despite a successful pathogen colonization (Schafer, 1971) could give insights regarding the tolerance of these lettuce cvs. against *V. dahliae*.

Most of the cvs. that expressed delayed FS are adapted to winter production in southern California and Arizona, which may explain the poor adaption and delayed maturity when

Fig. 3. Relative marginal effects (RMEs) for foliar severity (FS) and market maturity (MM) of 11 iceberg cultivars in (A) Davis Road (Salinas, CA) during 2013 and (B) Somavia Road (Chualar, CA) during 2014. Black dots are FS and grey bars are MM. The bars represent confidence intervals (CIs) at 95% probability for FS. CI for MM are omitted in the figure. Median MM and FS are shown for each cultivar in the in-set box.

Table 5. Head weight, head height, core length, and percentage of plants with tipburn of 11 iceberg cultivars in replicated field experiments in 2013 in Davis Road (Salinas, CA) and in 2014 in Somavia Road (Chualar, CA) in commercial fields.

Cultivar	Davis Road (2013)				Somavia Road (2014)			
	Head (g)	Ht (cm)	Core (cm)	TB (%)	Head (g)	Ht (cm)	Core (cm)	TB (%)
Anuenue	689 ABCD	15.71 AB	5.18 AB	12.5 CD	820 BCD	15.70 CD	9.17 AB	12.5 AB
BRG	416 E	11.63 C	6.29 A	45.0 ABC	434 D	11.51 E	8.59 B	39.7 AB
Climax ²	—	—	—	—	1,385 A	19.13 A	9.32 AB	61.9 A
Coyote	861 A	17.42 A	4.03 AB	65.0 AB	1,411 A	18.01 ABC	8.85 B	50.0 AB
El Dorado	794 ABC	15.13 AB	5.18 AB	22.5 BCD	838 BCD	14.90 D	8.27 BC	32.5 AB
Gabilan	802 ABC	14.79 B	3.43 B	10.0 CD	781 BCD	15.51 D	5.29 C	5.0 B
Green Lakes	636 BCDE	13.91 BC	3.20 B	15.0 CD	626 CD	13.89 DE	5.26 C	13.8 AB
Honcho II	690 ABCD	16.01 AB	4.75 AB	72.5 A	1134 ABC	18.53 AB	12.09 A	62.5 A
Tiber ³	850 AB	15.10 AB	4.31 AB	0.0 D	—	—	—	—
Yuma	551 DE	15.53 AB	3.28 B	10 CD	1,220 AB	18.24 AB	8.30 BC	2.5 B
Controls								
Salinas	625 CDE	14.66 B	4.53 AB	17.5 CD	1,013 ABC	16.34 BCD	9.23 AB	40.0 AB
RH11-1798	855 AB	15.58 AB	4.20 AB	15.0 CD	1,004 ABC	15.35 D	6.84 BC	20.0 AB
P > F	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0009

²Cultivar Climax was not planted in the 2013 trial.

³Cultivar Tiber was not planted in the 2014 trial.

Least square means followed by the same letter are not significantly different according to Tukey–Kramer test ($P < 0.01$).

grown in Coastal California. These winter-production-adapted cvs. are also genetically related through a few common ancestors, primarily cvs. Climax and Vanguard 75. Other winter-adapted cvs. such as ‘Annie’ and ‘Grizzly’ are also derived from ‘Climax’ and ‘Vanguard 75’ but did not exhibit delayed FS. The occurrence of genetic variation for delayed FS within a population of related cvs. with similar adaptation implies that delayed FS is not strictly related to adaptation or maturity. Further genetic studies are needed to confirm the utility of this characteristic to breed improved cvs.

Cultivars with delayed FS were generally not adapted to Coastal California due to poor head weight, delayed maturity, tall core height, or high tipburn incidence. Heading in iceberg lettuce is a photoperiod and temperature sensitive process (Still, 2007). Growing low-desert and winter-adapted cvs. during the summer in Coastal California likely exposed them to temperature and photoperiod regimes to which they are not adapted. Consequently, the cvs. with delayed FS do not appear to be immediately useful for iceberg lettuce production in the Salinas Valley. At this time, the only iceberg germplasm that combines verticillium wilt resistance with adaptation to Coastal California production conditions are USDA breeding lines that carry the *Verticillium resistance 1* gene (Hayes et al., 2015).

In conclusion, there appears to be genetic variation for FS in iceberg lettuce. Cultivars that demonstrated delayed onset of FS were nonetheless susceptible to *Verticillium* wilt as determined by RD. Combining delayed onset of FS with other sources of partial resistance to race 2 may further reduce symptoms.

Literature Cited

- Blackhurst, F.M. and R.K.S. Wood. 1963. Verticillium wilt of tomatoes—further experiments on the role of pectic and cellulolytic enzymes. *Ann. Appl. Biol.* 52:89–96.
- Brunner, E., S. Domhof, and F. Langer. 2002. Nonparametric analysis of longitudinal data in factorial experiments. Wiley, New York, NY.
- Chen, P., B. Lee, and J. Robb. 2004. Tolerance to a non-host isolate of *Verticillium dahliae* in tomato. *Physiol. Mol. Plant Pathol.* 64:283–291.
- Fradin, E.F. and B.P.H.J. Thomma. 2006. Physiology and molecular aspects of verticillium wilt diseases caused by *V. dahliae* and *V. albo-atrum*. *Mol. Plant Pathol.* 7:71–86.
- Gayoso, C., F. Pomar, E. Novo-Uzal, F. Merino, and O. Martinez de Ilarduya. 2010. The V-mediated resistance response of the tomato to *Verticillium dahliae* involves H₂O₂, peroxidase and lignins and drives PAL gene expression. *BMC Plant Biol.* 10:232.
- Gurung, S., D.P.G. Short, Z.K. Atallah, and K.V. Subbarao. 2014. Clonal expansion of *Verticillium dahliae* in lettuce. *Phytopathology* 104:641–649.
- Hayes, R.J., K. Maruthachalam, G.E. Vallad, S.J. Klosterman, I. Simko, Y.G. Luo, and K.V. Subbarao. 2011a. Iceberg lettuce breeding lines with resistance to verticillium wilt caused by race 1 isolates of *Verticillium dahliae*. *HortScience* 46:501–504.
- Hayes, R.J., K. Maruthachalam, G.E. Vallad, S.J. Klosterman, and K.V. Subbarao. 2011b. Selection for resistance to verticillium wilt caused by Race 2 isolates of *Verticillium dahliae* in accessions of lettuce (*Lactuca sativa* L.). *HortScience* 46:201–206.
- Hayes, R.J., L.K. McHale, G.E. Vallad, M.J. Truco, R.W. Michelmore, S.J. Klosterman, K. Maruthachalam, and K.V. Subbarao. 2011c. The inheritance of resistance to verticillium wilt caused by race 1 isolates of *Verticillium dahliae* in the lettuce cultivar La Brillante. *Theor. Appl. Genet.* 123:509–517.
- Hayes, R.J., G. Sandoya, I. Simko, Y.G. Luo, and K.V. Subbarao. 2015. Notice of release of PI 673090, 673091, 673092, 673093, 673094, 673095, 673096, 673097, lettuce. U.S. Department of Agriculture, Agricultural Research Service, Washington, DC, 2008.
- Hayes, R.J., G.E. Vallad, Q.-M. Qin, R.C. Grube, and K.V. Subbarao. 2007. Variation for resistance to verticillium wilt in lettuce (*Lactuca sativa* L.). *Plant Dis.* 91:439–445.
- Maruthachalam, K., Z.K. Atallah, G.E. Vallad, S.J. Klosterman, R.J. Hayes, R.M. Davis, and K.V. Subbarao. 2010. Molecular variation among isolates of *Verticillium dahliae* and polymerase chain reaction-based differentiation of races. *Phytopathology* 100:1222–1230.
- Monterey County Crop Report. 2012. County of Monterey Agricultural Commissioner. 4 Apr. 2017. <<http://www.co.monterey.ca.us/Home/ShowDocument?id=1483>>.
- Robb, J., B. Lee, and R. Nazar. 2007. Gene suppression in a tolerant tomato–vascular pathogen interaction. *Planta* 226:299–309.
- Ryder, E.J. 1999. Lettuce, endive and chicory. Crop production science in horticulture series. CABI Publishing, New York, NY.
- Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. 23rd SAS Users Group Intl., p. 1243–1246. SAS Institute, Cary, NC.
- Schafer, J.F. 1971. Tolerance to plant disease. *Annu. Rev. Phytopathol.* 9:235–252.
- Shah, D.A. and L.V. Madden. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology* 94:33–43.
- Simko, I., R.J. Hayes, B. Mou, and J.D. McCreight. 2014. Lettuce and spinach, p. 53–86. In: S. Smith, B. Diers, J. Specht, and B. Carver (eds.). Yield gains in major U.S. field crops. Series. CSSA Special Publications. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, WI.
- Still, D. 2007. Lettuce, p. 380. In: C. Kole (ed.). Genome mapping and molecular breeding in plants. Springer-Verlag, Berlin, Heidelberg, Germany.
- Tan, G., K. Liu, J. Kang, K. Xu, Y. Zhang, L. Hu, J. Zhang, and C. Li. 2015. Transcriptome analysis of the compatible interaction of tomato with *Verticillium dahliae* using RNA-sequencing. *Front. Plant Sci.* 6:48.
- Vallad, G.E., Q.M. Qin, R. Grube, R.J. Hayes, and K.V. Subbarao. 2006. Characterization of race-specific interactions among isolates of *Verticillium dahliae* pathogenic on lettuce. *Phytopathology* 96:1380–1387.
- Vallad, G.E. and K.V. Subbarao. 2008. Colonization of resistant and susceptible lettuce cultivars by a green fluorescent protein-tagged isolate of *Verticillium dahliae*. *Phytopathology* 98:871–885.
- Veronese, P., M.L. Narasimhan, R.A. Stevenson, J.-K. Zhu, S.C. Weller, K.V. Subbarao, and R.A. Bressan. 2003. Identification of a locus controlling verticillium disease symptom response in *Arabidopsis thaliana*. *Plant J.* 35:574–587.