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

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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 at or 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 genotypes and 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 Brilliante’ 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 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...
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 discoloration, 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 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 conducted 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.

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

| Cultivar          | Reported adaptation<sup>a</sup>          | Adaptation data summarized from the vegetable cultivar list (http://cuke.hort.ncsu.edu/cucurbithome/vegcult/vegintro.html) and plant variety protection certificates (https://apps.ams.usda.gov/CMS/default.aspx). | Low desert is the Imperial Valley, CA, and Yuma Valley, AZ. The San Joaquin Valley is in California. |  |
|-------------------|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|---|
| Alaska            | Alaska, United States                    | Coastal California, San Joaquin Valley, and low desert<sup>b</sup>                                                                                                             | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Annie             | San Joaquin Valley and low desert<sup>b</sup> | Coastal California, San Joaquin Valley, and low desert<sup>b</sup>                                                                                                             | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Anuenue           | Low land Hawaii, United States           | Wisconsin, United States                                                                                                                                                        | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Batavia Reine des Glaces (BRG) | France, Europe | Florida, United States                                                                                                                                                           | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Bubba             | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Cibola            | Low desert                               | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Climax            | Low desert                               | Wisconsin, United States                                                                                                                                                        | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Coyote            | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Del Rio           | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Desert Storm       | Unknown                                   | Wisconsin, United States                                                                                                                                                        | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Dominguez 67      | Unknown                                   | Wisconsin, United States                                                                                                                                                        | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| El Dorado          | Coastal California                       | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Emperor           | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Floricrisp 1265    | Florida, United States                   | Florida, United States                                                                                                                                                           | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Gabilan           | Coastal California                       | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Green Lake         | San Joaquin Valley and low desert        | Wisconsin, United States                                                                                                                                                        | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Green Lightning    | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Grizzly            | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Head Master        | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Honcho II          | San Joaquin Valley low desert            | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Ithaca            | Northeast, United States                 | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Kikugawa           | Japan                                     | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Oswego             | Northeast                                | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Premiere           | San Juan Bautista, CA                    | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Salad Crisp        | Northeast, United States                 | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Shinano Hope       | Japan                                     | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Star Ray           | Unknown                                   | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Sun Devil          | San Joaquin Valley                       | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Valley Green       | Unknown                                   | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Valverde           | Lower Rio Grande Valley, TX              | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Vanguard 75        | Low desert                               | Lower Rio Grande Valley, TX                                                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Yuma               | San Joaquin Valley and low desert        | San Joaquin Valley and low desert                                                                                                                                              | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| Controls           |                                          |                                                                                                               |                                                                                                           |  |
| Salinas (susceptible) | Coastal California Tiber × (La Brillante × Pacific) | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |
| RH11-1798 (resistant)<sup>c</sup> | Coastal California, San Joaquin Valley, and low desert | Coastal California, San Joaquin Valley, and low desert                                                                                                                      | https://apps.ams.usda.gov/CMS/default.aspx.                                                                 |  |

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

<sup>b</sup>Low desert is the Imperial Valley, CA, and Yuma Valley, AZ. The San Joaquin Valley is in California.

<sup>c</sup>Breeding line released as RH12-3197 (Hayes et al., 2015).

### Table 2. Experiments conducted in this research.

| Expt. Location | Expt. Location | Expt. Location | Expt. Location | Expt. Location | Expt. Location | Expt. Location | Expt. Location | Expt. Location |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  | USDA, Salinas  |
| 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    | 16 May 2012    |
| Three time points | Three time points | Three time points | Three time points | Three time points | Three time points | Three time points | Three time points | Three time points | Three time points |
| 33             | 33             | 33             | 33             | 33             | 33             | 33             | 33             | 33             | 33             |
| RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          | RCBDA          |
| RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   | RDI, RDS, MM   |

<sup>d</sup>RDI = root discoloration incidence; RDS = root discoloration severity; FS = foliar symptoms; MM = market maturity; Horticultural = head weight, head height, core height, and tipburn incidence; RCBDA = 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 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 VERT2012-1, 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 × 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 RDs 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

| Cultivar | 2012 RDI | 2013 RDI | 2014 RDI | Multi-yr avg |
|----------|----------|----------|----------|-------------|
|          | Median RME 95% CI | Median RME 95% CI | Median RME 95% CI | Median RME 95% CI |
| Control  |          |          |          |             |
| Salinas  | 0.78 0.56–0.90 | 0.79 0.69–0.86 | 0.67 0.36–0.87 |             |
| RH11-1798 | 0.02 0.02–0.04 | 0.02 0.01–0.02 | 0.01 0.00–0.03 |             |
| Cultivars |          |          |          |             |
| Anuenue  | 0.11 0.04–0.27 | 0.11 0.05–0.24 | 0.41 0.33–0.51 |             |
| Honcho II | 0.25 0.19–0.33 | 0.21 0.20–0.47 | 0.46 0.40–0.50 |             |
| BRG      | 0.31 0.18–0.48 | 0.32 0.20–0.47 | 0.42 0.36–0.49 |             |
| Green Lakes | 0.32 0.24–0.41 | 0.32 0.20–0.47 | 0.41 0.35–0.49 |             |
| Valverde | 0.47 0.36–0.55 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Yuma     | 0.60 0.44–0.74 | 0.53 0.38–0.52 | 0.41 0.34–0.48 |             |
| Kikugawa | 0.51 0.38–0.64 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Salad Crisp | 0.52 0.38–0.64 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Green Lightning | 0.68 0.53–0.78 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Vanguard 75 | 0.57 0.38–0.53 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Desert Storm | 0.58 0.44–0.74 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Cibola   | 0.57 0.38–0.53 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Emperor  | 0.53 0.38–0.53 | 0.43 0.29–0.46 | 0.41 0.34–0.48 |             |
| Floricrisp 1265 | 0.61 0.44–0.74 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Oswego   | 0.65 0.44–0.74 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Shinao Hope | 0.63 0.44–0.74 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Grizzly  | 0.62 0.44–0.74 | 0.53 0.38–0.48 | 0.41 0.34–0.48 |             |
| Ibicba   | 0.72 0.53–0.83 | 0.66 0.44–0.67 | 0.41 0.34–0.48 |             |
| Ithaca   | 0.80 0.63–0.90 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Star Ray | 0.80 0.63–0.90 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Sun Devil | 0.80 0.63–0.90 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Valley Green | 0.80 0.63–0.90 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Annie    | 0.78 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Del Rio  | 0.78 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Dominguez 67 | 0.78 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Climax   | 0.75 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Head Master | 0.75 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| El Dorado | 0.92 0.75–0.98 | 0.80 0.63–0.78 | 0.41 0.34–0.48 |             |
| Coyote   | 0.92 0.75–0.98 | 0.80 0.63–0.78 | 0.41 0.34–0.48 |             |
| Gabian   | 0.90 0.75–0.98 | 0.80 0.63–0.78 | 0.41 0.34–0.48 |             |
| Picholine | 0.82 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |
| Experiment total | 0.78 0.63–0.89 | 0.75 0.53–0.64 | 0.41 0.34–0.48 |             |

*Cultivar Vanguard 75 was not planted in 2012.

*Cultivars 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. ($P < 0.0001$)
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 × 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

| Cultivars          | 2013 field exp. | 2014 field exp. |
|--------------------|-----------------|-----------------|
|                    | Evaluation time points¹ | Evaluation time points¹ |
|                    | 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 |
| Climax²           | 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 Ray¹          | 0.15 | 0.58 | 0.72 | 0.20 | 0.91 |
| Shino Nohe         | 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 |
| Gabilán            | 0.15 | 0.70 | 0.97 | 0.67 | 1.06 |

¹Evaluation time points correspond to before, at, and after peak market maturity in 2013 and at and after peak market maturity in 2014.

²Cultivars Star Ray and Climax were discarded in 2014 because of poor germination and seed contamination.

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
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 *F. dahliae* and *V. albo-atrum*. Mol. Plant Pathol. 7:71–86.

Gayoso, C., F. Pomar, E. Novo-Uzal, F. Merino, and O. Martinez de Iarduya. 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. 6:496.

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.