

CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2014 - March 31, 2015

BIOLOGY AND EPIDEMIOLOGY OF VERTICILLIUM WILT OF LETTUCE AND SPINACH

Krishna V. Subbarao

Department of Plant Pathology

University of California, Davis

SUMMARY

There were many objectives in last year's proposal but due to personnel departures, work could only be completed on four objectives. Two of the objectives were resistance breeding-related and progress on these will be in Ryan Hayes' report. We recently described five new species of *Verticillium* including *V. isaacii* and *V. klebahnii*, both of which occur in agricultural soils in coastal CA, and have been isolated from asymptomatic and diseased spinach and lettuce plants. Little is known about their pathogenicity and virulence on lettuce and a broader range of other crops important to coastal California that in turn can impact lettuce production. Four isolates each of *V. isaacii* and *V. klebahnii* along with *V. dahliae* races 1 and 2 were inoculated on eight crops (artichoke, cauliflower, eggplant, lettuce, pepper, tomato, spinach and strawberry) in a greenhouse experiment. After 8 wk, plants were assessed for disease severity to determine the relative host ranges of *Verticillium* isolates. Additionally, 13 lettuce lines resistant to race 1 and partially resistant to race 2 of *V. dahliae* were screened against *V. isaacii* and *V. klebahnii* to evaluate their responses. Three of four *V. isaacii* and four of four *V. klebahnii* isolates tested were non-pathogenic on all crops tested except for those indicated below. One *V. isaacii* isolate caused wilt on artichoke and lettuce cv. Salinas, and most isolates of both species caused varying degrees of Verticillium wilt on strawberry. Lettuce lines resistant to *V. dahliae* race 1 and partially resistant to *V. dahliae* race 2 also exhibited resistance to all of the isolates of *V. isaacii* and *V. klebahnii*. Thus, at least some isolates of *V. isaacii* and *V. klebahnii* have the potential to become significant pathogens of coastal CA crops. However, resistance developed against *V. dahliae* also offers resistance to the pathogenic isolates of these new species, at least in lettuce. Some isolates of *V. isaacii* and *V. klebahnii* when inoculated two weeks in advance of the pathogenic *V. dahliae*, suppress Verticillium wilt by more than 50%. These isolates are being evaluated further for their biocontrol potential.

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**PROJECT TITLE: BIOLOGY AND EPIDEMIOLOGY OF
VERTICILLIUM WILT OF LETTUCE**

PRINCIPAL INVESTIGATOR: Krishna V. Subbarao
Department of Plant Pathology
University of California, Davis

**COOPERATING PERSONNEL: Patrik Inderbitzin, Suraj
Gurung, Dylan Short, Alex Putman
and German Sandoya**
Department of Plant Pathology,
University of California, Davis

Steven T. Koike
U. C. Cooperative Extension, Salinas, CA

Ryan Hayes
USDA-ARS, Salinas

OBJECTIVES:

- A. Evaluation of the pathogenicity of *V. klebahnii* and *V. isaacii* relative to *V. dahliae* on lettuce and other hosts.
- B. Evaluate the effectiveness of *V. dahliae* race 1 resistance identified in lettuce against *V. klebahnii*, *V. isaacii* and *V. dahliae*.
- C. Create a race 2-infested plot at the USDA Station.
- D. Continue the breeding program to develop race 1 resistance in crisphead, leaf, and other lettuce types.

PROCEDURES AND RESULTS:

Objective A. Evaluation of the pathogenicity of *V. klebahnii* and *V. isaacii* relative to *V. dahliae* on lettuce and other hosts.

Methods.

Four isolates each of *V. isaacii* (Cello-A-Tri-P (spinach), PD 341 (lettuce), PD 661 (lettuce) and PD 752 (spinach)) and *V. klebahnii* (PD 347 (artichoke), PD 407 (lettuce), PD 458 (lettuce) and PD 659 (lettuce)), and two isolates of *V. dahliae* representing race 1 (Ls. 16) and 2 (Ls. 17) were tested on artichoke (cv. 5473/91 black), cauliflower (cv. snowball), eggplant (cv. Long purple), lettuce cv. La Brillante (resistant to race 1 of *V. dahliae*), lettuce cv. Salinas (susceptible to both *V. dahliae* races), pepper (cv. California wonder), spinach (cv. Hector), strawberry (cv.

Albion) and tomato (cv. Beefsteak) in the greenhouse. Experiments were conducted two times. Thirty seeds of each host were sown in 200-well plug trays filled with Sunshine Mix #4. Three weeks after sowing, seedlings were transferred into 0.5-liter foam-insulated cups filled with a pasteurized sand-potting soil mixture (2:1 vol/vol). Strawberry transplants were first established in trays containing the potting mix; after dormancy was broken and the plants had grown leaves and new roots, plants were then transplanted for inoculation. Each host-isolate treatment combination was replicated three times with 10 plants in each replication and was arranged in a randomized complete block design. Inoculum from all *V. dahliae*, *V. isaacii* and *V. klebahnii* isolates were prepared. Seedlings were inoculated at 4 wks after sowing by saturating the soil in each well with a 5 to 10-ml suspension containing 1×10^7 conidia/ml in sterile distilled water. Two additional inoculations were performed on all plants with the corresponding inoculum at one and two weeks after the first inoculation. Control plants for each host were mock inoculated with sterile distilled water and were kept in a separate block. Plants were grown on greenhouse benches for approximately ten wks after the first inoculations and were evaluated for Verticillium wilt disease severity. Verticillium wilt severity was rated 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%; 4 = 76 to 100% discoloration and no foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt.

Symptomatic root and crown tissues were washed, surface sterilized using distilled water and 5% bleach, dissected, and plated on NP-10 medium. After 10 to 14 days incubation at 22 to 24° C, plates were examined under a dissecting microscope for the presence of microsclerotia, verticillate conidiophores and hyaline conidia, the distinguishing characteristics of *Verticillium* species.

Results.

Analysis of variance indicated no significant differences between the two experiments (Table 1), thus, the data from the two experiments were combined. Analysis of variance indicated no significant differences among isolates of *V. isaacii*, *V. klebahnii*, and *V. dahliae*. Most of the *V. klebahnii* and *V. isaacii* isolates did not cause Verticillium wilt symptoms on most hosts evaluated. Correspondingly, significant interactions were observed among hosts and isolate \times host interactions (Table 1).

Pathogenicity on artichoke. Artichoke plants inoculated with *V. dahliae* isolates (Ls. 16 and Ls. 17) exhibited root discoloration (Fig. 1A). Plants were significantly stunted and had dry leaves relative to non-inoculated plants. Similarly, *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD 347, PD 407, PD 458 and PD 659) also produced root discoloration. *Verticillium isaacii* isolate Cello-A-Tri-P produced higher disease severities than the *V. dahliae* race 1 isolate Ls.16 (Table 2). Overall, mean ratings of disease severity caused by isolates of *V. dahliae* were higher than those caused by *V. isaacii* and *V. klebahnii* (Table 2). *Verticillium dahliae* and *V. isaacii* were successfully re-isolated from the discolored roots of the infected artichoke plants.

Pathogenicity on cauliflower. Cauliflower plants inoculated with *V. dahliae* isolates (Ls. 16 and Ls. 17), *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD 347, PD 407, PD 458 and PD 659) exhibited no or negligible root discoloration (Table 2). Plants looked healthy and disease severity was no different from that on control plants (data not shown). Median disease severity for all isolates and species varied between 0.0 and 0.58 (Table 2).

Pathogenicity on eggplant. Eggplants inoculated with *V. dahliae* isolate Ls. 17 exhibited severe root discoloration in most plants as well as stunting and dried leaves relative to non-inoculated plants. However, *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD 347, PD 407, PD 458 and PD 659) produced little or no root discoloration. Overall, mean ratings of disease severity caused by isolates of *V. dahliae* were higher than those caused by *V. isaacii* and *V. klebahnii*, with the highest median disease severity of 4.15 for plants inoculated with *V. dahliae* Ls. 17 (Table 2).

Pathogenicity on lettuce cvs. Salinas and La Brillante. Lettuce cv. Salinas inoculated with *V. dahliae* isolates Ls. 16 and Ls. 17 exhibited root discoloration (Fig. 1B) as did the plants inoculated with *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD 347, PD 407, PD 458 and PD 659). Of the *V. isaacii* isolates, Cello-A-Tri-P induced disease severity similar to *V. dahliae* isolate Ls. 16 (Fig. 1B). Further, *V. dahliae* and *V. isaacii* were successfully re-isolated from the discolored tap roots of the infected cv. Salinas. Overall, median ratings of disease severity produced by isolates of *V. dahliae* were greater than those caused by *V. isaacii* and *V. klebahnii*. Roots of cv. La Brillante inoculated with *V. dahliae* isolate Ls. 17 were discolored and the plants displayed wilting symptoms, however, there was no vascular discoloration symptom when inoculated with *V. dahliae* isolate Ls. 16. Even though plants of cv. La Brillante inoculated with *V. isaacii* and *V. klebahnii* isolates displayed vascular discoloration, they had low disease severity ratings (Table 2). In general, median ratings of disease severity produced by isolates of *V. dahliae* were greater than those caused by *V. isaacii* and *V. klebahnii* (Table 2).

Pathogenicity on pepper. All of the *V. dahliae*, *V. isaacii* or *V. klebahnii* isolates evaluated in this study caused negligible to no vascular discoloration on pepper (Table 2). Plants looked healthy and disease severity scores between inoculated and non-inoculated control plants were not statistically different (data not shown).

Pathogenicity on spinach. Spinach plants inoculated with *V. dahliae* isolates Ls. 16 and Ls. 17 showed root discoloration. However, none of the plants inoculated with *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) or *V. klebahnii* (PD 347, PD 407, PD 458 and PD 659) exhibited symptoms associated with Verticillium wilt. Overall, median disease ratings of *V. dahliae* were higher than those caused by *V. isaacii* and *V. klebahnii* (Table 2).

Pathogenicity on strawberry. All plants inoculated with *V. dahliae*, *V. isaacii* and *V. klebahnii* showed Verticillium wilt symptoms to varying degrees (Fig. 1C). The disease severity ratings for all isolates of *V. isaacii* and *V. klebahnii* were above the minimum severity rating of 2.0 associated with isolates pathogenic on individual hosts. Overall, median disease severity ratings caused by the race 2 isolate of *V. dahliae* were higher than those produced by *V. isaacii* and *V. klebahnii*. However, all isolates of *V. isaacii* and *V. klebahnii* including the race 1 isolate of *V. dahliae* caused lesser disease than *V. dahliae* race 2 isolate Ls. 17 (Table 2).

Pathogenicity on tomato. On tomato cv. Beefsteak, only *V. dahliae* isolate Ls. 17 caused vascular discoloration and wilting symptoms. Isolate Ls. 16 showed only slight discoloration and no wilting was observed (Table 2). However, none of the *V. isaacii* and *V. klebahnii* isolates caused any symptoms associated with Verticillium wilt (Table 2).

Contrasts between *V. isaacii*, *V. klebahnii* and the two races of *V. dahliae*. Except for the contrast between race 1 of *V. dahliae* (Ls. 16 vs. *V. isaacii* in Table 3) and *V. isaacii*, all other

contrasts between *V. isaacii* and *V. klebahnii* and the two races of *V. dahliae* were significant, suggesting that disease severities caused by these two species were either higher or lower than those of the two races of *V. dahliae* (Table 3). Except for one isolate of *V. isaacii* on artichoke and all isolates of both *V. isaacii* and *V. klebahnii* on both cultivars of lettuce, the disease severities were lower than those caused by both races of *V. dahliae* (Tables 2 and 3). In strawberry, however, nearly all isolates of *V. isaacii* and *V. klebahnii* caused as much or higher disease severities than the race 1 isolate of *V. dahliae* (Tables 2 and 3). By far, the most virulent isolate on all hosts was race 2 (Ls. 17) of *V. dahliae* (Tables 2 and 3).

Objective B. Evaluate the effectiveness of *V. dahliae* race 1 resistance identified in lettuce against *V. klebahnii*, *V. isaacii* and *V. dahliae*.

Methods

Eight *V. dahliae* race 1-resistant cvs. Annapolis, Eruption, Defender, Pavane, Little Gem, Sentry, Infantry, Merlot, and four *V. dahliae* race 2-partially resistant accessions 169511, 171674, 204707, and 226641 were tested against two isolates of *V. isaacii* (Cello-A-Tri-P and PD341), two isolates of *V. klebahnii* (PD458 and PD659), and a race 1 (Ls. 16) and race 2 (Ls. 17) isolate of *V. dahliae*. Isolate Cello-A-Tri-P was previously known to be pathogenic while isolates PD 341, PD 458 and PD 659 were determined to be pathogenic on strawberry from the pathogenicity assays described above.

All plant growth conditions, inoculum preparation, inoculation, and disease scoring were the same as described above. Lettuce cultivars - isolate combinations were arranged in a randomized complete block design with three replications and each replication included 10 plants of each lettuce line. The experiment was repeated twice. Verticillium wilt severity was assessed as previously 10 wks after the first inoculation in each experiment. Root discoloration was rated 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%; 4 = 76 to 100% discoloration and no foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt.

Results.

Verticillium isaacii isolate PD 341 and *V. klebahnii* isolates (PD 458 and PD 659) failed to produce Verticillium wilt symptoms on susceptible cv. Salinas. However, *V. isaacii* isolate Cello-A-Tri-P induced obvious disease symptoms on cv. Salinas, but failed to produce any disease symptoms on the other *V. dahliae*-resistant and partially resistant lettuce cultivars (Table 4).

Table 1. Statistical analysis of variance (ANOVA) based on the effect of individual isolates of *Verticillium dahliae*, *V. isaacii* and *V. klebahnii* on the ranked means of *Verticillium* wilt severity on different hosts across two independent experiments

Effect	df _{Num}	df _{Den}	ATS	P value
Experiment	1.37	1	8.85	0.2189
Isolate	8.99	1	32	0.1364
Host	6.56	302	99.07	<0.0001
Isolate × Host	59.1	302	4.13	<0.0001

Table 2. Median and maximum disease severity and relative marginal effects along with 95% confidence intervals (CI) for *Verticillium* wilt severity ratings in relation to different hosts and isolates of *Verticillium dahliae*, *Verticillium isaacii* and *Verticillium klebahnii*

Hosts	Isolates ^a	Disease severity ^b		Relative marginal effect ^c	
		Median	Maximum	Estimate	95% Confidence interval
Artichoke (cv. 5473/91 black)	Ls.16	1.43	2.50	0.69	(0.57, 0.79)
	Ls.17	2.75	3.50	0.92	(0.83, 0.94)
	Cello-A-Tri-P	2.00	2.43	0.71	(0.57, 0.81)
	PD 341	0.92	1.29	0.46	(0.32, 0.60)
	PD 661	0.47	1.14	0.33	(0.20, 0.49)
	PD 752	0.31	1.40	0.36	(0.24, 0.52)
	PD 347	0.69	2.17	0.45	(0.24, 0.68)
	PD 407	0.32	1.67	0.37	(0.17, 0.65)
	PD 458	0.21	1.86	0.34	(0.19, 0.55)
	PD 659	0.34	2.29	0.37	(0.18, 0.63)
Cauliflower (cv. Snowball)	Ls.16	0.37	0.70	0.59	(0.39, 0.75)
	Ls.17	0.57	1.00	0.62	(0.36, 0.82)
	Cello-A-Tri-P	0.05	0.60	0.42	(0.24, 0.62)
	PD 341	0.58	1.30	0.65	(0.42, 0.81)
	PD 661	0.00	1.22	0.37	(0.19, 0.62)
	PD 752	0.05	0.33	0.36	(0.23, 0.51)
	PD 347	0.50	1.25	0.64	(0.40, 0.82)
	PD 407	0.00	1.33	0.41	(0.20, 0.67)
	PD 458	0.27	0.44	0.47	(0.31, 0.64)
	PD 659	0.15	1.22	0.48	(0.26, 0.70)
Eggplant (cv. Long purple)	Ls.16	1.17	2.14	0.78	(0.64, 0.86)
	Ls.17	4.15	5.00	0.95	(0.63, 0.99)
	Cello-A-Tri-P	0.05	0.80	0.32	(0.17, 0.54)

	PD 341	0.39	1.29	0.54	(0.36, 0.70)
	PD 661	0.10	0.60	0.37	(0.20, 0.59)
	PD 752	0.26	0.56	0.43	(0.26, 0.62)
	PD 347	0.16	0.30	0.34	(0.22, 0.49)
	PD 407	0.15	0.56	0.37	(0.21, 0.58)
	PD 458	0.31	0.89	0.5	(0.33, 0.68)
	PD 659	0.11	0.70	0.41	(0.23, 0.62)
Lettuce (cv. Salinas)	Ls.16	3.00	3.50	0.81	(0.67, 0.89)
	Ls.17	4.50	4.67	0.85	(0.74, 0.91)
	Cello	3.26	3.96	0.69	(0.54, 0.80)
	PD 341	1.00	3.00	0.41	(0.23, 0.62)
	PD 661	1.75	3.13	0.53	(0.32, 0.73)
	PD 752	0.75	2.13	0.29	(0.14, 0.52)
	PD 347	1.04	1.78	0.31	(0.17, 0.51)
	PD 407	1.05	2.33	0.36	(0.21, 0.55)
	PD 458	1.25	2.17	0.46	(0.28, 0.66)
	PD 659	0.95	1.50	0.3	(0.20, 0.43)
Lettuce (cv. La Brillante)	Ls.16	0.00	0.00	0.00	(0.00, 0.00)
	Ls.17	2.93	4.78	0.87	(0.75, 0.92)
	Cello-A-Tri-P	1.19	1.38	0.63	(0.41, 0.79)
	PD 341	1.13	1.30	0.59	(0.45, 0.72)
	PD 661	1.00	1.50	0.32	(0.19, 0.48)
	PD 752	1.04	1.60	0.32	(0.29, 0.49)
	PD 347	1.36	1.50	0.42	(0.23, 0.65)
	PD 407	1.25	1.86	0.47	(0.24, 0.72)
	PD 458	0.66	1.13	0.18	(0.12, 0.30)
	PD 659	1.42	1.50	0.47	(0.30, 0.65)
Pepper (cv. California Wonder)	Ls.16	0.13	0.20	0.65	(0.46, 0.80)
	Ls.17	0.00	0.00	0.35	(0.30, 0.40)
	Cello-A-Tri-P	0.00	0.40	0.53	(0.33, 0.71)
	PD 341	0.00	0.00	0.35	(0.30, 0.40)
	PD 661	0.00	0.10	0.47	(0.34, 0.61)
	PD 752	0.00	0.00	0.35	(0.30, 0.40)
	PD 347	0.00	0.56	0.55	(0.33, 0.75)
	PD 407	0.33	0.44	0.71	(0.46, 0.86)
	PD 458	0.11	0.60	0.69	(0.45, 0.85)
	PD 659	0.00	0.00	0.35	(0.30, 0.40)
Spinach (cv. Hector)	Ls.16	4.10	5.00	0.93	(0.87, 0.94)
	Ls.17	3.70	4.20	0.86	(0.81, 0.89)
	Cello-A-Tri-P	0.00	0.20	0.22	(0.15, 0.31)
	PD 341	1.50	3.00	0.65	(0.48, 0.79)
	PD 661	0.00	0.80	0.32	(0.36, 0.48)
	PD 752	0.00	0.10	0.30	(0.32, 0.46)
	PD 347	0.40	1.40	0.46	(0.47, 0.63)
	PD 407	0.50	0.60	0.42	(0.31, 0.56)

	PD 458	0.50	1.20	0.49	(0.32, 0.62)
	PD 659	0.20	1.40	0.37	(0.42, 0.54)
Strawberry (cv. Albion)	Ls.16	2.20	2.60	0.39	(0.20, 0.44)
	Ls.17	4.25	5.00	0.93	(0.89, 0.95)
	Cello-A-Tri-P	2.13	2.40	0.40	(0.27, 0.50)
	PD 341	2.00	2.40	0.31	(0.30, 0.47)
	PD 661	2.38	3.40	0.50	(0.31, 0.69)
	PD 752	2.22	3.60	0.46	(0.25, 0.69)
	PD 347	2.85	3.40	0.71	(0.62, 0.78)
	PD 407	2.55	3.00	0.57	(0.43, 0.70)
	PD 458	2.10	2.80	0.35	(0.21, 0.52)
	PD 659	2.20	3.40	0.47	(0.30, 0.67)
Tomato (cv. Beefsteak)	Ls.16	1.46	2.80	0.81	(0.58, 0.90)
	Ls.17	2.81	3.50	0.92	(0.87, 0.94)
	Cello-A-Tri-P	0.00	0.43	0.45	(0.33, 0.58)
	PD 341	0.00	0.17	0.44	(0.33, 0.56)
	PD 661	0.00	0.00	0.38	(0.34, 0.41)
	PD 752	0.00	0.22	0.44	(0.33, 0.56)
	PD 347	0.00	0.00	0.38	(0.34, 0.41)
	PD 407	0.00	0.00	0.38	(0.34, 0.41)
	PD 458	0.00	0.00	0.38	(0.34, 0.41)
	PD 659	0.00	0.25	0.44	(0.33, 0.57)

^a *V. dahliae* isolates are Ls.16 and Ls.17 (lettuce), *V. isaacii* isolates are Cello-A-Tri-P (spinach), PD 341 (lettuce), PD 661 (lettuce), and PD 752 (spinach), and *V. klebahnii* isolates are PD 347 (artichoke), PD 407 (lettuce), PD 458 (lettuce), and PD 659 (lettuce).

^b Disease severity of Verticillium wilt was assessed visually on an ordinal 0 to 5 scale, where 0 = no vascular discoloration, 1 = 1 to 25% of the vascular tissue showing discoloration, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100% discoloration with no foliar symptoms, and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt (Vallad et al. 2006). Analysis of variance indicated no significant differences between two experiments, and therefore averaged disease severity from the two experiments are shown. Median values were calculated from wilt severity values averaged over inoculated plants within replications.

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

Table 3. Orthogonal contrasts between *Verticillium isaacii* and *V. klebahnii* and type isolates for races 1 (Ls.16) and 2 (Ls.17) of *V. dahliae* on hosts in which the two former species caused Verticillium wilt

Contrasts	Numerator DF	Denominator DF	F	P > F
Artichoke				
Ls.16 vs <i>V. isaacii</i>	1	48	6.26	0.0158
Ls.16 vs. <i>V. klebahnii</i>	1	48	12.98	0.0007
Ls.16 vs. Ls.17	1	48	7.02	<0.0109
Ls.17 vs <i>V. isaacii</i>	1	48	64.08	<0.0001
Ls.17 vs <i>V. klebahnii</i>	1	48	119.11	<0.0001
Lettuce - Salinas				
Ls.16 vs <i>V. isaacii</i>	1	48	14.18	0.0005
Ls.16 vs. <i>V. klebahnii</i>	1	48	34.15	<0.0001
Ls.16 vs. Ls.17	1	48	0.32	0.5717
Ls.17 vs <i>V. isaacii</i>	1	48	33.85	<0.0001
Ls.17 vs <i>V. klebahnii</i>	1	48	103.87	<0.0001
Lettuce – La Brillante				
Ls.16 vs <i>V. isaacii</i>	1	48	6.94	0.0113
Ls.16 vs. <i>V. klebahnii</i>	1	48	11.76	0.0013
Ls.16 vs. Ls.17	1	48	130.63	<0.0001
Ls.17 vs <i>V. isaacii</i>	1	48	18.72	<0.0001
Ls.17 vs <i>V. klebahnii</i>	1	48	26.76	<0.0001
Strawberry				
Ls.16 vs <i>V. isaacii</i>	1	66	1.75	0.1910
Ls.16 vs. <i>V. klebahnii</i>	1	66	5.80	0.0188
Ls.16 vs. Ls.17	1	66	56.83	<0.0001
Ls.17 vs <i>V. isaacii</i>	1	66	144.90	<0.0001
Ls.17 vs <i>V. klebahnii</i>	1	66	56.29	<0.0001

Table 4. Median and maximum disease severity and relative marginal effects along with 95% confidence intervals (CI) for *Verticillium* wilt severity ratings in relation to different isolates of *Verticillium isaacii*, *V. klebahnii* and *V. dahliae* on lettuce cultivars resistant to *Verticillium dahliae* race 1 and partially resistant to *Verticillium dahliae* race 2 isolates

Isolates ^a	Cultivars ^b	Relative marginal effects ^d		
		Median	Estimate	95% Confidence Interval
Cello-A-Tri-P	169511 ^b	0.00	0.35	(0.32, 0.37)
	171674	0.00	0.47	(0.34, 0.62)
	204707	0.00	0.49	(0.33, 0.64)
	226641	0.00	0.41	(0.30, 0.53)
	Annapolis	0.20	0.55	(0.38, 0.71)
	Defender	0.70	0.72	(0.55, 0.84)
	Eruption	0.00	0.35	(0.32, 0.37)
	Infantry	0.00	0.35	(0.32, 0.37)
	La Brillante	0.20	0.56	(0.38, 0.72)
	Little Gem	0.20	0.55	(0.38, 0.71)
	Merlot	0.00	0.47	(0.34, 0.62)
	Pavane	0.00	0.47	(0.34, 0.62)
	Salinas	2.70	0.83	(0.82, 0.84)
	Sentry	0.00	0.35	(0.32, 0.37)
PD 341	169511	0.00	0.47	(0.33, 0.63)
	171674	0.00	0.35	(0.32, 0.37)
	204707	0.00	0.49	(0.33, 0.65)
	226641	0.00	0.41	(0.30, 0.54)
	Annapolis	0.00	0.41	(0.29, 0.54)
	Defender	0.00	0.41	(0.29, 0.54)
	Eruption	0.00	0.35	(0.32, 0.37)
	Infantry	0.00	0.49	(0.33, 0.65)
	La Brillante	0.00	0.35	(0.32, 0.37)
	Little Gem	0.00	0.35	(0.32, 0.37)
	Merlot	0.00	0.41	(0.30, 0.53)
	Pavane	0.00	0.41	(0.30, 0.53)
	Salinas	0.00	0.49	(0.33, 0.65)
	Sentry	0.00	0.35	(0.32, 0.37)
PD 458	169511	0.00	0.47	(0.32, 0.63)
	171674	0.00	0.41	(0.29, 0.54)
	204707	0.00	0.35	(0.32, 0.37)
	226641	0.00	0.35	(0.32, 0.37)
	Annapolis	0.00	0.47	(0.33, 0.63)

	Defender	0.00	0.35	(0.32, 0.37)
	Eruption	0.00	0.42	(0.29, 0.57)
	Infantry	0.00	0.35	(0.32, 0.37)
	La Brillante	0.00	0.35	(0.32, 0.37)
	Little Gem	0.00	0.35	(0.32, 0.37)
	Merlot	0.00	0.49	(0.32, 0.65)
	Pavane	0.00	0.35	(0.32, 0.37)
	Salinas	0.20	0.56	(0.37, 0.73)
	Sentry	0.00	0.47	(0.32, 0.63)
PD 659	169511	0.00	0.47	(0.32, 0.63)
	171674	0.00	0.35	(0.32, 0.37)
	204707	0.00	0.35	(0.32, 0.37)
	226641	0.00	0.35	(0.32, 0.37)
	Annapolis	0.20	0.54	(0.37, 0.70)
	Defender	0.00	0.35	(0.32, 0.37)
	Eruption	0.00	0.35	(0.32, 0.37)
	Infantry	0.00	0.35	(0.32, 0.37)
	La Brillante	0.00	0.35	(0.32, 0.37)
	Little Gem	0.00	0.35	(0.32, 0.37)
	Merlot	0.00	0.35	(0.32, 0.37)
	Pavane	0.00	0.35	(0.32, 0.37)
	Salinas	0.00	0.49	(0.32, 0.65)
	Sentry	0.00	0.47	(0.32, 0.63)
Ls.16	169511	0.00	0.35	(0.32, 0.37)
	171674	0.00	0.35	(0.32, 0.37)
	204707	0.00	0.35	(0.32, 0.37)
	226641	0.00	0.50	(0.32, 0.67)
	Annapolis	0.00	0.41	(0.29, 0.54)
	Defender	0.00	0.41	(0.29, 0.54)
	Eruption	0.00	0.35	(0.32, 0.37)
	Infantry	0.00	0.35	(0.32, 0.37)
	La Brillante	0.00	0.35	(0.32, 0.37)
	Little Gem	0.00	0.41	(0.29, 0.54)
	Merlot	0.00	0.42	(0.29, 0.57)
	Pavane	0.00	0.35	(0.32, 0.37)
	Salinas	4.70	0.99	(0.98, 0.99)
	Sentry	0.00	0.35	(0.32, 0.37)
Ls.17	169511	1.20	0.66	(0.45, 0.82)
	171674	2.00	0.67	(0.45, 0.84)
	204707	3.10	0.87	(0.85, 0.89)

226641	4.00	0.93	(0.91, 0.94)
Annapolis	3.80	0.90	(0.86, 0.93)
Defender	4.20	0.93	(0.89, 0.96)
Eruption	3.80	0.91	(0.88, 0.92)
Infantry	3.80	0.91	(0.87, 0.94)
La Brillante	4.30	0.93	(0.90, 0.95)
Little Gem	4.00	0.92	(0.90, 0.94)
Merlot	3.70	0.90	(0.87, 0.93)
Pavane	4.20	0.93	(0.89, 0.95)
Salinas	4.50	0.96	(0.92, 0.98)
Sentry	4.00	0.91	(0.88, 0.93)

^a Isolate Cello-A-Tri-P (*V. isaacii*) was collected from a spinach plant in CA. Isolates PD341 (*V. isaacii*), PD458 and PD659 (*V. klebahnii*) were collected from a lettuce field in CA. *Verticillium dahliae* isolates Ls.16 (race 1) and Ls.17 (race 2) were collected from CA and were used as controls.

^b Lettuce cultivars 169511, 171674, 204707 and 226641 are partially resistant to *V. dahliae* race 2 isolates (Hayes et al. 2011a). Similarly, Annapolis, Defender, Eruption, Infantry, La Brillante, Little Gem, Merlot, Pavane, Salinas and Sentry are resistant to *V. dahliae* race 1 isolates (Hayes et al. 2007).

^c Disease severity of Verticillium wilt was assessed visually on an ordinal 0 to 5 scale, where 0 = no vascular discoloration, 1 = 1 to 25% of the vascular tissue showing discoloration, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100% discoloration with no foliar symptoms, and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt (Vallad et al., 2006). Median values were calculated from wilt severity values averaged over inoculated plants within replications.

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

Fig. 1. Vascular discoloration in artichoke (A), lettuce (B), and strawberry (C) caused by the race 1 and 2 strains of *Verticillium dahliae* (Vd.Ls16 and Vd.Ls17) and an isolate of *V. isaacii* (Cello-A-Tri-P) relative to an uninoculated control plant.

