

**Project Title:** Race Characterization of *Verticillium* Isolates Recovered from Lettuce Growing Regions

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**Abstract:**

Based on recent research findings (Krishna Subbarao, UC Davis; CLGRB funding), it is clear that the *Verticillium* wilt pathogen (*Verticillium dahliae*) found in coastal California lettuce and other crops consists of different races. Race 1 is the predominant race; it is not known to what extent non-race 1 isolates occur. For this project we (a) purified *V. dahliae* isolates obtained from lettuce, strawberry, and other coastal crops affected by *Verticillium* wilt; (b) characterized isolates to race using molecular methods developed by the Subbarao research team; and (c) mapped the findings to document distribution of races 1, 2, and 3. In this study, 131 plant samples from 85 field locations are analyzed, resulting in 646 purified *Verticillium* isolates. *Verticillium dahliae* race 1, found throughout the surveyed central coast region, is the most commonly recovered race (76%) in this study. Race 2 represents 7% of isolates; most of these isolates come from lettuce, though race 2 is also recovered from artichoke and tomato. Race 3 is identified as 18% of isolates; most race 3 isolates come from strawberry, but also are found in pepper and cabbage. Isolates (from pepper and maple tree) recovered from four locations are confirmed to be *V. dahliae* but do not register a race. These isolates could possibly belong to a novel, new race; however, they could also be variants that lack the molecular characteristics of known *V. dahliae* races. Of special note is that the TriCal Diagnostics lab detected *Verticillium* wilt of lettuce in Santa Maria for the first time in 2021; this 2022 survey project again found lettuce *Verticillium* wilt in this growing region, which previously did not have the disease.

**Objectives:**

Conduct an extensive survey of *Verticillium dahliae* isolate races recovered from lettuce and other coastal crops. Explore the extent of races 2 and 3 occurring in coastal California fields.

## **Procedures:**

Sample collection and processing: For this project, most samples are obtained from TriCal Diagnostics clients who use our disease diagnostic services. If *Verticillium* wilt is suspected, the roots and main stems of whole plant samples are thoroughly washed, surface sanitized in 1% sodium hypochlorite, and then aseptically dissected. Symptomatic, discolored pieces from vascular tissues are transferred to NP-10 semi-selective medium (Koike et al. 1994) to recover *Verticillium* isolates. Following our regular practice, these samples are also tested for other fungal (*Fusarium*, *Pythium*, *Sclerotinia*, *Botrytis*, *Thielaviopsis*, others) and virus (INSV, TSWV, LDaV, others) pathogens as appropriate based on symptoms. Additional samples are collected by Koike, who visited lettuce field sites following reports of severe *Verticillium* wilt cases.

Isolate purification and DNA extraction: For positive *Verticillium* cultures, two to four isolates per sample are transferred to PDA to allow for aerial mycelial growth. Two weeks after inoculation, mycelial mats are collected and DNA extracted using a slightly modified protocol for Qiagen's Qiacube DNeasy Blood and Tissue program. Mycelial mats are ground in CTAB at a 1:2 ratio; 350  $\mu$ L of extract is centrifuged; the supernatant is decanted into 2 mL sample tubes for extraction on the Qiacube Connect equipment. CTAB is used as an extraction buffer instead of AL buffer and P3 buffer from Qiagen's DNeasy Plant Mini Kit used instead of ethanol. All downstream steps follow the manufacturer's recommendation.

Molecular characterization: *Verticillium dahliae* species and race are molecularly confirmed using four primer sets and protocols provided by the Subbarao lab (Inderbitzin et al. 2013; Wang et al. 2021; CLGRB report 2021). Isolates identified as *V. dahliae* race 2, race 3, and "unidentified" are re-tested to verify results. Isolates that do not amplify with any of these primer sets are sequenced with PCR primers amplifying the internal transcriber space (ITS) region ITS1/ITS4 (White et al. 1990). Sequences are compared to type material sequences on the NCBI database (nBLAST) for species identification.

Isolate preservation: Isolates identified as *V. dahliae* race 2, race 3, and "unidentified" are further purified via single-sporing. These single-spore cultures are sent to the Subbarao lab for further genomic analysis and future pathogenicity tests.

Isolate distribution: Maps are constructed to depict the survey findings according to geographic region, race of *Verticillium dahliae*, and host crop.

## **Results and Discussion:**

Sample collection and processing: From April to November 2022, TCD received 131 samples with suspected *Verticillium* disease; these opportunistic samples are from 85 sites (Figures 1 and 2, Table 1). The survey is opportunistic because most samples are submitted by growers and PCAs based on disease outbreaks. Therefore, some sites are sampled more often than others. For some samples, a larger number of isolates is purified and preserved. For these reasons, percentages below are calculated based on site rather than isolate or sample number to avoid sampling bias.

Most of the samples are from Monterey County (75%) with additional ones from Santa Cruz (10%), Santa Barbara (7%), and Santa Clara (5%) counties; one sample each is collected from San Mateo, San Luis Obispo, and San Diego counties (Figures 1 and 2).

The majority of the samples are lettuce (65%) with other samples being strawberry (10%), napa cabbage (5%), pepper (5%), Brussels sprouts (4%), artichoke (4%), cauliflower (3%), and tomato (3%) (Figure 2). A Japanese maple tree infected with *Verticillium* wilt was submitted from San Mateo County. We could not recover *Verticillium* from eight samples. Of the remaining 123 samples from 80 sites, 646 isolates are purified and DNA extracted for molecular characterization.

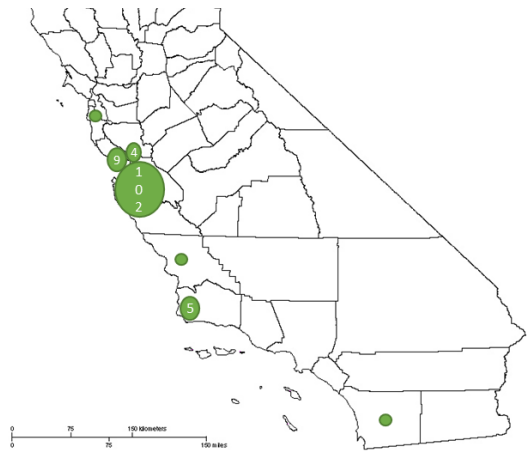


Figure 1: Number of plant samples as distributed among California coastal counties.

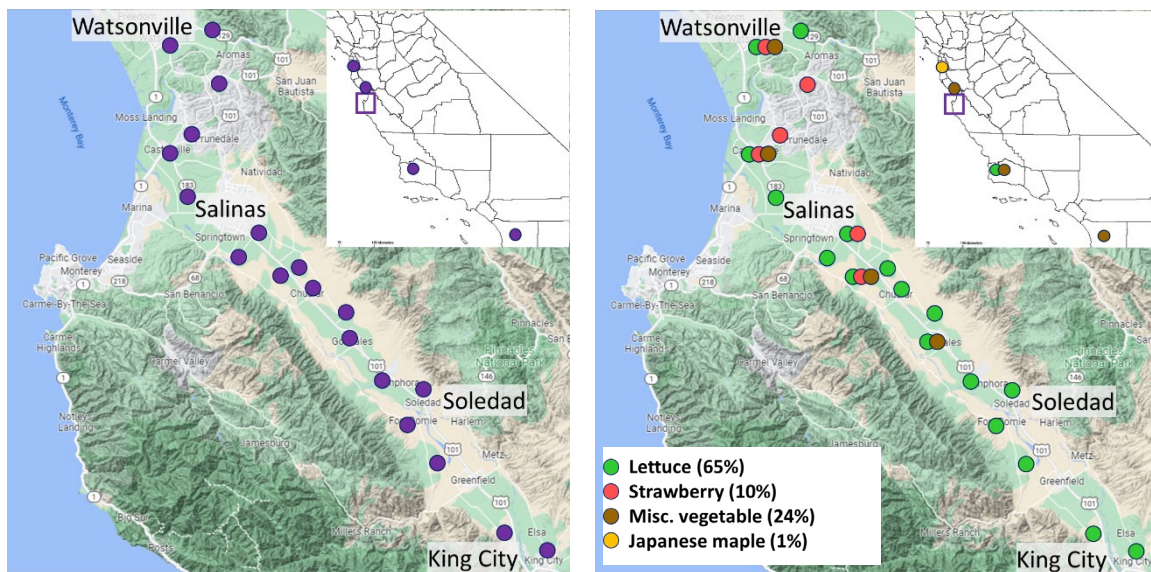


Figure 2: Left) General location of collection sites within Monterey, Santa Cruz, and Santa Clara counties (purple dots); Right) Hosts sampled at each location. Black and white inserted California maps indicate the central coast sample region (purple square) and other collection sites in the state.

**Molecular characterization:** *Verticillium dahliae* race 1 is the most commonly recovered race (76%) in this study and is found throughout the surveyed central coast region (Figure 3, Table 1). Race 2 is identified from 7% of the isolates; most of the race 2 isolates come from lettuce, though race 2 is also recovered from artichoke and tomato. Race 3 is identified from 18% of isolates; most race 3 isolates come from strawberry, though it is also found in pepper and cabbage.

Isolates (from pepper and maple tree) collected from four sites are confirmed to be *V. dahliae* but did not register a race. These isolates could possibly belong to a novel, new race. Another possibility is that these isolates could be similar to Vd.Ls.17, a previously described race 2 type isolate that lacks the molecular characteristics of known *V. dahliae* races (Subbarao, CGLRB Report 2021). Whole genome analysis will be needed to characterize such isolates.

Race 2, race 3, some race 1, and novel isolates are purified and given to the Subbarao lab for further study. In total, 49 isolates from 18 samples representing 17 sites are single-spored and sent to this collaborator.

Our survey also recovers a number of non-*V. dahliae* isolates. From lettuce we identify *V. isaacii*, and *V. klebahnii*; from brassica crops we find *V. alfalfae*. It is notable that for the second season in a row, we confirm Verticillium wilt on lettuce from Santa Maria, and recover *V. dahliae* from lettuce grown in that area.

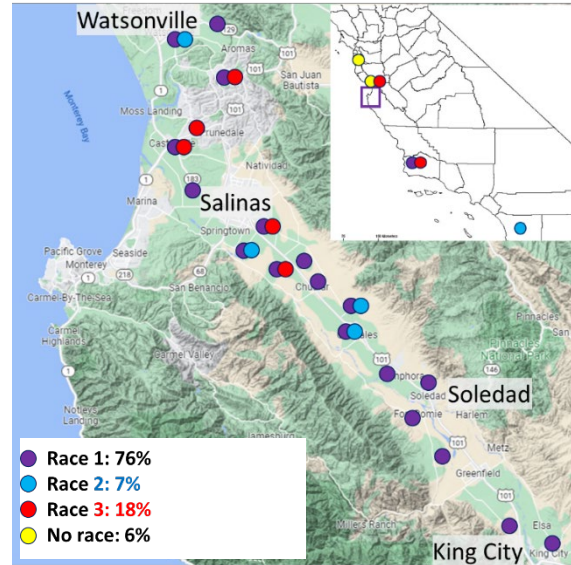


Figure 3: *V. dahliae* races identified at sample locations. Black and white inserted California map indicates the central coast sample region (purple square) and other CA collection sites.

County	<i>V. dahliae</i> race	Host	Isolates	Sites
San Mateo	Novel?	Japanese maple	2	1
Santa Cruz	1, 2	lettuce, strawberry, tomato	27	8
Santa Clara	3, Novel?	pepper	16	4
Monterey	1, 2, 3	lettuce, strawberry, cabbage, Brussels sprouts, cauliflower, artichoke	554	60
San Luis Obispo		cabbage	3	1
Santa Barbara	1, 2, 3	lettuce, cabbage	39	5
San Diego	2	tomato	4	1
TOTALS			645	80

Table 1: Number of isolates, number of collection sites, plant hosts, and *Verticillium dahliae* race identifications by California county.

## Acknowledgments

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## References:

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