Prevalence of Race 2 Strains of *Verticillium dahliae* Causing Verticillium Wilt of Mint in Oregon

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Introduction

Verticillium dahliae is a plant pathogenic fungus that causes Verticillium wilt, which is the most detrimental disease of commercially grown peppermint (*Mentha piperita*) in Oregon. Symptoms in peppermint include asymmetrical growth, reddening, chlorosis, necrosis, stunted growth, wilt, and premature plant death. Two pathogenic races of *V. dahliae* have been identified. Race 1 isolates contain the *Ave1*gene. Several plants that are resistant to race 1 have been identified, including lettuce and tomato. These resistant plants contain a gene which encodes for plant immune-receptors that can recognize the avirulence gene in race 1 strains of *V. dahliae* (de Jonge et al. 2012). Race 2 isolates of *V. dahliae* do not possess the avirulence gene and therefore do not trigger an immune response in plants containing the resistance gene. Several studies have reported an increased prevalence of race 2 relative to race 1, likely owing to its success in colonizing a greater variety of plants in crop rotations (Short et al. 2014).

Despite being the top mint producing area in the country, there has been relatively little work done to investigate the races of *V. dahliae* infecting mint in the Pacific Northwest. One study tested 16 *V. dahliae* isolates collected from Washington mint and all 16 isolates were determined to be race 2. However, it is not known which race(s) of *V. dahliae* cause Verticillium wilt in commercial peppermint production fields of Oregon. We hypothesize that race 2 *V. dahliae* strains are responsible for Verticillium wilt in peppermint grown in Oregon. The objectives of this study were to: (i) determine which race of *V. dahliae* is infecting Oregon peppermint; and (ii) determine which race is predominately responsible for causing Verticillium wilt in important crop plants other than peppermint.

Methods and Materials

A total of 65 isolates of *V. dahliae* collected from symptomatic peppermint grown in commercial peppermint fields in Oregon over the last two years were used in this study. The 65 isolates collected from peppermint in Oregon included 25 isolates from central Oregon, 2 isolates from the Grande Ronde Valley, and 38 isolates from the Willamette Valley. An additional 96 isolates from mint and other hosts/sources collected from commercial fields across the United States were also included. The hosts/sources of the additional isolates are listed in Table 2.

All isolates were grown on potato dextrose agar for 5-10 days at room temperature in the dark. Mycelia of each isolate were grown in Czapek Dox broth, retrieved, rinsed with sterile water, and lyophilized. DNA from approximately 20 mg of lyophilized tissue from each sample was

obtained using the glass bead breakage method with phenol and chloroform extraction. The concentration of DNA was diluted to 2 ng/µl prior to PCR.

Identification of *V. dahliae* races was performed using two previously developed race-specific primer pairs as previously described (Short et al. 2014). PCR mixtures consisted of 12 µl GoTaq 2X Master Mix (Promega Corporation, Madison, WI), 1µl of each primer from an original concentration of 10 µM, 10 µl of water, and 1 µl of genomic DNA for a total reaction volume of 25 µl. The amplicons were subjected to gel electrophoresis in 1.5% (wt/vol) agarose gels at 90V for 90 min. The three controls were water (negative control), isolate Ls16 (race 1 control), and isolate Ls17 (race 2 control). The race 1 control is expected to generate a band approximately 900-bp in size and the race 2 control is expected to produce a band approximately 256-bp in size (Short et al. 2014). Both PCR reactions (race 1 and race 2) were repeated for 24 isolates to confirm reproducibility and validate the results.

Results and Discussion

Of the total 161 isolates included in this study, 98% were found to be race 2. All 65 isolates of *V. dahliae* collected from Oregon peppermint in 2014 and 2015 were classified as race 2 based on PCR using race-specific primers (Fig. 1; Table 1). Additionally, all 35 isolates of *V. dahliae* isolated from mint in various production areas also were classified as race 2. This is consistent with a previous study which only observed race 2 strains among 16 isolates from mint grown in Washington (Short et al. 2014). A total of 58 out of 61 isolates from other hosts were classified as race 2. One isolate from pistachio and two isolates from tomato were classified as race 1 (Table 2). These results, along with the prevalence of *V. dahliae* race 2 strains observed in association with other crop hosts of the Pacific Northwest, implies the importance of developing peppermint cultivars that are resistant to race 2 strains of *V. dahliae*.

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References

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Tables

Table 1. Number of *Verticillium dahliae* race 1 and race 2 isolates recovered from infected peppermint samples collected from three locations in Oregon during 2014 and 2015

	Number of isolates	
Location	**Race 1**	**Race 2**
Central Oregon	0	25
Grande Ronde Valley	0	2
Willamette Valley	0	38
Total	0	65

Table 2. Number of *Verticillium dahliae* race 1 and race 2 isolates recovered from various hosts and states in the U.S.

		Number of isolates	
Host	**State**	**Race 1**	**Race 2**
Peppermint	**CA, ID, IN, MI, MT, OR, WA**	0	25
Native spearmint	**WA**	0	5
Scotch spearmint	**IN, WA**	0	5
	Subtotal	0	35
Ash	**ID**	0	1
Black Raspberry	**WA**	0	1
Blackberry	**ND, WA**	0	2
Cherry	**ID**	0	1
Cotton	**CA, ID**	0	2
Hyssop	**WA**	0	1
Maple	**ME, OR, WA**	0	3
Pepper	**AZ, CA**	0	2
Pistachio	**CA**	1	0
Potato plant	**NY, OH, OR, WA, WI**	0	12
Potato Seed Tuber	**ID, ME, MI, MT, OR, PA, WA, WI**	0	18
Potato Seed Tuber Tare Soil	**OR, PA, WA**	0	4
Red Raspberry	**ID**	0	1
Skullcap	**WA**	0	2

Spinach	**WA**	0	3
Strawberry	**ID**	0	1
Sugarbeet	**ID**	0	2
Tomato	**ID, NY**	2	0
Watermelon	**WA**	0	2
	Subtotal	3	58
	Total	3	93

Figures

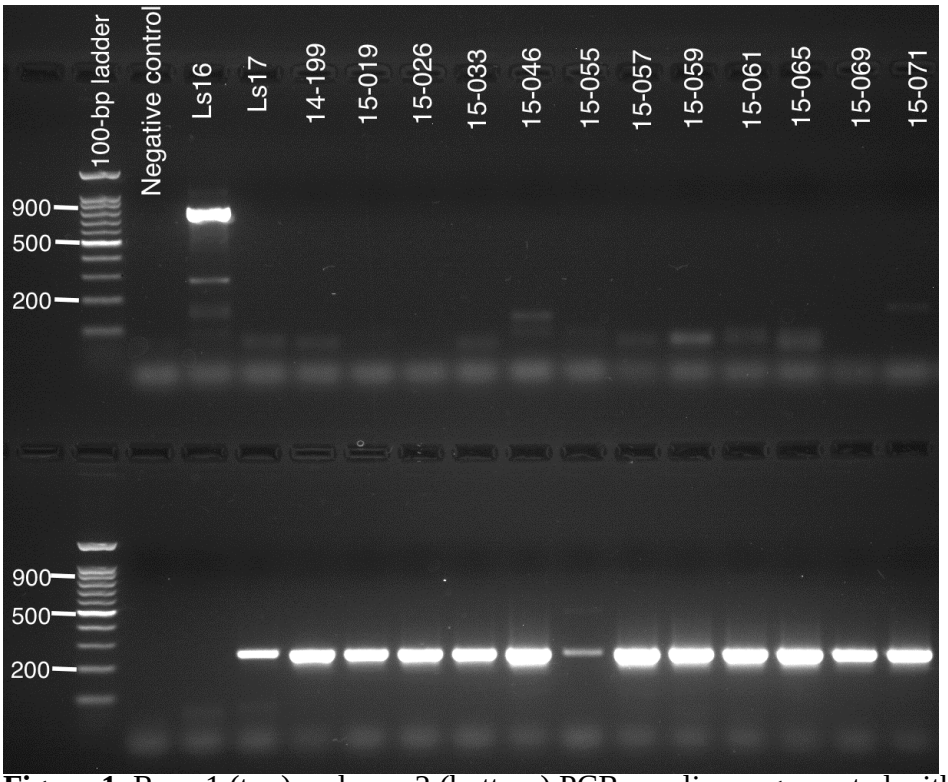

Figure 1. Race 1 (top) and race 2 (bottom) PCR amplicons generated with *Verticillium dahliae* race-specific primers. Isolate Ls16 and Ls17 were collected from lettuce and used as race 1 and race 2 positive controls, respectively. The race 1 control is expected to generate a band approximately 900-bp in size and the race 2 control is expected to produce a band approximately 256-bp in size. The remaining isolates were collected from peppermint grown in Oregon between 2014 and 2015.