Verticillium dahliae Populations from Mint and Potato Are Genetically Divergent with Predominant Haplotypes

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ABSTRACT

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In total, 286 *Verticillium dahliae* isolates from mint, potato, and other hosts and substrates were characterized for mating type, vegetative compatibility group (VCG), and multilocus microsatellite haplotype to determine population genetic structure among populations infecting mint and potato. Populations from mint and potato fit a clonal reproductive model, with all isolates a single mating type (*MAT1-2*) and multiple occurrences of the same haplotypes. Haplotype H02 represented 88% of mint isolates and was primarily VCG2B, while haplotype H04 represented 70% of potato isolates and was primarily VCG4A. Haplotypes H02 and H04 typically caused severe disease on mint and potato, respectively, in greenhouse assays regardless of host origin. Principal coordinate analysis and analysis of molecular variance indicated that mint and potato populations were significantly genetically diverged ($P = 0.02$), and identification of private alleles and estimation of migration rates suggested restricted gene flow. Migration was detected between infected potato plants and seed tubers, infested tare soil, and field soils. Genetic differentiation of *V. dahliae* from mint and potato may be due to the occurrence of a single mating type and differences in VCG. Populations of *V. dahliae* in potato and mint were characterized by the presence of aggressive, clonally reproducing haplotypes which are widely distributed in commercial mint and potato production.

Verticillium wilt is an economically significant disease of many dicotyledonous crops, including mint (*Mentha* L.) and potato (*Solanum tuberosum* L.). Mint and potato are important crops in the United States and especially in the Pacific Northwest (Idaho, Oregon, and Washington States). The Columbia Basin region is a semiarid, irrigated region located in Washington and Oregon states and is a leading producer of high-quality mint oil produced from peppermint, scotch spearmint, and native spearmint, with >23,000 ha under production in these two states in 2011. The area is also a leading grower of potato in the United States, with >80,000 ha harvested in 2011. In the Columbia Basin, mint and potato can be grown in close proximity or in rotation with each other. Verticillium wilt is a persistent problem in both mint and potato production in the region.

Verticillium dahliae (Kleb.), causal agent of Verticillium wilt, is a soilborne fungus with a wide host range that includes >200 dicotyledonous plants. The fungus can survive for many years in the soil in the form of microsclerotia, where it remains dormant until it germinates in response to root exudates. Despite its wide host range, *V. dahliae* exhibits a range of aggressiveness on different host plants, suggesting host specialization or host adaptation (8). Differences in aggressiveness among *V. dahliae* populations associated with mint and potato are well documented (11,16,24,33,39).

The pathogen is only known to reproduce asexually, although mating type idiomorphs in a heterothallic arrangement have been described, which suggests that meiotic recombination may be possible (43). The presence of both mating types could potentially lead to sexual reproduction and the production of new sources of inoculum in the form of ascospores. Various vegetative compatibility groups (VCGs) exist in *V. dahliae*, whereby isolates are grouped based on the ability of nitrate-nonutilizing (*nit*) mutants to undergo hyphal anastomosis with known tester strains (23). VCGs are thought to limit gene flow among populations of *V. dahliae* and may contribute to genetic drift and differentiation (27). However, parasexual recombination can occur through hyphal anastomosis between like VCGs. Genetic recombination, through sexual, asexual, or a combination of both processes, can lead to greater genotypic diversity and a better ability to survive and respond to dynamic environments and control measures. The genetic and genotypic diversity of *V. dahliae* populations infecting different hosts is likely a result of the population diversity present in the soil and the presence or absence of host-adapted genotypes, the number of mating types and VCGs present, and the role of other sources of inoculum such as infected propagative materials.

Correlations between VCG subgroups and pathogenicity, phenotype, or geographic distributions have been observed and may be important in Verticillium wilt epidemiology (26). However, such correlations do not always occur and all known VCGs have the potential to infect a wide range of hosts under greenhouse conditions (8,35). Isolates from mint are typically VCG2B and highly aggressive on mint, suggesting the presence of a mint-adapted VCG2B pathotype (11,16,30,35). In North America, VCG4A isolates of *V. dahliae* are primarily found associated with potato, followed by VCG4B and VCG2 (24,33,39,40). Both VCG4 and VCG2 often cause the most severe symptoms on potato, and VCG4A isolates of *V. dahliae* from potato exhibit the greatest aggressiveness on potato. Strong host-specific interactions may exert high selection pressure on the pathogen, resulting in differentiation among pathogen populations by host and limiting the diversity of populations on mint compared with potato. This may be especially true for vascular wilt pathogens, which must successfully invade the vascular system of hosts to cause disease, form resting structures, and complete the disease cycle. The occurrence of highly aggressive VCG2B and VCG4A isolates

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from mint and potato may indicate the presence of specific populations with particular specialization on these hosts.

Knowledge regarding the genetic and genotypic diversity of *V. dahliae* infecting mint and potato will provide additional insights into the epidemiology and management of Verticillium wilt in these two important hosts. Microsatellites, or simple sequence repeats, are putatively neutral markers which are widely used to detect genetic and genotypic diversity in populations (4). Previous studies using microsatellite markers have detected genetic and genotypic differentiation among *V. dahliae* populations from different hosts and geographic regions (3,7) and, in one study, recombination was detected among populations (3). However, information regarding the mating type and genetic diversity of *V. dahliae* isolates infecting mint and potato in North America is lacking. We hypothesize that populations of *V. dahliae* from mint will have reduced genotypic variability relative to populations from potato due to the more limited production of mint (7 U.S. states compared with 50 states for potato), the predominance of VCG2B on the host, and the occurrence of a host-adapted phenotype. In contrast, potato may exhibit higher levels of genotypic diversity than mint due to the occurrence of multiple VCGs and the wide-scale production of potato across North America.

This study placed a particular emphasis on *V. dahliae* populations impacting the Columbia Basin region of the U.S. Pacific Northwest. In this region, approximately half of the mint fields are grown in rotation with potato while ≈10% of potato fields are cropped in rotation with mint (32), and the potential exists for *V. dahliae* inoculum associated with mint production to subsequently cause Verticillium wilt in potato and vice versa. In addition, >156,300 metric tons of seed potato were planted in the Columbia Basin in 2007 (25), and infected seed tubers or infested tare soil associated with seed lots can provide potential sources of new strains of *V. dahliae* to be introduced into Columbia Basin fields (13,33). The primary objective of this research was to quantify the genetic and genotypic diversity and differentiation among *V. dahliae* populations from mint and potato in North America using microsatellites, mating types, and VCG analysis. The genetic and genotypic diversity of *V. dahliae* isolates from infected seed tubers and infested soils associated with certified potato seed lots was also estimated because they represent a potential source of infection and infestation in commercial potato production. Isolates from field soils associated with potato production in Washington and Ohio were also included. Pathogenicity assays were performed in the greenhouse to determine the aggressiveness of various *V. dahliae* haplotypes on these two hosts.

MATERIALS AND METHODS

Fungal isolates. For this study, 286 isolates were used. Isolates were stored on sterile filter paper at –20°C and maintained on 1% potato dextrose agar (potato dextrose agar at 10 g/liter and agar at 15 g/liter). The mint population comprised 104 isolates from infected mint, including peppermint (n = 74), Scotch spearmint (n = 12), and native spearmint (n = 18). The potato population consisted of 119 isolates isolated from potato, certified seed potato, or soil associated with certified seed potato. Mint isolates were mostly sampled from the Columbia Basin of Washington in 1996 (n = 85). Isolates from mint in the Columbia Basin were obtained from 32 fields located in five counties. Up to three monoconidial isolates were obtained from different plants in each field. Additional isolates from other mint-producing areas of the United States (Idaho, Indiana, Michigan, Montana, and Oregon) and years (pre-1996, 2006, and 2007) were included for comparison with the Washington isolates. Twenty isolates of *V. dahliae* were sampled from infected potato plants in Idaho, North Dakota, New York, Ohio, Pennsylvania, Washington, and Wisconsin and represented a range of collection years (pre-1977 through 2010). Isolates of *V. dahliae* were obtained from infected seed tubers produced in several states (Idaho, Maine, Michigan, Montana, Nebraska, New York, Oregon, Pennsylvania, South Dakota, Washington, and Wisconsin; n = 72) in 1995, 1996, and 2007 to 2010, as well as from infested soil adhering to seed tubers (Idaho, Montana, North Dakota, and Oregon; n = 17) and from bags and trucks used to transport certified seed lots (n = 10) between 2009 and 2010. *V. dahliae* isolates from field soils associated with potato production were also included (n = 20). *V. dahliae* isolates from other hosts and various states (n = 43) were included to provide an outgroup and represent the potential genetic and genotypic diversity of *V. dahliae*. Isolate designations, VCG, host and geographic origins, collection years, and sources are listed in Table 1. VCGs of the majority of isolates were determined by other workers or in previous studies (10,11,23,32,33) but VCGs of 79 isolates were characterized in this study using reference strains provided by Dr. R. Rowe (Ohio Agricultural Research and Development Center, The Ohio State University) as previously described (12,23).

Amplification of mating type idiomorphs. DNA was extracted from lyophilized mycelia using a glass bead disruption technique (10). The quantity and quality of DNA was measured using agarose gel electrophoresis and a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Eluted DNA was stored at –20°C until needed. Mating type of *V. dahliae* isolates was determined using a multiplex polymerase chain reaction (PCR) assay with primer pairs VdMAT1-1a/VdMAT1-1b and VdMAT1-2a/VdMAT1-2b (43). These primers amplify the *MAT1-1* (≈600 bp of product) and *MAT1-2* (≈400 bp of product) idiomorphs, respectively. PCR reaction mixtures included 10 ng of *V. dahliae* DNA, 1× GoTaq Colorless Mastermix (Promega Corp., Madison, WI), and 200 nM each primer in 10 µl reactions. Amplifications were performed in Bio-Rad DNA Engine thermocyclers (Bio-Rad Laboratories, Hercules, CA), using the following cycling parameters: denaturing at 94°C for 3 min, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 1 min. A final extension was carried out at 72°C for 3 min. All reactions were repeated once. The PCR products were electrophoresed on a 2% (wt/vol) agarose gel containing 0.5× Tris-borate-EDTA and visualized using ethidium bromide.

Microsatellite amplification. Ten microsatellite markers (VD1, VD2, VD3, VD4, VD8, VD10, VD26, VD27, VD74, and VD96), which were previously developed and shown to be polymorphic in *V. dahliae* populations infecting other hosts (2,3), were used in the study. Repeat motifs were 3 to 10 bp in length and previously localized to four different chromosomes using the *V. dahliae* genome sequence of strain VdLs17, which can be found at the Verticillium Group Database (http://www.broad.mit.edu/annotation/genome/verticillium_dahliae/MultiHome.html) (2). Genotyping was performed using fluorescently labeled M13 primers as described by Schuelke (37), with modifications. PCR reagents included: 500 pg of *V. dahliae* DNA, 1× GoTaq colorless PCR reaction buffer (Promega Corp.), 400 nM dNTP mix, 2.5 mM $MgCl_2$, 1.5 U of GoTaq polymerase, 250 nM reverse primer, 50 nM forward primer amended at the 5′ end with the universal M13 primer sequence (CACGACGTTGTAAAACGAC), and 200 nM universal M13 primer labeled with one of four fluorophores: FAM or HEX (Applied Biosystems, Foster City, CA), or ATTO 550 or ATTO 565 (ATTO-TEC, Siegen, Germany). Amplifications were performed independently for each locus in Bio-Rad DNA Engine thermocyclers (Bio-Rad Laboratories), using the following cycling parameters: denaturing at 95°C for 2 min, followed by 35 cycles of denaturing at 95°C for 10 s, annealing at 58°C for 10 s, and extension at 72°C for 30 s. A final extension was carried out at 72°C for 5 min. Four differentially labeled PCR products were multiplexed for fragment analysis. Each labeled PCR product (3 µl) was combined in 13 µl of sterile,

TABLE 1. Isolates used in this study and their corresponding host origins, states and years of isolation, mating type (*MAT*) idiomorphs, vegetative compatibility groups (VCG), multilocus haplotypes, and sources[a]

Sample group, isolate	Host	State[b]	Year	*MAT* idiomorph	VCG	Multilocus haplotype	Source
Mint							
17	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
19	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
20	Native spearmint	WA	1996	*MAT1-2*	2A/B	H02	L. Douhan
46	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
47	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
48	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
52	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
53	Native spearmint	WA	1996	*MAT1-2*	2B	H31	L. Douhan
54	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
55	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
56	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
57	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
73	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
74	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
103	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
104	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
121	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
124	Native spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
152	Peppermint	ID	1996	*MAT1-2*	2B	H29	L. Douhan
155	Peppermint	ID	1996	*MAT1-2*	4A	H04	L. Douhan
13	Peppermint	IN	1996	*MAT1-2*	2B	H02	L. Douhan
8	Peppermint	MI	1996	*MAT1-2*	2B	H30	L. Douhan
9	Peppermint	MI	1996	*MAT1-2*	ND	H02	L. Douhan
10	Peppermint	MI	1996	*MAT1-2*	2B	H02	L. Douhan
29	Peppermint	MT	1996	*MAT1-2*	2B	H02	L. Douhan
134	Peppermint	MT	1996	*MAT1-2*	2B	H02	L. Douhan
136	Peppermint	MT	1996	*MAT1-2*	2B	H02	L. Douhan
156	Peppermint	MT	1996	*MAT1-2*	2B	H02	L. Douhan
150	Peppermint	OR	1996	*MAT1-2*	2B	H02	L. Douhan
151	Peppermint	OR	1996	*MAT1-2*	2B	H28	L. Douhan
157	Peppermint	OR	1996	*MAT1-2*	2B	H02	L. Douhan
97-098-1	Peppermint	OR	1996	*MAT1-2*	2B	H02	L. Douhan
1	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
5	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
24	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
25	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
27	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
32	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
34	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
35	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
36	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
37	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
38	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
41	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
43	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
44	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
45	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
49	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
50	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
51	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
58	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
60	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
61	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
62	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
65	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
67	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
68	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
69	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
70	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
71	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
72	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
77	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
78	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
79	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan

(*continued on next page*)

[a] ND = not determined and Unk = unknown.

[b] WA = Washington, ID = Idaho, IN = Indiana, MI = Michigan, MT = Montana, OR = Oregon, ND = North Dakota, NY = New York, PA = Pennsylvania, WI = Wisconsin, OH = Ohio, SD = South Dakota, ME = Maine, N/A = not applicable, AZ = Arizona, CA = California, and TX = Texas.

[c] Isolate was obtained from the same seed lot as the isolate above.

[d] Previously characterized as VCG2B but characterized as VCG4B in this study.

[e] Previously characterized as VCG2B but characterized as VCG4A in this study.

TABLE 1. (*continued from preceding page*)

Sample group, isolate	Host	State[b]	Year	*MAT* idiomorph	VCG	Multilocus haplotype	Source
80	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
82	Peppermint	WA	1996	*MAT1-2*	2B	H30	L. Douhan
84	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
92	Peppermint	WA	1996	*MAT1-2*	ND	H02	L. Douhan
93	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
94	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
95	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
96	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
97	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
101	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
102	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
106	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
107	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
109	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
111	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
112	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
113	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
114	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
115	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
116	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
119	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
128	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
140	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
141	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
142	Peppermint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
Bi.Gr.	Peppermint	MT	pre-1997	*MAT1-2*	ND	H02	T. Davis
V-16	Peppermint	IN	1991	*MAT1-2*	2B	H33	R. Green
M.pi-06	Peppermint	WA	2006	*MAT1-2*	2B	H02	J. Dung
15	Scotch spearmint	IN	pre-1996	*MAT1-2*	2B	H27	L. Douhan
21	Scotch spearmint	WA	1996	*MAT1-2*	2B	H30	L. Douhan
22	Scotch spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
66	Scotch spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
98	Scotch spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
105	Scotch spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
126	Scotch spearmint	WA	1996	*MAT1-2*	2B	H35	L. Douhan
127	Scotch spearmint	WA	1996	*MAT1-2*	ND	H02	L. Douhan
129	Scotch spearmint	WA	1996	*MAT1-2*	2B	H34	L. Douhan
130	Scotch spearmint	WA	1996	*MAT1-2*	2B	H34	L. Douhan
145	Scotch spearmint	WA	1996	*MAT1-2*	2B	H02	L. Douhan
M.gr-07	Scotch spearmint	WA	2007	*MAT1-2*	2B	H02	J. Dung
Potato							
240	Potato plant	WA	pre-1977	*MAT1-2*	4B	H21	J. Davis
V-25	Potato plant	ID	pre-1983	*MAT1-2*	4A	H04	L. Sorenson
317	Potato plant	ND	pre-1983	*MAT1-2*	4A/B	H04	G. Secor
318	Potato plant	ND	pre-1983	*MAT1-2*	4A	H04	G. Secor
V-9-86	Potato plant	NY	pre-1983	*MAT1-2*	4A/B	H09	V. Stockwell
V-13-86	Potato plant	PA	pre-1983	*MAT1-2*	2A/B	H26	V. Stockwell
Par-4	Potato plant	WI	1983	*MAT1-2*	4B	H09	R. Rowe
P-3	Potato plant	OH	1984	*MAT1-2*	4B	H08	R. Rowe
P-7	Potato plant	OH	1984	*MAT1-2*	4A	H22	R. Rowe
AF 10-7	Potato plant	ID	pre-1994	*MAT1-2*	4B	H07	C. Strausbaugh
AF 2-3	Potato plant	ID	pre-1994	*MAT1-2*	4A	H04	C. Strausbaugh
235	Potato plant	WA	1995	*MAT1-2*	4B	H08	R. Rowe
239	Potato plant	WA	1995	*MAT1-2*	4B	H07	R. Rowe
274	Potato plant	WA	1995	*MAT1-2*	4B	H08	R. Rowe
Vd.St-1	Potato plant	ID	pre-1997	*MAT1-2*	4A	H04	W. Schnathorst
233	Potato plant	Unk	pre-1997	*MAT1-2*	4A	H11	R. Rowe
W.1721.2009	Potato plant	WA	2009	*MAT1-2*	4A	H04	J. Dung
TSPS.1.2010	Potato plant	WA	2010	*MAT1-2*	4A	H04	J. Dung
TSPS.2.2010	Potato plant	WA	2010	*MAT1-2*	4A	H04	J. Dung
VSP 712	Potato plant	WA	Unk	*MAT1-2*	2B	H25	L. du Toit
653	Seed tuber	ID	1995	*MAT1-2*	4A	H04	R. Rowe
W-83	Seed tuber	MT	1995	*MAT1-2*	4A	H04	R. Rowe
W-201	Seed tuber	MT	1995	*MAT1-2*	4B	H08	R. Rowe
W-152	Seed tuber	OR	1995	*MAT1-2*	4B	H13	R. Rowe
W-90	Seed tuber	SD	1995	*MAT1-2*	4A	H04	R. Rowe
W-140	Seed tuber	WA	1995	*MAT1-2*	4B	H16	R. Rowe
W-87	Seed tuber	WA	1995	*MAT1-2*	4A	H05	R. Rowe
W-100	Seed tuber	WY	1995	*MAT1-2*	4A	H04	R. Rowe
11-11	Seed tuber	ME	1996	*MAT1-2*	4B	H06	R. Rowe
66-12	Seed tuber	ME	1996	*MAT1-2*	4A	H20	R. Rowe
21-18	Seed tuber	MI	1996	*MAT1-2*	4A	H06	R. Rowe
30-6	Seed tuber	NE	1996	*MAT1-2*	4A	H04	R. Rowe

(*continued on next page*)

TABLE 1. (*continued from preceding page*)

Sample group, isolate	Host	State[b]	Year	*MAT* idiomorph	VCG	Multilocus haplotype	Source
99-1	Seed tuber	NY	1996	*MAT1-2*	4A/B	H09	R. Rowe
101-1	Seed tuber	NY	1996	*MAT1-2*	4B	H06	R. Rowe
102-1	Seed tuber	NY	1996	*MAT1-2*	4B	H06	R. Rowe
1-3	Seed tuber	PA	1996	*MAT1-2*	4A	H08	R. Rowe
83-1	Seed tuber	WI	1996	*MAT1-2*	4A	H11	R. Rowe
S.15	Seed tuber	ID	2007	*MAT1-2*	4A	H04	J. Dung
SC.19	Seed tuber	ID	2007	*MAT1-2*	4A	H04	J. Dung
TN.26	Seed tuber	ID	2007	*MAT1-2*	4A	H04	J. Dung
TN.40[c]	Seed tuber	ID	2007	*MAT1-2*	4A	H04	J. Dung
TN.66[c]	Seed tuber	ID	2007	*MAT1-2*	4A	H04	J. Dung
L7.T31	Seed tuber	ID	2008	*MAT1-2*	4A	H04	J. Dung
L8.T25	Seed tuber	ID	2008	*MAT1-2*	4A	H04	J. Dung
L10.T15	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L10.T17[c]	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L10.T26[c]	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L10.T35[c]	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L10.T7[c]	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L9.T12	Seed tuber	MT	2008	*MAT1-2*	4A	H04	J. Dung
L2.T20	Seed tuber	Unk	2008	*MAT1-2*	ND	H08	J. Dung
L3.T19	Seed tuber	Unk	2008	*MAT1-2*	4A	H04	J. Dung
09-29.6.A	Seed tuber	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-41.15.5	Seed tuber	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-41.8.3[c]	Seed tuber	ID	2009	*MAT1-2*	4A	H04	J. Dung
B.A-296	Seed tuber	ID	2009	*MAT1-2*	4A	H04	J. Dung
L7.2009	Seed tuber	ID	2009	*MAT1-2*	4B	H07	J. Dung
09-29.3	Seed tuber	MT	2009	*MAT1-2*	4A	H04	J. Dung
09-29.9[c]	Seed tuber	MT	2009	*MAT1-2*	4A	H04	J. Dung
09-54.5C	Seed tuber	MT	2009	*MAT1-2*	4A	H04	J. Dung
09-55.8	Seed tuber	MT	2009	*MAT1-2*	4A	H04	J. Dung
NG1.9	Seed tuber	MT	2009	*MAT1-2*	4A	H04	J. Dung
S.1-Umat-15.09	Seed tuber	WA	2009	*MAT1-2*	4A	H04	J. Dung
UG4.11	Seed tuber	WA	2009	*MAT1-2*	4A	H04	J. Dung
UG4.16[c]	Seed tuber	WA	2009	*MAT1-2*	4A/B	H04	J. Dung
UG4.18[c]	Seed tuber	WA	2009	*MAT1-2*	4A	H04	J. Dung
LS8-A	Seed tuber	WI	2009	*MAT1-2*	4B	H07	J. Dung
LS8-B[c]	Seed tuber	WI	2009	*MAT1-2*	4A	H09	J. Dung
12.A.2010	Seed tuber	ID	2010	*MAT1-2*	4A	H04	J. Dung
59.A.2010	Seed tuber	ID	2010	*MAT1-2*	4A	H04	J. Dung
30.A.2010	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
30.B.2010[c]	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
48.A.2010	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
48.B.2010[c]	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
52.A.2010	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
52.C.2010[c]	Seed tuber	MT	2010	*MAT1-2*	4A	H04	J. Dung
20.C.2010	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
20.D.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H23	J. Dung
20.F.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4B	H15	J. Dung
21.C.2010	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
21.D.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H05	J. Dung
21.F.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H05	J. Dung
21.H.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
21.I.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
29.A.2010	Seed tuber	OR	2010	*MAT1-2*	4B	H09	J. Dung
38.A.2010	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
38.B.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
38.C.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4B	H14	J. Dung
38.J.2010[c]	Seed tuber	OR	2010	*MAT1-2*	4A	H04	J. Dung
49.B.2010	Seed tuber	OR	2010	*MAT1-2*	4B	H07	J. Dung
55.A.2010	Seed tuber	Unk	2010	*MAT1-2*	4A	H04	J. Dung
3.1.2010	Seed tuber	Unk	2010	*MAT1-2*	4A	H04	J. Dung
09-41.8.1	Seed tuber soil	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-41.8.2[c]	Seed tuber soil	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-45.2009	Seed tuber soil	ID	2009	*MAT1-2*	4A	H19	J. Dung
09-55.7.1	Seed tuber soil	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-55.7.2[c]	Seed tuber soil	ID	2009	*MAT1-2*	4A	H04	J. Dung
Col.8	Seed tuber soil	ID	2009	*MAT1-2*	4A	H04	J. Dung
09-48A.3.1	Seed tuber soil	Unk	2009	*MAT1-2*	4A	H04	J. Dung
18.A.2010	Seed tuber soil	ID	2010	*MAT1-2*	4A	H04	J. Dung
18.B.2010[c]	Seed tuber soil	ID	2010	*MAT1-2*	4A	H04	J. Dung
28.2010	Seed tuber soil	ID	2010	*MAT1-2*	4A/B	H04	J. Dung
19.2010	Seed tuber soil	MT	2010	*MAT1-2*	4A	H04	J. Dung
23.2010	Seed tuber soil	MT	2010	*MAT1-2*	4A	H04	J. Dung
44.A.2010	Seed tuber soil	MT	2010	*MAT1-2*	4A	H04	J. Dung

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TABLE 1. (*continued from preceding page*)

Sample group, isolate	Host	State[b]	Year	*MAT* idiomorph	VCG	Multilocus haplotype	Source
57.A.2010	Seed tuber soil	ND	2010	*MAT1-2*	4A	H04	J. Dung
41.A.2010	Seed tuber soil	OR	2010	*MAT1-2*	4A	H04	J. Dung
6.A.2010	Seed tuber soil	Unk	2010	*MAT1-2*	4A	H04	J. Dung
6.B.2010[c]	Seed tuber soil	Unk	2010	*MAT1-2*	4A	H04	J. Dung
103.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
105.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
107.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
111.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
208.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
212 Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
401 Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
402.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
404.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
406.Tare	Loose tare soil	N/A	2010	*MAT1-2*	4A	H04	J. Dung
Field soil							
S-180	Soil	OH	1984	*MAT1-2*	4A/B	H06	R. Rowe
S-221	Soil	OH	1984	*MAT1-2*	2B	H32	R. Rowe
S-39	Soil	OH	1984	*MAT1-2*	4B	H12	R. Rowe
S-55	Soil	OH	1984	*MAT1-2*	4A	H04	R. Rowe
S-80	Soil	OH	1984	*MAT1-2*	2B	H32	R. Rowe
CB 51-4	Soil	OR	1998	*MAT1-2*	4A	H04	D. Johnson
CB 65-2	Soil	OR	1998	*MAT1-2*	4B	H08	D. Johnson
CB 1-2	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 18-1	Soil	WA	1998	*MAT1-2*	4B	H10	D. Johnson
CB 19-2	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 22-4	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 2-3	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 27-3	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 4-2	Soil	WA	1998	*MAT1-2*	4A	H18	D. Johnson
CB 4-6	Soil	WA	1998	*MAT1-2*	4B	H08	D. Johnson
CB 71-1	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 72-3	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 74-4	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 76-1	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
CB 86-7	Soil	WA	1998	*MAT1-2*	4A	H04	D. Johnson
Other hosts							
Vd-Agastache	Hyssop	Unk	Unk	*MAT1-2*	3	H45	D. Johnson
Ash	Ash	Unk	Unk	*MAT1-2*	4B	H08	P. Hamm
49-08	Blackberry	OR	2008	*MAT1-2*	4A	H04	J. Weiland
08-139	Blackberry	WA	Unk	*MAT1-2*	4A	H04	P. Hamm
601	Cherry	WA	pre-1997	*MAT1-2*	4A	H04	D. Johnson
DX-2	Cotton	AZ	pre-1983	*MAT1-2*	1	H01	S. Alcorn
T9	Cotton	CA	pre-1983	*MAT1-2*	1	H01	P. Nicot
V-44	Cotton	TX	pre-1983	*MAT1-2*	1	H01	J. Puhalla
V-EMS(481)	Elm	OH	pre-1984	*MAT1-2*	1	H44	L. Schreiber
01-83	Maple	OR	1983	*MAT1-2*	2B	H02	J. Weiland
06-166	Maple	Unk	Unk	*MAT1-2*	4A	H04	P. Hamm
182-08	Maple	OR	2008	*MAT1-2*	4A	H41	J. Weiland
PCW	Pepper	CA	pre-1983	*MAT1-2*	3	H03	J. Puhalla
Ca.35	Pepper	CA	1996	*MAT1-2*	ND	H36	K. Subbarao
PH	Pistachio	CA	pre-1983	*MAT1-2*	2A	H40	J. Puhalla
08-128	Black raspberry	WA	Unk	*MAT1-2*	4A	H04	P. Hamm
08-139	Red raspberry	WA	Unk	*MAT1-2*	4A	H04	P. Hamm
VSP 08-33 A3	Skullcap	WA	2008	*MAT1-2*	ND	H02	L. du Toit
VSP 08-33 C4	Skullcap	WA	2008	*MAT1-2*	ND	H02	L. du Toit
09-123.1	Skullcap	WA	2009	*MAT1-2*	2B	H02	L. du Toit
09-123.2	Skullcap	WA	2009	*MAT1-2*	2B	H02	L. du Toit
09-123.3	Skullcap	WA	2009	*MAT1-2*	2B	H02	L. du Toit
09-123.4	Skullcap	WA	2009	*MAT1-2*	ND	H02	L. du Toit
09-123.5	Skullcap	WA	2009	*MAT1-2*	ND	H02	L. du Toit
09-123.6	Skullcap	WA	2009	*MAT1-2*	2B	H02	L. du Toit
SL-2	Skullcap	WA	2009	*MAT1-2*	ND	H02	J. Dung
SL-3	Skullcap	WA	2009	*MAT1-2*	2B	H02	J. Dung
VD1 VSP695	Spinach	WA	2001	*MAT1-2*	2B	H42	L. du Toit
VD5 VSP699	Spinach	WA	2001	*MAT1-2*	4B[d]	H07	L. du Toit
VD4 VSP698	Spinach	WA	2002	*MAT1-2*	4B	H39	L. du Toit
VSP263	Spinach	WA	2003	*MAT1-2*	4B	H07	L. du Toit
VSP268	Spinach	WA	2003	*MAT1-2*	4A[e]	H07	L. du Toit
VSP678	Spinach	WA	2004	*MAT1-2*	2B	H43	L. du Toit
Fca.29	Strawberry	CA	1996	*MAT1-2*	4B	H17	K. Subbarao
F608	Sugar beet	ID	2007	*MAT1-2*	4A	H04	C. Strausbaugh
F611	Sugar beet	ID	2007	*MAT1-2*	4A	H04	C. Strausbaugh
F612	Sugar beet	ID	2007	*MAT1-2*	4A	H04	C. Strausbaugh

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TABLE 1. (*continued from preceding page*)

Sample group, isolate	Host	State[b]	Year	*MAT* idiomorph	VCG	Multilocus haplotype	Source
F616	Sugar beet	ID	2007	*MAT1-2*	4A	H04	C. Strausbaugh
F625	Sugar beet	ID	2007	*MAT1-2*	2B	H02	C. Strausbaugh
461	Tomato	OH	1984	*MAT1-1*	2	H37	R. Rowe
VMD-4	Tomato	NY	pre-1983	*MAT1-2*	2A/B	H38	M. Lacy
97-147-2	Watermelon	OR	Unk	*MAT1-2*	4A	H04	P. Hamm
381	Watermelon	OH	1981	*MAT1-2*	2A/B	H24	S. Miller

distilled water for a total volume of 25 µl. In total, 3 µl of the resulting mixture of amplicons was combined with 10 µl of Hi-Di formamide and 0.03 µl of LIZ-665 size standard (Applied Biosystems), and denatured at 95°C for 5 min. Sizes of the fluorescently labeled fragments were determined using capillary electrophoresis on an ABI 3730*xl* DNA Analyzer (Applied Biosystems) at the United States Department of Agriculture Wheat Genetics, Quality, Physiology, and Disease Research Lab in Pullman, WA. Fragment analysis was performed in GeneMarker (SoftGenetics LLC, State College, PA). All PCR reactions and fragment analyses were independently replicated using the same DNA extractions to assess data reproducibility. In addition, a subset of isolates representing all 45 multilocus haplotypes observed in this study were genotyped using independently grown cultures, DNA extracts, PCR reactions, and fragment analyses.

Assessment of homoplasy among microsatellite loci. Representative fragments of each size class amplified for each locus were sequenced to verify allelism and provide an assessment of homoplasy in the data set (18). All putative alleles were sequenced at least once but, if an allele accounted for ≥10% of the observed loci, attempts were made to sequence the allele from at least four different isolates from various hosts and geographic locations. Sequencing was performed in 25 µl reactions consisting of 200 µM dNTPs, 2.5 mM $MgCl_2$, 200 nM each primer, 1 U of GoTaq polymerase (Promega Corp.), and 10 ng of template DNA in 1× GoTaq buffer under the PCR conditions described above. The quality and quantity of DNA was determined using a NanoDrop 1000 spectrophotometer and by gel electrophoresis. Two 3-µl aliquots of each PCR product were treated with ExoSAP-IT (USB Corporation, Cleveland), prepared as previously described (12), and sequenced in both directions by Elim Biopharmaceuticals, Inc. (Hayward, CA).

Estimates of genetic diversity and genotypic richness, evenness, and diversity. Nei's expected heterozygosity (H_{exp}), which is equivalent to genetic diversity in haploid organisms and defined as the probability that two randomly selected haplotypes are different (29), was calculated using the software Arlequin, ver. 3.5 (19). H_{exp} was also calculated for each locus in Arlequin using full and clone-corrected data sets. Heterozygosity values from mint and potato sample groups were compared using the Mann-Whitney U test. Rarefaction curves were estimated in Multilocus, ver. 1.3b to estimate mean genotypic diversity expected in relation to the number of loci analyzed using 1,000 random samples (1). The observed number of genotypes (g_{obs}) was obtained based on the total number of unique multilocus haplotypes observed in each sample group. The number of haplotypes expected in each sample group after correcting for the smallest sample size (g_{exp}) was calculated using 10,000 jackknife replicates in GenoDive, ver. 1.1 (28). Multilocus genotypic diversity was estimated using Shannon-Wiener's H', Stoddart and Taylor's G, and the index N_1, while multilocus genotypic evenness was estimated using the indices G/g_{obs}, E_1, and E_5. Indices of multilocus genotypic diversity, evenness, and 95% confidence intervals were calculated using a modified <jackboot> macro and 2,000 bootstrap replicates in SAS (version 9.2; SAS Institute, Cary, NC), with the exception of G/g_{obs} (21). Genotypic indices were considered significantly different if there were no overlaps of 95% confidence intervals. Subsequent analyses were conducted using clone-corrected data and default software parameters under the stepwise mutation model, unless otherwise noted (31).

Genetic differentiation between populations from mint and potato. A matrix of genetic distances between multilocus haplotypes was estimated in GenoType (28). Pairwise genetic distances were plotted using principal coordinate analysis (PCoA) in GeneAlex, ver. 6.41 (34) to visualize genetic clusters among isolates from different sample groups and VCGs. Minimum spanning networks of VCG2B isolates from mint and VCG4 isolates from potato were estimated from the genetic distance matrix using MINSPNET (20), plotted in HapStar, ver. 0.5 (42), and visualized using the open-source vector graphics editor Inkscape, ver. 0.48 (http://www.inkscape.org).

Slatkin's R_{st} was estimated (38) and pairwise comparisons of isolates collected from infected potato plants, infected certified seed tubers, and infested tare soil from certified seed lots were performed to determine whether isolates associated with potato could be grouped together. Pairwise comparisons of populations from mint, potato, other hosts, and infested field soils associated with commercial potato production were also conducted. All pairwise comparisons were performed using the sequential Bonferroni method (36) and 16,000 permutations in Arlequin. Analysis of molecular variance (AMOVA) was performed to test the null hypothesis that *V. dahliae* populations associated with mint and potato were not genetically differentiated. AMOVA was also conducted to test the null hypothesis that VCG4A and VCG4B populations of *V. dahliae* from potato are not significantly differentiated. AMOVA and pairwise comparisons of Slatkin's R_{st} were performed in Arlequin using full and clone-corrected sets with 16,000 permutations.

Private alleles and migration rate estimates. The number of private alleles, scaled for the smallest clone-corrected population size ($n = 9$), was estimated for VCG2B haplotypes from mint and VCG4A and VCG4B haplotypes from potato using rarefaction curves in ADZE, ver. 1.0 (41). The program Migrate, ver. 3.0 (5,6), was used to calculate maximum likelihood estimations of the mutation-scaled population size (θ) and mutation-scaled migration rates (M) among isolates associated with potato (plants, seed tubers, tare soil, and field soils). Migration rates between VCG2B haplotypes from mint and VCG4A and VCG4B haplotypes from potato were also determined. Five runs were performed using a continuous Brownian motion model and heating applied at four temperatures (1.0, 1.5, 3.0, and 9.0). The run which resulted in the smallest likelihood value is reported. Five short chains, consisting of 1,000 samples sampled at increments of 20, were performed and 1,000 trees were recorded. Five long chains were performed with 5,000 samples, a burn-in of 10,000, and 5,000 trees were recorded.

Aggressiveness assays. Eighteen isolates were selected for aggressiveness assays on mint and potato based on multilocus haplotype, VCG, and host origin. Mint and potato assays were performed separately and repeated once. All treatments were replicated four times and blocked in the greenhouse. Experiments were arranged in the greenhouse as a randomized complete block design. Inoculum, consisting of a conidial suspension (10^6 CFU/ml), was prepared as previously described (16). A

VCG2B isolate from mint (isolate 111) and a VCG4A isolate from potato (isolate 653) were included as positive controls. Isolates 111 and 653 were previously shown to be pathogenic on mint (16) and potato (11,15), respectively, and were characterized according to multilocus haplotype in this study. Water-inoculated negative controls were included in all experiments.

Scotch spearmint (*Mentha* × *gracilis*) cuttings were rooted in LC1 potting mix for 4 weeks prior to inoculation. Plants were inoculated using a soil drench of conidial suspension to a final concentration of 10^5 CFU/cm^3 soil. Verticillium wilt symptoms were evaluated weekly beginning at 4 weeks post inoculation until 8 weeks postinoculation, using the following disease severity index (DSI): 0 = no visible symptoms; 1 = mild chlorosis <10% of plant; 2 = distinct chlorosis 10 to 25% of plant; 3 = asymmetrical apical growth, chlorosis 25 to 50% of plant or stunting (<75% height of control plants); 4 = chlorosis ≥50% of plant or severe stunting (<50% height of control plants); 5 = necrosis ≥50% of plant; and 6 = dead or nearly dead plant. DSI ratings were converted to area under the disease progress curve (AUDPC) using the formula $\Sigma_i^{n-1}[(Y_i + Y_{i+1})/2](t_{i+1} - t_i)$, where Y_i = disease rating at the ith observation, t_i = time (days post inoculation) at the ith observation, and n = number of observations.

Certified seed tubers ('Russet Norkotah') were cut into pieces (≈57 g) and planted into 3.8-liter pots containing LC1 potting mix. After 7 weeks, plants were inoculated as described above. Total chlorosis and necrosis were recorded at 4, 5, and 6 weeks post inoculation and values were combined into areas under senescence progress curves (AUSPC) using the formula $\Sigma_i^{n-1}[(Y_i + Y_{i+1})/2](t_{i+1} - t_i)$, where Y_i = cumulative senescence (percent chlorosis and percent necrosis) at the ith observation, t_i = time (days post inoculation) at the ith observation, and n = number of observations.

Analysis of variance was conducted on AUDPC and AUSPC data in SAS using PROC MIXED. Data were analyzed as a one-way factorial design with trials and blocks as random effects. Multiple pairwise comparisons against the water-inoculated control treatments were performed using Dunnett's test (17).

RESULTS

Mating type analysis. All but one isolate amplified a 400-bp band in the *MAT*-specific PCR assay and were designated as mating type *MAT1-2*. The only exception was isolate 461 from tomato, which amplified a 600-bp band and was designated as *MAT1-1*.

Assessment of homoplasy among microsatellite loci. Sequence analysis of representative fragments of each size class amplified from each locus confirmed that similar-sized fragments were allelic. Differences in fragment sizes were primarily due to expected expansions and contractions in microsatellite repeat motifs. Sequences of VD4 alleles were only obtained from three to four isolates due to the repeated difficulty in obtaining usable sequences from this locus. Sequence analysis confirmed that small differences in fragment sizes (1 to 2 bp) detected using fragment analysis were caused by insertions or deletions in the microsatellite flanking regions but these did not affect interpretation of alleles. Potential compound microsatellites were observed in VD26 [$(GCAGAGAG_{n1})$ $(GCACAGAG_{n2})$] and VD27 [$(CAATGCCTCG_{n1})$ $(CCATGCCTCG_{n2})$]. Because the inclusion of these polymorphisms did not result in the identification of any new haplotypes, they were also disregarded in the analyses. In addition, homoplasy was identified in two VD1 (CCTG) alleles which had identical fragment sizes but possessed one to three single-nucleotide polymorphisms that were either G-C or C-G transversions or G-A transitions. The transversions (G-C) and transitions (G-A) observed in VD1 resulted in the loss or gain of CCTG repeats, and slightly different results could be obtained depending on the method of scoring (fragment analysis versus sequence analyses). For consistency, results from fragment analysis were used for marker VD1. Furthermore, these polymorphisms were not included in the analyses due to the relatively small number of potentially homoplasious alleles (1.4% of loci analyzed), the large amount of variability observed over all 10 loci, and the difficulty in incorporating such data with microsatellites into an appropriate mutation model (18).

Estimates of genetic diversity and genotypic richness, evenness, and diversity. Genetic diversity, based on Nei's heterozygosity (H_{exp}) (29), was greater among the potato population (H_{exp} = 0.51) compared with the mint population (H_{exp} = 0.22) (Table 2) but this difference was not significant (P = 0.70). The potato population also had greater H_{exp} values compared with the mint population at the allelic level (Table 3) but the differences were also not significant using either full (P = 0.10) or clone-corrected (P = 0.21) data sets. Within the potato population, isolates from potato plants exhibited the greatest gene diversity (H_{exp} = 0.83), followed by isolates from potato seed tubers (H_{exp} = 0.53) and seed tuber soil (H_{exp} = 0.12). Only one multilocus haplotype was observed among isolates obtained from seed lot soil. Among isolates from mint, *V. dahliae* isolates from native spearmint exhibited the least gene diversity (H_{exp} = 0.11), followed by isolates from peppermint (H_{exp} = 0.16) and isolates from scotch spearmint (H_{exp} = 0.67).

Plots of the mean number of genotypes expected in relation to the number of loci scored reached saturation, indicating that a sufficient number of loci were analyzed (Fig. 1). Overall, genotypic richness (g_{obs}) was greater in the potato population (g_{obs} = 18) compared with the population from mint (g_{obs} = 10) (Table 2). When corrected for the smallest sample size (n = 20), estimated genotypic richness was greater among populations from potato (g_{exp} = 6.0) compared with mint (g_{exp} = 3.2). The population consisting of isolates sampled from other hosts exhibited the highest g_{obs} (g_{obs} = 18) even when corrected for the smallest sample size (g_{exp} = 10.2), whereas the population from field soils exhibited g_{obs} and g_{exp} values of 7 for both uncorrected and corrected sample sizes. The multilocus haplotypes of isolates characterized in this study are summarized in Table 1.

Potato and mint populations exhibited relatively lower indices of evenness compared with the populations from other hosts and field soils (Table 2). A single haplotype, designated H04, was associated with potato in Idaho, Montana, North Dakota, Nebraska, Oregon, South Dakota, Washington, and Wyoming. This haplotype accounted for 70% of isolates from potato. Haplotype H04 was also isolated from *V. dahliae*-infected peppermint and sugar beet (*Beta* sp.) in Idaho; maple (*Acer* sp.), raspberry, blackberry (*Rubus* spp.), and watermelon (*Citrullus* sp.) in Oregon; and cherry (*Prunus* sp.) in Washington, as well as from field soils in Ohio, Oregon, and Washington. All isolates designated as haplotype H04 belonged to VCG4A, with the exception of two isolates which were characterized as VCG4B and one isolate which belonged to VCG4A/B. Haplotypes H06, H07, and H08 were found to contain isolates belonging to both VCG4A and VCG4B subgroups. Isolates characterized as H09 were found to belong to VCG4A, VCG4B, and VCG4A/B subgroups. In all, 88% of mint isolates were represented by a single VCG2B haplotype (H02). Haplotype H02 was sampled from mint in Indiana, Oregon, Michigan, Montana, and several counties in Washington. This haplotype was also recovered from maple in Oregon, sugar beet in Idaho, and skullcap (*Scutellaria* sp.) in Washington. All H02 haplotypes belonged to VCG2B, with the exception of a single isolate from native spearmint which was characterized as VCG2A/B. One haplotype (H30) was sampled from peppermint in Michigan and Scotch spearmint in Washington. Indices of evenness were greatest and similar among populations consisting of isolates from other hosts and field soils (Table 2).

Overall, indices of genotypic diversity (H', N_1, and G) were significantly greater among populations associated with potato

compared with mint (Table 2). The reference populations consisting of isolates obtained from other hosts and field soils exhibited relatively high and moderate indices of genotypic diversity, respectively (Table 2).

Genetic differentiation between populations from mint and potato. PCoA was used to identify clusters of isolates based on genetic similarity. Mint and potato haplotypes clustered into distinct genetic groups (Fig. 2). Haplotypes from potato could be further subdivided into two groups. The first potato group consisted entirely of haplotypes characterized as VCG4A. The second group consisted mostly of haplotypes belonging to VCG4B intermixed with haplotypes belonging to VCG4A and VCG4A/B. As indicated above, some haplotypes were represented by multiple isolates that were determined to belong to different VCG4 subgroups (Table 1). VCG2A/B isolates from potato and watermelon clustered with VCG2B isolates from mint but VCG2A/B isolates from pistachio and tomato clustered more closely with VCG4B isolates from potato. Isolates belonging to VCG1 and VCG3 were separate but clustered more closely with isolates from potato characterized as VCG4A. Minimum spanning networks of haplotypes from mint and haplotypes from potato were distinctly different (Fig. 3). Haplotype H02 was located at the center of the mint network, with several closely related haplotypes diverging from the predominant clone with no evidence of reticulation. However, reticulation was observed in the minimum spanning network among haplotypes scored as VCG4B and VCG4A/B.

Pairwise comparisons of Slatkin's R_{st} and AMOVA were used to test the hypothesis that *V. dahliae* populations from mint and potato were not genetically differentiated. Isolates from infected potato plants, infected certified seed tubers, infested tare soil associated with certified seed lots, and field soils associated with commercial potato production were not significantly different ($R_{st} \leq 0.12$) (Table 4). Mint and potato populations were significantly different from each other using pairwise comparisons of Slatkin's R_{st} ($R_{st} = 0.70$) (Table 5). The population from mint was also significantly different from field soil ($R_{st} = 0.58$) but was not significantly different from isolates from other hosts ($R_{st} = 0.11$). In all, >94% of the observed genetic variability could be explained by genetic differentiation between mint and potato popu-

TABLE 2. Number of *Verticillium dahliae* isolates sampled; number of haplotypes observed; Nei's expected heterozygosity; and indices of genotypic richness, evenness, and diversity[a]

			Indices of genotypic richness[d]		Indices of genotypic evenness[e]			Indices of genotypic diversity[f]		
Sample group	n[b]	H_{exp}[c]	g_{obs}	g_{exp}	G/g_{obs}	E_1	E_5	H'	N_1	G
Mint	104	0.22		2.12		0.26	0.34	0.60	1.82	1.28
		(0.16–0.27)	10	(3.19)	0.13	(0.16–0.36)	(0.30–0.37)	(0.35–0.85)	(1.37–2.27)	(1.10–1.45)
Native spearmint	18	0.11				0.31	0.49	0.21	1.24	1.12
		(0.01–0.21)	2	1.55	0.56	(0.06–0.56)	(0.35–0.63)	(–0.09–0.52)	(0.87–1.61)	(0.88–1.36)
Peppermint	74	0.16				0.23	0.36	0.41	1.50	1.18
		(0.10–0.21)	6	1.78	0.20	(0.11–0.35)	(0.31–0.42)	(0.16–0.66)	(1.33–1.87)	(1.02–1.34)
Scotch spearmint	12	0.67				0.77	0.65	1.23	3.44	2.57
		(0.56–0.81)	5	4.49	0.51	(0.54–0.99)	(0.43–0.87)	(0.72–1.74)	(2.01–4.86)	(1.21–3.93)
Potato	119	0.51	1	3.75		0.48	0.34	1.39	4.00	2.02
		(0.45–0.57)	8	(6.00)	0.12	(0.39–0.57)	(0.30–0.38)	(1.09–1.68)	(2.91–5.08)	(1.56–2.48)
Plants	20	0.83				0.85	0.85	1.86	6.43	4.65
		(0.75–0.90)	9	5.94	0.52	(0.72–0.98)	(0.72–0.98)	(1.45–2.27)	(4.35–8.50)	(2.54–6.77)
Seed tubers	72	0.53				0.53	0.38	1.37	3.93	2.11
		(0.46–0.60)	13	3.91	0.18	(0.41–0.65)	(0.32–0.44)	(1.02–1.72)	(2.66–5.19)	(1.47–2.75)
Seed tuber soil	17	0.12				0.32	0.50	0.22	1.25	1.12
		(0.02–0.22)	2	1.59	0.56	(0.06–0.58)	(0.35–0.64)	(–0.09–0.54)	(0.86–1.64)	(0.87–1.38)
Seed lot soil	10	...	1	1.00	...	...	...	...	...	...
Field soil	20	0.64		4.55		0.70	0.54	1.37	3.92	2.56
		(0.52–0.76)	7	(7.00)	0.37	(0.51–0.90)	(0.36–0.71)	(0.88–1.86)	(2.29–5.55)	(1.20–3.93)
Other hosts	43	0.86		6.32		0.80	0.58	2.30	9.98	6.23
		(0.82–0.90)	18	(10.24)	0.35	(0.71–0.88)	(0.47–0.69)	(1.95–2.66)	(7.17–12.79)	(3.86–8.60)
Total	286	…	45	…	…	…	…	…	…	…

[a] Values in parenthesis represent 95% confidence intervals calculated using the SAS macro <jackboot.sas> and 2,000 bootstrap replicates.
[b] Number of individuals.
[c] H_{exp}, Nei's expected heterozygosity calculated using Arlequin version 3.5.
[d] Genotypic richness indices: g_{obs}, number of multilocus haplotypes observed; g_{exp}, number of multilocus haplotypes expected for a sample size of 10 isolates and 20 isolates (parentheses), calculated using GenoDive version 1.1 and 10,000 jackknife replicates.
[e] Genotypic evenness indices: $E_1 = H'/[\ln(g_{max})]$, where g_{max} is the maximum number of expected genotypes; $E_5 = [(G - 1)/(N_1 - 1)]$.
[f] Genotypic diversity indices: H' = Shannon-Wiener diversity index; $N_1 = e^{H'}$; and G = Stoddart-Taylor genotypic diversity index.

TABLE 3. Nei's expected heterozygosity (H_{exp}) estimated for each microsatellite locus and *Verticillium dahliae* sample group[a]

	Locus										
Group	VD1	VD2	VD3	VD4	VD96	VD8	VD10	VD26	VD27	VD74	Mean[b]
Mint	0.06	0.08	0.04	0.06	0.06	0.04	0.15	0.02	0.02	0.02	0.05 ± 0.04
	(0.36)	(0.51)	(0.36)	(0.51)	(0.38)	(0.38)	(0.76)	(0.20)	(0.20)	(0.20)	(0.38 ± 0.17)
Potato	0.34	0.38	0.03	0.37	0.39	0.03	0.03	0.03	0.41	0.38	0.24 ± 0.18
	(0.70)	(0.78)	(0.21)	(0.60)	(0.72)	(0.22)	(0.22)	(0.21)	(0.69)	(0.71)	(0.50 ± 0.26)
Field soil	0.61	0.62	0.19	0.57	0.61	0.19	0.19	0.19	0.42	0.57	0.41 ± 0.20
	(0.81)	(0.81)	(0.29)	(0.52)	(0.81)	(0.29)	(0.29)	(0.29)	(0.67)	(0.52)	(0.53 ± 0.23)
Other hosts	0.72	0.79	0.49	0.63	0.71	0.72	0.72	0.47	0.74	0.74	0.67 ± 0.11
	(0.76)	(0.83)	(0.39)	(0.62)	(0.77)	(0.88)	(0.80)	(0.37)	(0.78)	(0.76)	(0.69 ± 0.18)

[a] Results in parentheses represent analyses conducted using clone-corrected data.
[b] Mean and standard deviation.

lations using AMOVA, and this difference was statistically significant (P = 0.03) (Table 6). When clone-corrected data were employed in AMOVA, >76% of the overall genetic variation was due to differences between mint and potato sample groups, and this was also significant (P = 0.03). AMOVA was also used to test the hypothesis that *V. dahliae* isolates belonging to VCG4A and VCG4B were not genetically differentiated. Although genetic differences between VCG4A and VCG4B accounted for 73% of the total variation, VCG4 subgroups from potato were not significantly different (P = 0.34) (Table 7). Only 20% of the overall genetic variability was due to differences in VCG4 subgroups when clone-corrected data ere used for AMOVA (P = 0.33).

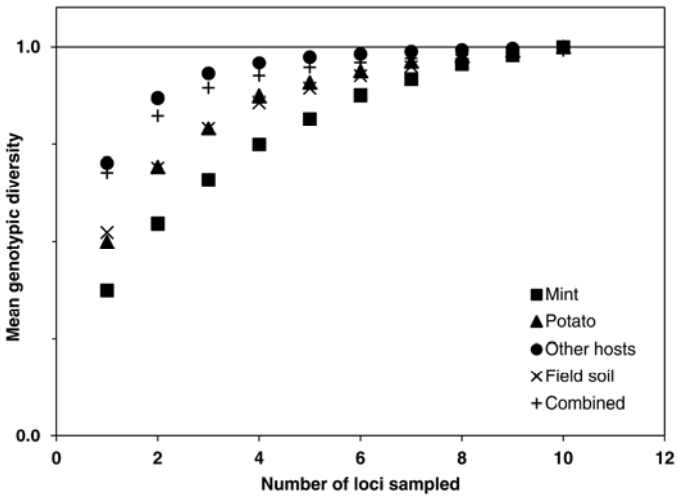

Fig. 1. Plots of mean genotypic diversity versus the number of loci sampled among *Verticillium dahliae* populations from mint, potato, other hosts, field soils, and all four sample groups combined.

Private alleles and migration rate estimates. When scaled for sample size (n = 9), VCG2B isolates obtained from mint possessed ≈1.70 ± 0.37 private alleles per locus compared with 0.30 ± 0.15 and 0.22 ± 0.14 private alleles among VCG4A and VCG4B isolates associated with potato, respectively (Table 8). Estimates of mutation-scaled population sizes and migration rates were conducted even though several populations comprised <10 haplotypes after clone correction. Migration was detected from VCG4B to VCG4A subgroups but not the converse or between VCG2B and either VCG4 subgroup (Table 8). More than two migrants per generation from tare soil to potato were estimated but not vice versa, and more than two migrants per generation were detected between field soils and potato (Table 9). More than one migrant per generation was estimated between infected seed tubers and plants.

Aggressiveness assays. The two positive control isolates used in the aggressiveness assays, isolate 111 from mint and isolate 653 from potato, were characterized as H02 and H04 haplotypes, respectively. Only five isolates caused Verticillium wilt symptoms on mint that were significantly different than asymptomatic water-inoculated controls. The mint-associated haplotype H02 caused significantly greater Verticillium wilt symptoms on mint ($P \leq$ 0.05) in both trials, even when the haplotype was sampled from other hosts such as maple and sugar beet (Fig. 4). Both VCG2 and VCG4A isolates from watermelon caused significant Verticillium

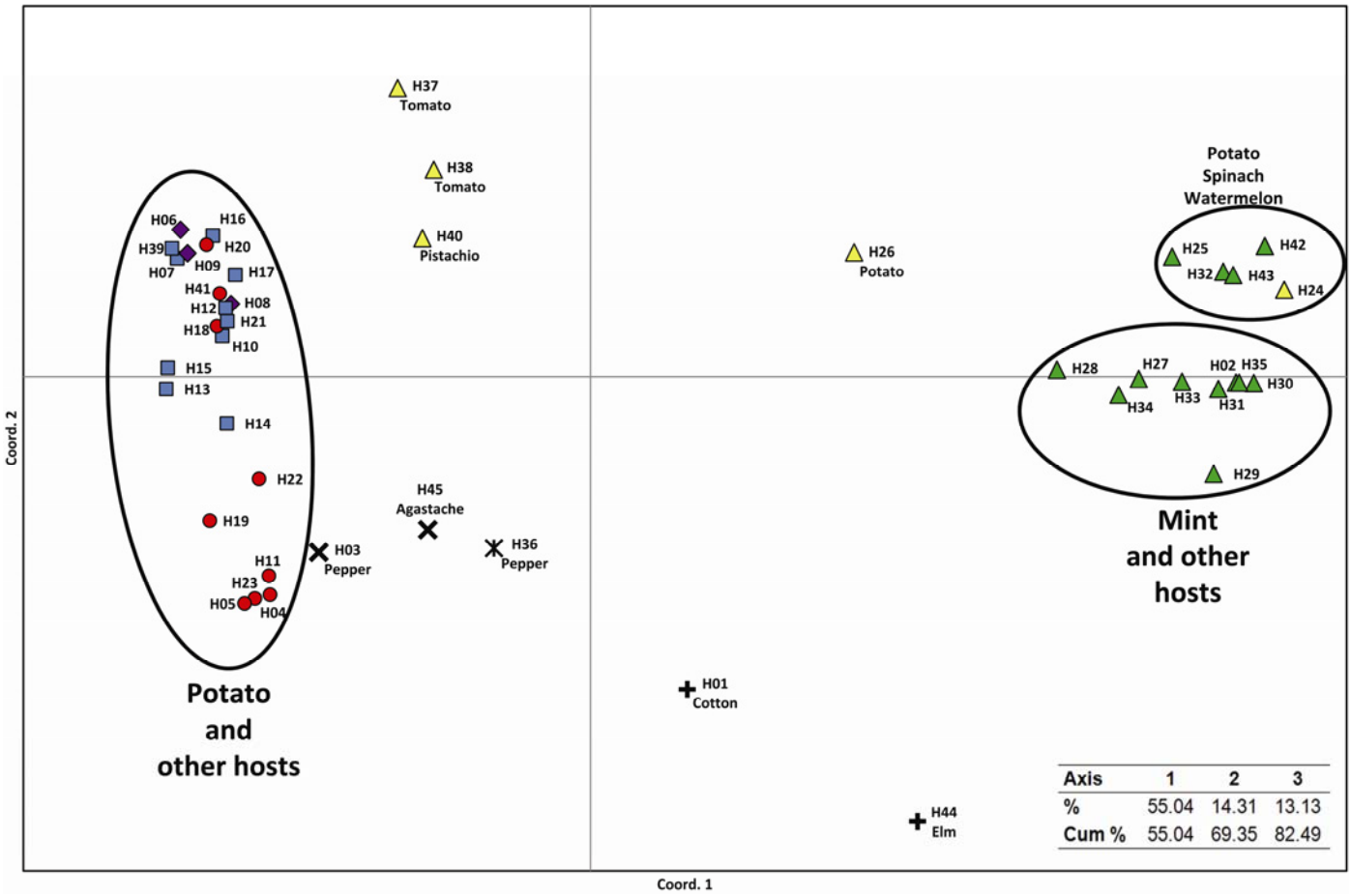

Fig. 2. Principal coordinate analysis of *Verticillium dahliae* microsatellite haplotypes from different hosts and vegetative compatibility groups (VCGs). Haplotypes correspond to those listed in Table 1. Vegetative compatibility groups are represented by the following: black cross = VCG1; yellow triangle = VCG2 and VCG2A; green triangle = VCG2B; black X = VCG3; red circle = VCG4A; blue square = VCG4B; purple diamond = VCG4A/B; and black star = not determined.

wilt symptoms on mint in both trials ($P \leq 0.05$). In all, 9 of 19 isolates caused significant ($P \leq 0.05$) Verticillium wilt symptoms in potato. The potato-associated haplotype H04 isolated from potato, maple, sugar beet, and tare soil caused significantly greater Verticillium wilt symptoms on potato compared with controls in both trials. In addition, VCG4B isolates from spinach and strawberry and VCG2 isolates from pistachio and tomato caused significantly greater disease symptoms on potato ($P \leq 0.05$). In general, most isolates were able to cause moderate to severe disease on potato and only mild to moderate symptoms on mint (Fig. 4).

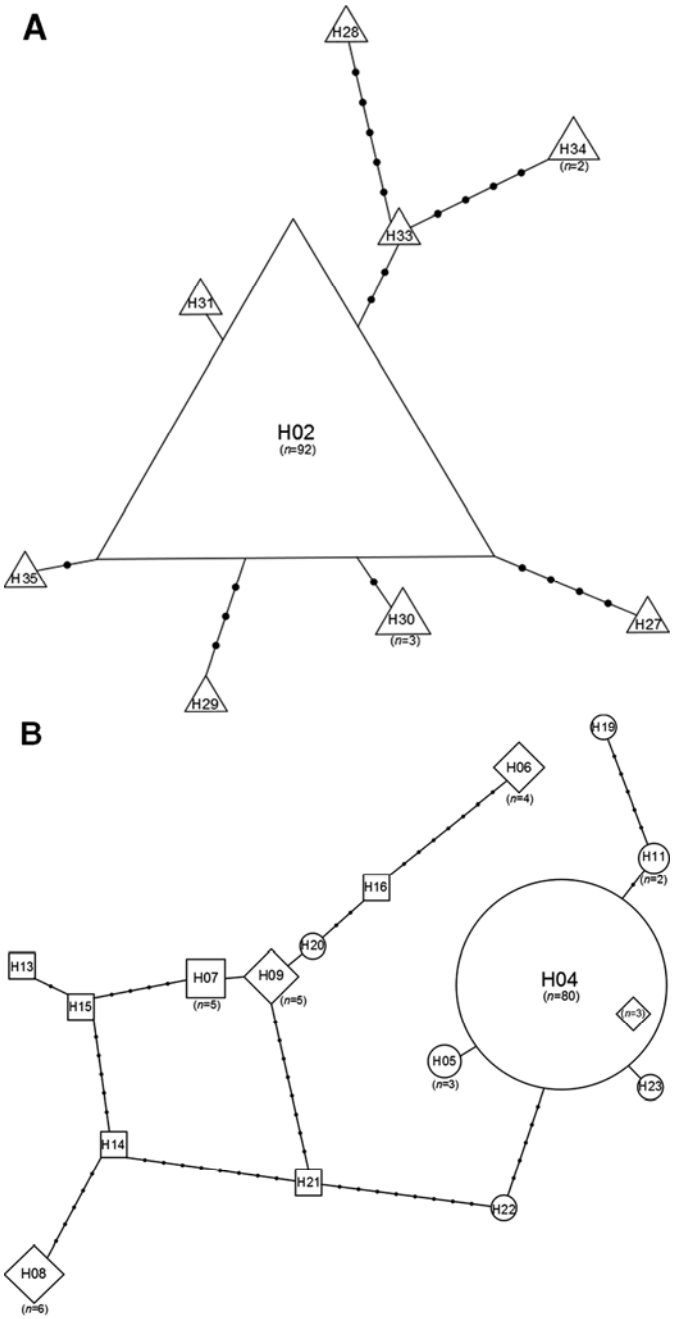

Fig. 3. Minimum spanning networks of **A,** *Verticillium dahliae* haplotypes from mint belonging to vegetative compatibility group (VCG)2B (triangles) and **B,** *V. dahliae* haplotypes from potato belonging to VCG4A (circles), VCG4B (squares), and VCG4A/B (diamonds). Distances between haplotypes are based on genetic distances calculated using the stepwise mutation model. Sizes of shapes are scaled according the haplotype frequencies. Black dots represent one mutation step. Haplotype codes refer to multilocus haplotypes as listed in Table 1.

DISCUSSION

In total, 286 *V. dahliae* isolates associated with mint, potato, and other hosts were analyzed for mating type, VCG, and using 10 microsatellite markers previously shown to be polymorphic in populations from California and Wisconsin (3). *V. dahliae* populations from mint and potato appear to fit a clonal reproductive model, with all isolates being a single mating type and multiple occurrences of multilocus haplotypes over time and space. The mint and potato populations each consisted of a different predominant multilocus haplotype. This is in contrast with a previous study using isolates from California and Wisconsin, in which most isolates were assigned to a unique haplotype and recombination was detected. The different results obtained in this study may be due to the fewer number of microsatellites markers used, since the use of more markers increases the probability of assigning isolates to unique haplotypes, or may be a result of differences in crops and cropping systems between the Columbia Basin and coastal California.

The predominant mint and potato haplotypes were primarily assigned to vegetative compatibility groups VCG2B and VCG4A, respectively, and aggressiveness assays demonstrated that these haplotypes frequently caused severe disease on their respective hosts, even when isolated from a different host. However, haplotype, VCG, and aggressiveness did not appear to be linked in this study, because several haplotypes were characterized as different VCGs or exhibited differences in aggressiveness on mint and potato. Significant genetic differentiation was observed between *V. dahliae* populations from mint and potato, and gene flow estimates, together with the existence of private alleles, indicated restricted gene flow between the two populations. Mint populations appeared to be the most genetically divergent in this study and contained more than twice the number of private alleles as the potato and field soil sample groups despite its low relative genotypic diversity and evenness. Several factors likely contribute to the genetic differentiation of *V. dahliae* from potato and mint, including the occurrence of a single mating type, as well as differences in VCG and host adaptation in *V. dahliae* populations from these hosts. The genetic structure observed may also be a due to a historical geographic separation of hosts and their associ-

TABLE 4. Pairwise comparisons of Slatkin's R_{ST} values for *Verticillium dahliae* sampling groups from infected potato plants, infected certified seed tubers, infested tare soil from certified seed lots, and field soils associated with commercial potato production[a]

Sample group	Plants	Seed tuber	Tare soil
Plants	…	…	…
Seed tubers	0.12021	…	…
Tare soil	–0.05572	0.03815	…
Field soil	–0.08772	0.10495	–0.05655

[a] An R_{ST} value of 0 indicates no separation, $0 < R_{ST} < 0.05$ indicates negligible differentiation, $0.05 \leq R_{ST} < 0.25$ indicates moderate differentiation, $0.25 \leq R_{ST} < 1$ indicates high differentiation, and $R_{ST} = 1$ indicates complete differentiation.

TABLE 5. Pairwise comparisons of *Verticillium dahliae* populations from different hosts and sources using Slatkin's R_{ST}[a]

Sample group	Mint	Potato	Field soil
Mint	…	…	…
Potato	0.69616[b]	…	…
Field soil	0.57953[b]	–0.01858	…
Other hosts	0.10721	0.16990	0.04527

[a] An R_{ST} value of 0 indicates no separation, $0 < R_{ST} < 0.05$ indicates negligible differentiation, $0.05 \leq R_{ST} < 0.25$ indicates moderate differentiation, $0.25 \leq R_{ST} < 1$ indicates high differentiation, and $R_{ST} = 1$ indicates complete differentiation.

[b] Significant at $P < 0.0083$ (0.05/6).

ated pathogen populations although, currently, both crops can be grown in close proximity or even in rotation with each other in many U.S. states.

Although not statistically significant, mint populations had lower gene diversity than populations from potato. The greater diversity of *V. dahliae* populations associated with potato in North America may be due to the fact that potato is grown in more states and on more hectares compared with mint. In addition, large-scale potato production has been occurring over a longer period of time in North America compared with mint, which may have led to greater diversity over time. As expected, the population of isolates from other hosts that was used as the outgroup exhibited the greatest amount of diversity and evenness, which was likely an artifact of the diverse sampling of hosts, VCGs, and geography. The predominant mint and potato haplotypes were represented among isolates collected from other hosts, indicating the potential for these haplotypes to infect hosts other than mint and potato.

Previous studies demonstrated that VCG2B is the predominant VCG isolated from mint. In this study, the most common haplotype from mint, H02, was consistently characterized from VCG2B isolates and accounted for 88% of the mint isolates sampled. This haplotype was sampled from mint in several mint-producing states (Indiana, Oregon, Michigan, Montana, and several counties in Washington) and in samples collected more than a decade apart, indicating that this haplotype is well established in commercial mint production. Several closely related haplotypes appear to be mutational derivatives of this haplotype, as indicated by the minimum spanning network. Overall, these results indicate that a single haplotype is common and widespread among isolates of *V. dahliae* in mint, consistent with previous studies concluding that a host-adapted pathotype of *V. dahliae* is present in mint production (11,30). Mint is vegetatively propagated and the potential exists for *V. dahliae* populations to persist clonally in this host. In addition, mint is a perennial crop which may contribute to the annual increase of host-adapted genotypes in fields over time. Although propagative materials for mint production start as disease-free tissue-culture or greenhouse-grown plants, rhizomes used for propagative materials are typically increased in

TABLE 6. Analysis of molecular variance of *Verticillium dahliae* grouped by host of isolation[a]

Source of variation	df	Sum of square	Variance components	Percent	Φ	*P* value
Among mint and potato	1	40,689.647	365.27398	94.04	0.93883	0.02725 ± 0.00140
	(1)	(4,418.803)	(253.26827)	(76.85)	(0.76854)	(0.02625 ± 0.00120)
Among sample groups within mint and potato	5	372.435	2.31944	0.60	0.100161	0.00219 ± 0.00039
	(5)	(413.124)	(1.78348)	(0.54)	(0.02338)	(0.28840 ± 0.00361)
Within sample groups	216	4,500.780	20.83694	5.36	0.94038	0.00000 ± 0.00000
	(31)	(2,309.257)	(74.49217)	(22.60)	(0.76854)	(0.00000 ± 0.00000)
Total	222	45,562.861	388.43037			
	(37)	(7,141.184)	(329.54392)	…	…	…

[a] Results in parentheses represent analyses conducted using clone-corrected data.

TABLE 7. Analysis of molecular variance of *Verticillium dahliae* within and among vegetative compatibility group (VCG) 4 subgroups (VCG4A and VCG4B)[a]

Source of variation	df	Sum of square	Variance components	Percent	Φ	*P* value
Among VCG4 subgroups	1	1,213.012	39.25300	73.22	0.73221	0.33777 ± 0.00373
	(1)	(136.668)	(8.27648)	(19.99)	(0.19985)	(0.33046 ± 0.00396)
Among sample groups within VCG4 subgroups	2	78.578	2.22744	4.16	0.15516	0.06455 ± 0.00185
	(2)	(77.894)	(1.5097)	(3.65)	(0.04556)	(0.32346 ± 0.00354)
Within sample groups	81	982.386	12.12822	22.62	0.77376	0.00000 ± 0.00000
	(19)	(600.917)	(31.62719)	(76.37)	(0.23630)	(0.05137 ± 0.00177)
Total	84	2,273.976	53.60866			
	(22)	(815.478)	(41.41338)	…	…	…

[a] Results in parentheses are from analyses conducted using clone-corrected data.

TABLE 8. Mean number of private alleles per locus, mutation-scaled population size (θ), and migration rates (*M*) between *Verticillium dahliae* populations from mint and potato belonging to different vegetative compatibility groups (VCGs)

Sample group	Private alleles/locus[a]	θ[b]	Mint (VCG2B)[b]	Potato (VCG4A)[b]	Potato (VCG4B)[b]
Mint (VCG2B)	1.70 (1.33–2.07)	1.3917 (1.0859–1.8324)	…	0.1291 (0.0657–0.2239)	0.0222 (0.0037–0.0693)
Potato (VCG4A)	0.30 (0.15–0.45)	0.6402 (0.4324–0.8757)	0.2298 (0.0883–0.4762)	…	1.9675 (1.4458–2.6521)
Potato (VCG4B)	0.22 (0.08–0.36)	1.0832 (0.8788–1.3797)	0.0387 (0.0067–0.1082)	0.5398 (0.3612–0.7691)	…

[a] Mean number of private alleles per locus, scaled for the smallest clone-corrected population size (n = 9), were calculated using a rarefaction approach in ADZE 1.0.

[b] Maximum likelihood estimates of migration rates (M = m/μ) and 95% confidence intervals (parentheses) were calculated using Migrate 3.0. Source and recipient populations are in columns and rows, respectively.

TABLE 9. Mutation-scaled population size (θ) and migration rates (*M*) between *Verticillium dahliae* isolates from infected potato plants, infected certified seed tubers, infested tare soil from certified seed lots, and field soils associated with commercial potato production[a]

Sample group	θ	Plants	Seed tubers	Tare soil	Field soil
Plants	1.0603 (0.8095–1.3946)	…	1.5393 (1.1330–2.0345)	2.5252 (1.9924–3.1608)	2.6831 (2.1248–3.3909)
Seed tubers	0.8750 (0.7233–1.0700)	1.0682 (0.7343–1.4480)	…	1.4131 (1.0724–1.8203)	0.4439 (0.2662–0.6870)
Tare soil	0.6211 (0.4925–0.8004)	0.7812 (0.5322–1.1328)	0.7851 (0.5475–1.0823)	…	1.1273 (0.8333–1.4902)
Field soil	0.8838 (0.6568–1.1792)	3.0168 (2.3669–3.7000)	0.3766 (0.1749–0.6564)	1.2333 (0.8925–1.6498)	…

[a] Maximum likelihood estimates of migration rates (M = m/μ) and 95% confidence intervals (parentheses) were calculated using Migrate 3.0. Source and recipient populations are in columns and rows, respectively.

the field prior to planting for commercial production, which can result in the introduction of *V. dahliae* into planting stock. Although many mint rootstock certification standards express a zero tolerance for Verticillium wilt in planting stock, there are acknowledged risks associated with undetected *V. dahliae* in planting materials, especially in those grown under less strict land requirements. Therefore, it is important that field increases of propagative materials for commercial production occur in soils free of *V. dahliae*, and especially in soils free of haplotypes known to be aggressive on mint.

The predominant haplotype associated with potato was H04 and this haplotype was most often characterized as VCG4A.

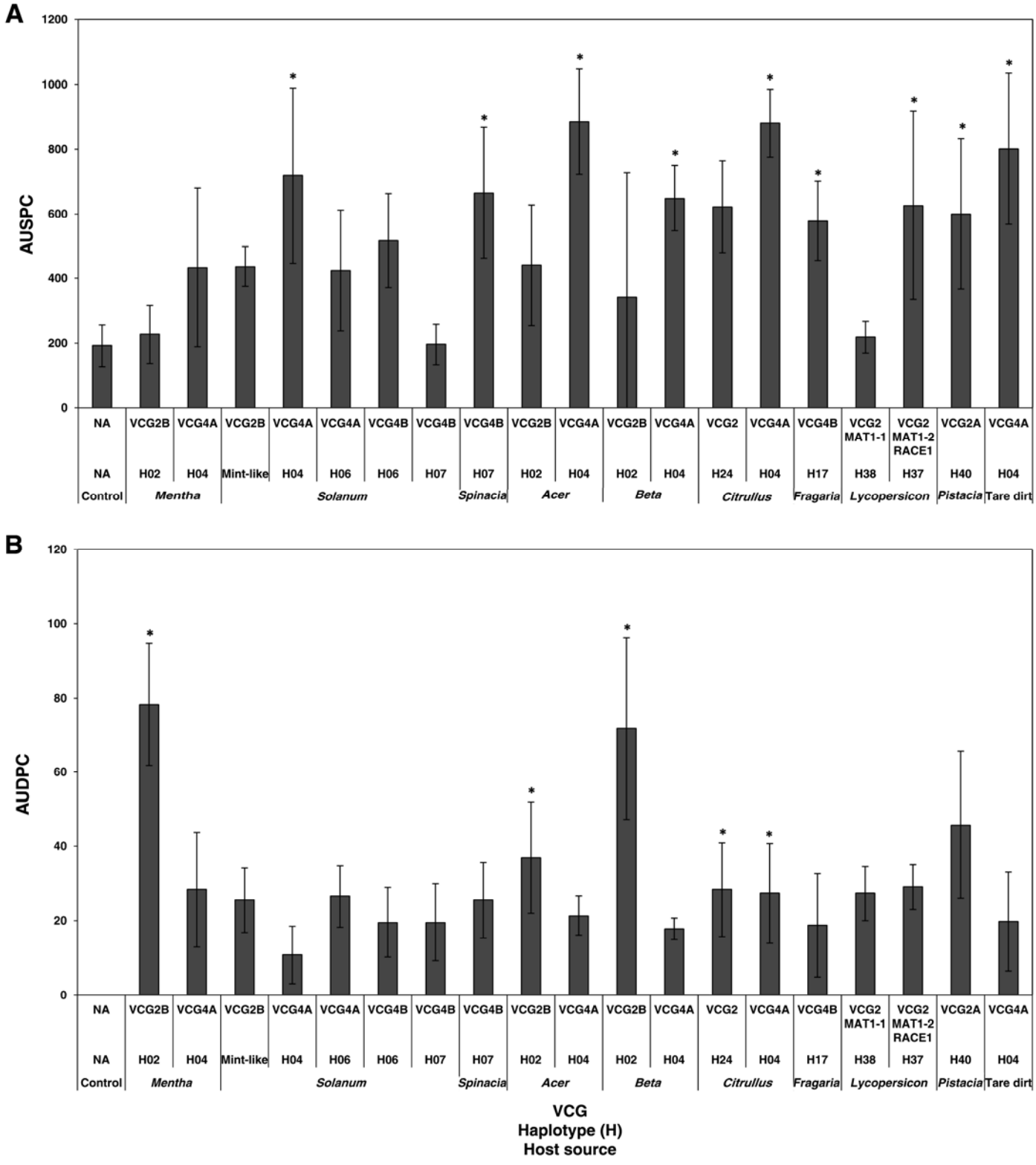

Fig. 4. A, Area under senescence progress curve (AUSPC) values of 'Russet Norkotah' potato and **B,** area under disease progress curve (AUDPC) values of Scotch spearmint following inoculations with different haplotypes of *Verticillium dahliae.* Haplotypes of *V. dahliae* were collected from different hosts and were characterized according to vegetative compatibility groups (VCGs). Asterisks indicate a significant difference ($P \leq 0.05$) from water-inoculated control treatments using Dunnett's test.

Haplotype H04 appears to be common in North American commercial potato production, having been observed in samples collected from seven states and over a period of several decades, from before 1983 through 2010. This haplotype was also isolated from mint, indicating the potential for this potato-associated haplotype to infect mint. In addition, one isolate from watermelon which was characterized as H04 caused significant disease in spearmint and many isolates caused mild symptoms; therefore, growers should use caution when rotating between potato and mint. Haplotype H04 was the predominant haplotype associated with infected seed tubers, tare soils associated with certified potato seed lots, and field soils associated with commercial potato production. Seed tubers, which are used to vegetatively propagate potato for commercial planting, are produced in 15 states of the United States and shipped to production areas throughout North America and the world. In this study, many isolates from infected seed tubers and infested soil associated with certified seed tubers were haplotype H04. It should be noted that 65% of seed lots sampled originated from Idaho, Montana, and Oregon and many of the isolates characterized as H04 were from these three states. In addition, the majority of the seed tuber isolates characterized as haplotype H04 was collected between 2007 and 2011. However, >39% of seed tuber-producing hectares are located in Idaho, Montana, and Oregon and these results suggest that a clonal population of *V. dahliae* is widespread in seed tubers used for commercial planting. Haplotype H04 was also found in infected seed tubers produced in several states prior to 1997, demonstrating its association with potato for more than a decade. Nonetheless, isolates from other seed-producing areas were typically haplotypes other than H04, indicating that genetic variation exists in *V. dahliae* populations among seed tuber-producing states. Like mint, potato propagative materials are started as tissue-culture or greenhouse-grown plants but are increased in fields over several seasons, a necessary practice but one which can result in the annual increase of pathogens in later generations of seed tubers. Higher estimated migration rates were observed between field soil and plants and between tare soil and plants compared with seed tubers and plants, which is in agreement with previous studies concluding that field and tare soils are important sources of inoculum for Verticillium wilt of potato (13–15). However, the potential does exist for infected seed tubers to infest field soils and infect plants in subsequent seasons rather than in the current season. Infected seed tubers or infested soil associated with seed tubers may provide a means for the movement of *V. dahliae* and facilitate the migration of novel or host-adapted genotypes across long distances.

PCoA separated VCG1, VCG2B, and VCG4 isolates into distinct groups. However, two clusters of VCG2 isolates were observed using PCoA. The first group consisted of isolates from tomato (*Lycopersicon esculentum*) and pistachio (*Pistacia vera*) and was more closely related to the VCG4 group, while the other group consisted of isolates from potato and watermelon and was more closely related to the mint VCG2B group. These results support a previous study suggesting that two genetically distinct groups of VCG2 isolates exist within *V. dahliae*, with one lineage exhibiting a closer genetic relationship with VCG4B (9). VCG3 isolates clustered more closely with VCG4 isolates and moderate complementation was observed between one VCG3 isolate from *Agastache* spp. and VCG4A tester strains, suggesting that these two VCGs are genetically similar. Complementation between VCG3 and VCG4A tester strains in *V. dahliae* has been previously reported (40).

VCG4 subgroups from potato and seed potato were not significantly differentiated using AMOVA and migration estimates indicated that VCG4B contributes nearly two migrants per generation into VCG4A. A cluster of VCG4 isolates, consisting of VCG4A, VCG4B, and VCG4A/B isolates, was observed using PCoA in this study, indicating that VCG4A and VCG4B subgroups are not genetically isolated. The lack of differentiation among VCG4A and VCG4B isolates may be due to gene flow or a relatively recent divergence. Some VCG4 isolates from potato exhibited strong complementation with both VCG4 subgroups and were designated as VCG4A/B; therefore, the possibility exists for gene flow to occur between VCG4A and VCG4B isolates via hyphal anastomosis with VCG4A/B isolates. In addition, several VCG4 isolates associated with potato also exhibited weak complementation with VCG4A or VCG4B, which may also facilitate gene flow between these subgroups over time. These results suggest that vegetative incompatibility may not present an absolute barrier to gene flow among VCG4A and VCG4B subgroups.

All isolates assayed for mating type in this study possessed the *MAT1-2* idiomorph, with the exception of isolate 461 from tomato, which possessed the *MAT1-1* idiomorph. These results suggest that the potential for sexual reproduction is low in *V. dahliae* populations impacting mint and potato in North America. The *MAT1-2* idiomorph was found to be predominant in *V. dahliae* populations in a previous study, occurring in 143 of 154 isolates from various hosts (22). The *MAT1-1* idiomorph has been found in isolates from tomato collected in California and Brazil, as well as isolates from other solanaceous hosts such as potato and pepper. The *MAT1-2* idiomorph was also found to be prevalent in *V. dahliae* populations from Japan, though the *MAT1-1* idiomorph was found in isolates from tomato and a few other isolates from other hosts and countries (43).

A greater number of sampled isolates caused moderate to severe Verticillium wilt symptoms on potato compared with mint. The H04 haplotype, which was predominant in potato and most often characterized as VCG4A, generally caused the most severe symptoms on potato regardless of host origin, followed by haplotypes belonging to VCG2 and VCG4B. These results are consistent with previous work demonstrating a continuum of aggressiveness among *V. dahliae* isolates on potato (39). In contrast, the H02 mint haplotype caused severe symptoms on mint whereas other haplotypes and VCGs were generally not as aggressive on mint. A number of isolates characterized as haplotype H02 in this study were shown to be aggressive on different *Mentha* spp. and hybrids in the current and previous studies (11,16). This is in agreement with previous studies concluding that isolates from mint are highly aggressive on mint and cause Verticillium wilt symptoms on a limited range of hosts (11,16,30,35). Host selection, or interactions between host resistance and pathogen aggressiveness, may result in differences among *V. dahliae* populations in their abilities to colonize certain hosts, impacting the genetic diversity of pathogen populations found in different crops and field. A greater susceptibility to a wider range of *V. dahliae* isolates may increase the genotypic variation observed in *V. dahliae* populations sampled from potato plants compared with mint due to less host-selection pressure.

We hypothesized that differences in vegetative compatibility, aggressiveness, or mating type compatibility between *V. dahliae* populations from mint and potato would result in a reduction in gene flow and genetic and genotypic differentiation between pathogen populations affecting these two hosts. Populations of the pathogen from mint and potato were genetically differentiated, indicating that there is limited migration or gene flow between them. Differences in vegetative compatibility and the occurrence of only a single mating type likely contribute to genetic drift and differentiation among *V. dahliae* populations from mint and potato. Populations of *V. dahliae* in mint and potato were characterized by the presence of highly aggressive, clonally reproducing haplotypes, and these haplotypes are widely distributed and established in commercial mint and potato production. Pathogen dissemination via infected propagative materials may lead to the establishment and predominance of certain host-adapted strains, especially in vegetatively propagated hosts. In addition, the long survival of *V. dahliae* as microsclerotia in soils may also result in

the continued persistence of certain host-adapted pathotypes. This, coupled with limited gene flow among unlike VCGs and the lack of sexual reproduction, may contribute to the persistence and differentiation of host-adapted *V. dahliae* populations in some cropping systems.

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