Detection, quantification and vegetative compatibility of *Verticillium dahliae* in potato and mint production soils in the Columbia Basin of Oregon and Washington

Dennis A. Johnson, Department of Plant Pathology, Washington State University, Pullman **M. A. Omer**, USDA-ARS Toledo, OH **Philip B. Hamm**, Department of Botany and Plant Pathology, Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, and **Randall C. Rowe**, Department of Plant Pathology, The Ohio State University-OARDC, Wooster.

INTRODUCTION

Verticillium dahliae is a widespread soil-borne fungal plant pathogen that causes vascular wilt disease on many important crops. In potato, infection by *V. dahliae*, especially in the presence of the root-lesion nematode *Pratylenchus penetrans*, causes potato early dying (PED). PED leads to early decline of potato foliage and can result in a substantial reduction in yield and tuber size. In North America, yields can be reduced 10 to 15% in moderately infested fields, and 30 to 50% in heavily infested fields. Effective management strategies for PED utilize various crop rotations, cultivar resistance and sometimes pre-plant soil fumigation. *V. dahliae* survives in soil as microsclerotia that form in the decaying tissues of infected host crops. Since microsclerotia are the primary overwintering inoculum of *V. dahliae*, detecting the presence and populations of microsclerotia in soils targeted for potato production is important in disease risk assessment. A direct relationship between inoculum density of *V. dahliae* in soil and wilt severity or stem colonization, and tuber yield has been shown in potato**.** Disease threshold values for potato have been demonstrated to be in the range of 5-30 cfu/cm^3 of air-dried soil for *V. dahliae* microsclerotia alone and as low as 2-13 cfu/cm^3 of air-dried soil for *V. dahliae* microsclerotia in the presence of *P. penetrans* (4, 10). Determining pre-plant populations of *V. dahliae* in soil can be a useful tool in selecting fields that are either safe for planting potatoes or that may benefit from specific disease management procedures. A direct relationship between *V. dahliae* inoculum density and disease incidence and severity also has been reported for several other crops.

Peppermint and Scotch spearmint, two of the three mint species produced commercially for essential oils in western and midwestern U. S., are both highly susceptible to infection by *V. dahliae*. Native spearmint can be infected by *V. dahliae*, but is less susceptible to wilt damage than the other two. However, the oil of this species has more limited commercial use and thus it is not as desirable a crop. Verticillium wilt of mint is characterized by chlorosis of foliage and stunting and mortality of plants. Initial inoculum of *V. dahliae* in mint consists of microsclerotia present in soil prior to planting rhizomes to establish a new mint field, or planting of rhizomes already infected with the pathogen. Severe wilt symptoms and yield loss can occur in peppermint at 10 cfu/g of soil, a pathogen threshold similar to potato.

The use of vegetative compatibility analysis to define distinct sub-specific groups of isolates within *V. dahliae* has led to recognition that host-adapted pathotypes of *V. dahliae* exist that are more aggressive to potato and mint as well as cotton and several other crops. Isolates of *V. dahliae* from infected mint plants growing in the western and midwestern United States nearly all belonged to vegetative compatibility group (VCG) 2B (2). VCG 2B isolates are more aggressive to mint than those in VCG 4A (6). It is well documented that isolates infecting potato are almost exclusively in VCG 4A and VCG 4B (8) and that VCG 4A isolates are the most aggressive to potato (5, 10, 11). Furthermore, synergistic interactions of *V. dahliae* with *P. penetrans* have been shown to occur in peppermint and Scotch spearmint with VCG 2B (6), but in potato with VCG 4A (4, 10). Given the existence of host-adapted pathotypes of *V. dahliae* and their different pathogenic interactions among specific crops, questions can be raised regarding the utility of pre-plant soil assays to enumerate soil populations of this pathogen as part of a management program. Information about the VCG composition of the populations that are being detected may provide a clearer indication of the potential pathogenicity and aggressiveness of *V. dahliae* to a crop intended for planting rather than mere numerical estimates of overall soil population density.

The objectives of this study were: 1) To extensively sample soils from commercial production fields throughout the Columbia Basin prior to planting either potato or mint in these fields and determine the populations of *V. dahliae* propagules in these samples, and 2) To isolate pure cultures of *V. dahliae* from representative colonies of the fungus recovered from these soil samples and determine the vegetative compatibility group of each isolate. The goal was to determine the prevalence of specific VCGs of *V. dahliae* in soils to be cropped to potato or mint -- information that would be useful in the development of improved Verticillium wilt management strategies for these two crops in the Columbia Basin.

MATERIALS AND METHODS

Collection and preparation of soil samples

In September and October of 1997, soil samples were collected randomly from 87 commercial fields in the Columbia Basin of Washington and Oregon that were intended for potato production in 1998 (Table 1). Among these fields, 68 had been used for potato production within the past five years, two had been in mint production within the past five years and one had produced mint 15 yrs earlier, and the remainder had been in various other crops within the past five years. Fields were selected from the upper, middle and lower areas of the Columbia Basin in Washington and the lower Columbia Basin near Hermiston, Oregon in order to reflect the region’s variable geography, soil type and cropping history. Each sample was obtained by inserting a 2.5-cm-diameter soil auger to a depth of approximately 15-cm. Thirty soil cores were taken randomly while walking along a serpentine pattern through each field. Cores from each field were bulked and mixed as a single sample immediately after collection and designated by a field number. All samples were air-dried in paper bags for 2-4 weeks at approximately 50% relative humidity and 73^0 F to eliminate viable propagules sensitive to desiccation. Samples were then passed through a coarse sieve to remove dirt clods, stones and organic debris.

A second set of soil samples was collected in October and November of 1998 from 51 commercial fields in the Columbia Basin of Central Washington (Table 2) that were to be planted with either peppermint or Scotch spearmint crops in 1999. Among these fields, 45 had been used within the past 19 years for either spearmint or peppermint production and six had not been used previously for mint production. Thirty of the 51 fields had been previously used for potato production within the last 13 years. Four of the fields had not been used previously to grow either potatoes or mint. Fields were again selected to represent variability in geography, soil type, and cropping history. Soil samples were collected as above, bulked for each individual field, and then each bulked sample was spread on a greenhouse bench and air dried for one month. After drying, each bulked sample was mixed, passed through a coarse sieve and collected on a surface-disinfested plastic container.

Determination of populations of *V. dahliae* in soil samples and collection of isolates

Dried soil samples were assayed for *V. dahliae* propagules by dilution plating directly onto a semi-selective medium for Verticillium (NP-10 agar medium). Contents of each plastic bottle were manually shaken to assure complete mixing and then 10-g sub-samples were removed (five for potato soils and four for mint soils). Each sub-sample of soil was placed in a 250-ml Erlenmeyer flask containing 90 ml of autoclaved 0.1 % water agar and the suspension was stirred for 2 min on an electric stir plate. While stirring, 1-ml aliquots were removed with a pipette and evenly spread onto individual petri dishes containing NP-10 medium. Five replicate plates were used for each sub-sample for a total of 25 plates per potato soil sample and 20 per mint soil sample. Following 2-3 wks incubation, the surface of each plate was gently washed under running tap water to remove soil residues and plates were examined under a dissecting microscope. Colonies on each plate that were morphologically characteristic of *V. dahliae* were identified and counted. The average number of colonies per plate was used to calculate an estimate of the population density of *V. dahliae* in the soil collected from each sampled field.

Vegetative compatibility analysis of isolates

Isolates of *V. dahliae* obtained from soil collected from both potato and mint fields were tested to identify the vegetative compatibility group to which each belonged. Nitrate non-utilizing (nit) mutants were prepared from these isolates and then paired with appropriate VCG tester strains using the methods described by Omer et al (8). Zero to four nit mutants were recovered from each isolate and then each was paired with standard tester strains of known VCGs.

RESULTS

Recovery of *V. dahliae* from soil samples

V. dahliae was recovered from soil collected from 77 of the 87 sampled fields that were intended for potato production in 1998 (Table 1). Population densities of the pathogen were found to range from 0 to 169 propagules per gram of air-dried soil (ppg). Among these fields, 37% had 10 ppg or more -- a population density threshold likely to cause significant incidence of PED and associated yield reduction **(**3, 10). Six per cent of the fields had population densities of *V. dahliae* that exceeded 30 ppg (Fig. 1). Commercial fields sampled in this study were located in the upper, middle or lower regions of the Columbia Basin (Table 1). In the upper Columbia Basin where 45 commercial fields were sampled, *V. dahliae* was detected in 36 fields. Of these, 67% had population densities <10 ppg, 24% had 10-20 ppg and 9% had > 20 ppg. Only three fields from the middle Columbia Basin were sampled and *V. dahliae* was detected in all three. One had a population density <10 ppg and two had densities between 10-20 ppg. Among the 39 commercial fields sampled in the lower Columbia Basin, *V. dahliae* was detected in all but one. Of these 59% had population densities <10 ppg, 26% had 10-20 ppg and 15 % had > 20 ppg.

Verticillium dahliae was recovered from soil collected from 41 of the 51 sampled fields that were intended for mint production in 1999 (Table 2). Population densities of the pathogen were found to range from 0-75 ppg of air-dried soil. Among these fields, 33% had 10 ppg or more and 20% had population densities of *V. dahliae* that exceeded 30 ppg (Fig. 1). *Verticillium dahliae* was recovered from only one of the four fields that had not been used previously to grow mint or potato. Population density of *V. dahliae* in this field was 1 ppg of air-dried soil.

Vegetative compatibility analyses

Ninety six nit mutant isolates of *V. dahliae* were generated from isolates of the fungus recovered from soil samples obtained from 40 fields planned for potato production in 1998 (Table 1). Eighty-three isolates (86%) were assigned to VCG 4A, 11 isolates (12%) to VCG 4B and two isolates (2%) to VCG 2B. Sixty nit mutant isolates of *V. dahliae* were generated from isolates of the fungus recovered from soil samples obtained from 16 fields planned for mint production in 1999 (Table 2). Forty six isolates (77%) were assigned to VCG 4A, 8 isolates (13%) to VCG 4B and six isolates (10%) to VCG 2B. The VCG was not determined for the isolate collected from the field that had not previously been used to grow either mint or potato.

DISCUSSION

This study was undertaken to determine the prevalence and population densities of *V. dahliae* in commercial production soils in the Columbia Basin of Washington and Oregon prior to planting crops of either potatoes or mint and to determine which vegetative compatibility groups of the pathogen were present. Although fields were selected for sampling without regard to prior history of Verticillium wilt, there is no doubt that *V. dahliae* is widespread in Columbia Basin soils involved in production of both these crops. The pathogen was recovered from 85% of all soil samples collected and, in both years, about a third of the sampled fields had soil populations that exceeded 10ppg, an action threshold above which significant damage is likely to occur in both crops (3, 10). This same pattern was found in fields planned for potato production that were sampled in all three geographic areas of the Columbia Basin. Crop history records showed that of the 87 fields sampled that were to be planted to potato, 68 (78%) had been used for potato production in the previous five years. Among these fields, two had been used to produce mint in the previous five years and one had been in mint 13 years earlier. Crop history records of the 51 fields sampled that were to be planted to mint showed that 45 (88%) had been used to produce mint within the previous 19 years and 30 (59%) had been in potatoes in the previous 13 years. Other crops produced in these 138 fields included alfalfa, wheat, sweet corn and various vegetable crops that are not susceptible to vascular wilt caused by *V. dahliae*. The finding that *V. dahliae* was widespread in these fields was not surprising given their long-term use for production of one or both of these highly susceptible crops. Microsclerotia of the pathogen are produced abundantly in necrotic tissues of both potato and mint and are released into soil as residues of these tissues degrade (11). Microsclerotia of *V. dahliae* can remain viable in soil for at least 10 years (12) and, in addition, soil populations of the pathogen can be maintained over time on the roots of some non-host plants (9, 11).

Of necessity, growers producing either potatoes or mint must be concerned about the inoculum of *V. dahliae* present in production fields prior to planting either of these crops. Selection of production fields and pre-plant decisions on the use of expensive soil fumigation treatments and the selection of resistant cultivars often have been guided by various soil assay techniques designed to assess the population density of *V. dahliae* (13). However, it has been well established that host-adapted pathotypes exist within populations of *V. dahliae* and this may complicate such assays. Vegetative compatibility analysis has been used to identify strains that are highly aggressive to potato (5, 8), mint (2, 6) and several other crops (1, 7). Both potato and mint are vegetatively propagated crops with a single aggressive pathotype of *V. dahliae* predominating on each. It is likely that this pattern is maintained when the host is a crop that is reproduced clonally. In potato, VCG 4A is the most aggressive pathotype causing severe disease symptoms. Strains in VCG 4B and VCG 2 are capable of infecting potato, but result in generally mild to moderate symptoms (5). VCG 2B is the most aggressive pathotype infecting mint (2). Conversely to the situation with potato, isolates of VCG 4A from mint, potato and other crops were capable of infecting mint, but caused only mild to moderate symptoms (2). Vegetative compatibility analysis of the 156 isolates of *V. dahliae* that we tested from Columbia Basin soils revealed that 98% of those from fields planned for potato production and 90% of those from fields planned for mint production were in VCG 4. In the former set of fields, 86% were in VCG 4A, the pathotype most aggressive on potato. However, in the latter set of fields, 77% of the isolates were also in VCG 4A. The pathotype most aggressive on mint, VCG 2B, was found in only 2% of the fields planned for potato production, but in 10% of those planned for mint production.

Although it is difficult to draw definitive conclusions from these data regarding the risk that these two crops pose to each other in rotation with regard to infection by *V. dahliae*, it appears that prior potato production in a field is not likely to seriously endanger future plantings of mint since very few isolates of VCG 2B were found in fields where potatoes had been commonly grown. It may be that pathotype does not survive well in a soil where potato is the commonly grown Verticillium-susceptible crop. Isolates of VCG 2B were more commonly found in soils where mint had been cropped, but even these soils were still dominated by VCG 4A. Potatoes had been previously grown in over half of the sampled fields intended for mint production and the 4A pathotype likely increased substantially when potatoes were grown. It also may be that the 4A pathotype can maintain itself well on mint plants despite not causing significant damage to that crop. Thus, planting potatoes in a field recently used to produce mint may pose a significant risk to the potato crop if populations of the VCG 4A pathotype are at high populations.

It is clear from these data that pre- plant assessment of soil populations of *V. dahliae* without regard to the relative populations of various pathotypes present in a particular sample could lead to information not fully useful in IPM systems. A simple determination of the total population of *V. dahliae* could over estimate the actual disease potential, particularly when planning to grow mint where VCG 4A strains are not the issue (2). Since current soil assay procedures based on soil plating techniques (13) do not yield information on the relative populations of various pathotypes in a given sample, new DNA-based soil assay technologies are needed that have this capability. This would facilitate the development of more precise information that could be used as an effective management tool for Verticillium wilt in either crop (11), possibly leading to reduced or more efficient use of control measures such as soil fumigants (3).

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Table 1. Isolation and characterization of *Verticillium dahliae* (Vd) from soil samples collected in 1997 from fields intended for potato production in 1998 in the Columbia Basin of Washington and Oregon.

Location[y]	No. of fields sampled	No. of fields from which Vd recovered	Nit mutants generated: No. of Nit mutants	Nit mutants generated: No. of fields	No. of Vd isolates characterized: VCG[z] 4A	No. of Vd isolates characterized: VCG 4B	No. of Vd isolates characterized: VCG 2B
Upper Columbia Basin							
George	17	14	9	4	9	0	0
Mattawa	5	4	1	1	0	1	0
Moses Lake	10	8	10	5	9	1	0
Royal City	6	4	13	4	9	4	0
Quincy	5	5	6	2	6	0	0
Othello	1	0	0	0	0	0	0
Ephrata	1	1	2	1	0	0	2
Totals	45	36	41	17	33	6	2
Middle Columbia Basin							
Pasco	1	1	2	1	2	0	0
Prosser	2	2	4	2	3	1	0
Totals	3	3	6	3	5	1	0
Lower Columbia Basin							
Hermiston, OR	20	20	20	10	16	4	0
Patterson	19	18	29	10	29	0	0
Totals	39	38	49	20	45	4	0
Grand Totals	87	77	96	40	83	11	2

[y] Nearest town to sampled field. All fields in Washington except those near Hermiston, Oregon.
[z] Vegetative compatibility group.

Table 2. Isolation and characterization of *Verticillium dahliae* (Vd) from soil samples collected in 1998 from fields intended for mint production in 1999 in the Columbia Basin of Washington.

Location[y]	No. of fields sampled	No. of fields from which Vd recovered	Nit mutants generated		No. of Vd isolates characterized		
			No. of Nit mutants	No. of fields	VCG[z] 4A	VCG 4B	VCG 2B
Ephrata	1	1	0	0	0	0	0
George	15	11	20	5	17	1	2
Royal City	21	19	36	9	28	7	1
Vantage	2	2	2	1	1	0	1
Beverly	2	2	2	1	0	0	2
Othello	2	2	0	0	0	0	0
White Swan	8	4	0	0	0	0	0
Totals	51	41	60	16	46	8	6

[y] Nearest town to sampled field.
[z] Vegetative compatibility group

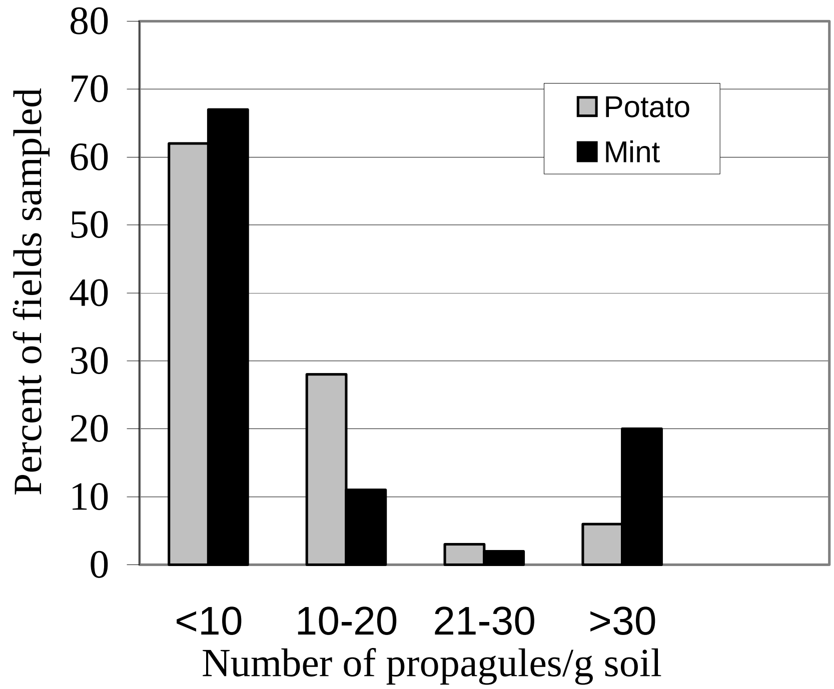

Fig. 1

Fig 1. Population densities of *Verticillium dahliae* propagules in soil samples collected from fields intended for potato and mint production in the Columbia Basin of Washington and Oregon.