Susceptibility of Weedy Hosts from Pacific Northwest Potato Production Systems to Crop-Aggressive Isolates of *Verticillium dahliae*

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Abstract

Verticillium wilt, caused by *Verticillium dahliae*, is a disease of dicotyledonous crops such as potato and has a wide host range and persistent, long-term survival structures called microsclerotia that can persist in soil for up to 14 years. Some *V. dahliae* isolates are particularly aggressive on a specific plant host while retaining the ability to infect a wide range of other hosts. Weeds can serve as hosts for *V. dahliae* but whether they serve as sources of inoculum for aggressive isolates of *V. dahliae* to crop hosts is unknown. The goal of this research was to quantify *V. dahliae* microsclerotia obtained from 16 weeds which were grown in the greenhouse. Potting medium was infested with one of eight *V. dahliae* isolates from potato, mint, sugar beet, sunflower, tomato, and watermelon. The isolates from mint and potato were aggressive on the host from which they were originally isolated. All 16 weeds were infected by at least

one *V. dahliae* isolate, although the number of microsclerotia produced from some infections was relatively low (≤ 5 microsclerotia/g of dry plant). Black nightshade yielded greater numbers of microsclerotia of the *V. dahliae* potato isolate than any other isolate in three of four trials in the greenhouse (second trial false discovery rate, adjusted $P \leq 0.0158$; third trial, $P \leq 0.0264$; and fourth trial, $P \leq 0.0193$). Litchi tomato yielded greater numbers of microsclerotia of the *V. dahliae* potato isolate than any other isolate in one of four trials (first trial, $P \leq 0.0149$). A *V. dahliae* isolate from tomato yielded greater numbers of microsclerotia in large crabgrass and wild oat in a second trial ($P \leq 0.0158$). Weeds, depending on the species, grown during and between potato crop rotations may increase the number of microsclerotia of the potato-aggressive isolates of *V. dahliae*.

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb., is one of the most important diseases of potato in North America (Agrios 2005; Omer et al. 2008). Microsclerotia function as the source of primary *V. dahliae* inoculum (Schnathorst 1981). Microsclerotia are usually found in the top 10 to 30 cm of soil (Jordan 1971; Taylor et al. 2005), and are distributed where infected plant residues decompose (Mace et al. 1981). Viable microsclerotia can persist in soil for 14 years (Wilhelm 1955), and may be found embedded in decaying host tissue or freely existing in soil (Mace et al. 1981).

Development of Verticillium wilt epidemics depends on the presence and number of microsclerotia in potato field soil prior to planting (Schnathorst 1981). Reducing the number of *V. dahliae* microsclerotia, or limiting their ability to germinate, is an important consideration prior to planting potato (Mace et al. 1981). Successful Verticillium wilt management hinges on the combined effects of chemical treatments (soil fumigants), suppressive soils, resistant cultivars (when available), and irrigation management (Johnson and Dung 2010; Pegg and Brady 2002). *V. dahliae* infection can occur whenever microsclerotia are present in soil, but epidemics of Verticillium wilt of potato typically arise when a minimum of 5 to 30 microsclerotia per gram of soil are present (Powelson and Rowe 1993). The disparity in Verticillium wilt severity and the number of microsclerotia in soil has been attributed to *V. dahliae* aggressiveness (Mace et al. 1981; Schnathorst 1981).

Certain *V. dahliae* isolates are considered more aggressive than other isolates when increasingly severe symptoms or greater numbers of microsclerotia are produced within one host compared with other plants in the host range (Douhan and Johnson 2001). Repeated infection of the same host by the same *V. dahliae* isolate is thought to accentuate aggressiveness. This phenomenon may occur over a relatively short period of time and within both annual and perennial crop hosts (Bhat et al. 2003; Fordyce and Green 1960).

Individual *V. dahliae* isolates that are aggressive to a particular host are called host-adapted pathotypes (Dung et al. 2013; Pegg

and Brady 2002). The term is sometimes abbreviated to pathotype for ease of explanation. Certain *V. dahliae* isolates that are grouped into pathotypes often fall into a vegetative compatibility group (VCG) (Katan 2000), although every isolate classified into a single VCG is not necessarily the same pathotype (Bhat and Subbarao 1999; Dung et al. 2013). Typing *V. dahliae* to VCG is conducted by complementation of nitrate-nonutilizing mutants that fuse with previously identified VCG tester cultures when cultured on minimal media with defined nitrogen sources (Joaquim and Rowe 1990, 1991).

Weeds present in the field are competitors for water, nutrients, sunlight, and space (Baker 1974). Hairy nightshade (*Solanum physalifolium*) at a density of one plants per 1-m row of potato can reduce U.S. No. 1 yield of ‘Russet Norkotah’ and ‘Russet Burbank’ potato by up to 27 and 10%, respectively (Hutchinson et al. 2011). One barnyard grass (*Echinochloa crus-galli*) plant per square meter of potato reduced marketable tuber yield by 19% if the barnyard grass emerged prior to the adjacent potato plants and continued to grow throughout the season in Michigan (Vangessel and Renner 1990).

Weedy hosts also can pose a problem by supporting pathogen populations (Demirci and Genc 2009; Linde et al. 2016) and by returning inoculum to the soil when residue decomposes at the end of the season (Woolliams 1966). Busch et al. (1978) emphasized that even immature weedy seedlings can host *V. dahliae* when distinctive *Verticillium* conidiophores were observed on 3-week-old seedlings. Aggressive pathotypes of a fungal pathogen may infect and persist in the environment through a potential weedy host and, possibly, maintain aggressiveness until the crop host is infected again. The hypothesis that an aggressive pathogen can persist in the environment through a weedy host was confirmed in the pathosystem involving *Rhynchosporium commune*, a fungal pathogen of weedy barley grass (*Hordeum* spp.), as well as barley (*Hordeum vulgare*). In this case, “barley grass acts as an important ancillary host to *R. commune*, harboring highly virulent pathogen types capable of transmission to barley.” This observation underlies the point that management of disease where pathogen aggressiveness can be maintained from a crop to weedy host and then back to the original crop must include an understanding of the maintenance of pathogen aggressiveness and, ultimately, control of the weed host (Linde et al. 2016).

Weedy hosts such as black nightshade and hairy nightshade are important to potato production in the Pacific Northwest because of their ability to directly compete for resources (Hutchinson et al. 2011) and serve as hosts for potato pathogens. The interaction of

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V. dahliae host-adapted pathotypes with weedy hosts has not been explored, despite the importance of aggressive isolates of *V. dahliae* to Verticillium wilt epidemics on potato. Complete understanding of the interaction of aggressive isolates of *V. dahliae* with weedy hosts is important to successful long-term management of Verticillium wilt of potato because locations where *Verticillium*-susceptible weedy hosts are prevalent in the potato field could present greater inoculum pressure from *V. dahliae* aggressive to potato in subsequent years. The objectives of this study were to (i) identify the susceptibility of 16 weeds from the Columbia basin to eight *V. dahliae* isolates and (ii) identify weedy hosts where the potato or mint pathotypes produce greater numbers of microsclerotia compared with other *V. dahliae* isolates.

Materials and Methods

Origin and preparation of *V. dahliae* isolates. *V. dahliae* isolates were previously characterized for aggressiveness to various hosts and VCG identity by Dung et al. (2013) (Tables 1 and 2). Inoculum was prepared by placing a single microsclerotium on semiselective media for *V. dahliae* (Butterfield and DeVay 1977) and allowing growth for 10 days before identification by morphology was confirmed, following the protocol from Inderbitzin et al. (2011). A subsection of the actively growing border of the colony was collected with a cork borer (7 mm in diameter) and agar disks were placed in Czapek-Dox broth (Sigma-Aldrich, St. Louis). Liquid cultures were shaken at 150 rpm at a room temperature of 21 to 23°C for 10 days. The CFU of conidial suspensions were standardized using a hemocytometer.

Quantification of *V. dahliae* CFU in weedy hosts and crops. Comparisons in the aggressiveness of *V. dahliae* isolates to 16 weedy hosts and three crop hosts (Tables 1 and 2) were made in four trials in a greenhouse from 2014 to 2016. A broad range of weeds was evaluated for *V. dahliae* susceptibility in the first two trials. The third and fourth trials focused on nightshades to further explore the interaction of the potato pathotype of *V. dahliae* and weedy nightshade hosts. In total, eight *V. dahliae* isolates were used in all four trials (Tables 1 and 2), with one noninoculated control treatment. Russet Norkotah potato was planted as a positive control in the third and fourth trials because of susceptibility to infection by *V. dahliae*, and the potato pathotype of *V. dahliae* was expected to produce more microsclerotia than other isolates within potato. ‘Night Shadow’ eggplant was expected to be susceptible to all *V. dahliae* isolates used in these experiments and was planted in the second, third, and fourth trials to demonstrate the ability of all isolates used in the study to infect a universally susceptible host.

Seed of the 16 weeds and two crop hosts (Tables 1 and 2) were germinated on moistened filter paper to determine whether seed was free

from *V. dahliae*. Germinated seed was visually assessed for the presence or absence of disease symptoms, sporulation, and microsclerotia associated with *V. dahliae*. ‘Alturas’, Russet Norkotah, and ‘Ranger Russet’ potato tubers were assayed for the absence of *V. dahliae* by slicing an approximately 0.5-cm-thick segment from the stolon end of the tuber, which was surface sterilized by submerging in 10% sodium hypochlorite (NaOCl, 6.0%; Independent Marketing Alliance, Houston, TX) for 3 min, rinsed in deionized water, and blotted dry. The segment from the stolon end of the tuber then was placed on NPX and incubated at room temperature (21 to 23°C) for 10 days, after which the presence or absence of *V. dahliae* microsclerotia was confirmed through visual observation of microsclerotia and sporulation using an Olympus SD-ILK dissecting microscope at $\times 7$ to $\times 20$ magnification (Olympus Optical Ltd., Tokyo). Potato tubers that did not test positive for microsclerotia were planted in 3.79-liter pots with soilless potting medium (Sunshine Advanced Growing Mix number 4; Sun Gro, Agawam, MA) and allowed to grow for 1 month. Soilless potting medium was assumed to be free of *V. dahliae*.

The experiment had 18 levels for host and 8 levels for pathogen (Tables 1 and 2) and pots were arranged in a completely randomized design with three replicates. Each potential weedy or crop host in each individual pot (experimental unit) was inoculated by dipping roots in a conidial suspension of a single *V. dahliae* isolate (Tables 1 and 2). Seedlings no greater than 8 cm in length were inoculated by submerging the hypocotyl and primary root in an agitated conidial suspension of one *V. dahliae* isolate at a concentration of approximately 1.0×10^6 CFU/ml for 2 to 3 s. Potential weedy and crop host seedlings were then directly transplanted into moistened soilless potting medium in 3.79-liter pots. Seedlings from the noninoculated control treatment were kept separate from seedlings inoculated with *V. dahliae* during the inoculation step in order to prevent cross-contamination. Seedlings that were inoculated with *V. dahliae* or were noninoculated were kept in the same greenhouse for the duration of the experiment. The greenhouse was maintained at a daytime average temperature of approximately 23°C and a nighttime average temperature of 15.5°C, with no supplemental lighting.

Plants were visually inspected for symptoms of Verticillium wilt once a week for 4 months postinoculation. Plants were harvested after 4 months of growth in the greenhouse and soilless potting mix was washed off; then, plants were blotted dry and slowly dried for 1 month at ambient temperatures of 21 to 24°C. Slow drying was necessary in order to facilitate the formation of microsclerotia and desiccation of conidia and hyphae. A dried stem section from the crown to upward of 16 cm and a root section from the crown downward to approximately 9 cm was harvested from each plant. Dried stems or roots were ground with an electric coffee grinder (Secura, Appleton, WA) until the particle size was approximately less than 4 mm². The removable cup from the grinder, as well as the lid, were sprayed with a mixture of 70% ethanol and 30% deionized water between plant samples in order to sterilize grinder surfaces contacted by plant matter.

Table 1. Weed plants and crop hosts and number of trials used to determine microsclerotia production when potential host plants were inoculated with *Verticillium dahliae* host-adapted isolates in four greenhouse trials from 2014 to 2016

Plant common name	Latin binomial	Number of trials
Annual bluegrass	<i>Poa annua</i>	2
Annual sowthistle	<i>Sonchus oleraceus</i>	2
Barnyard grass	<i>Echinochloa crusgalli</i>	2
Bittersweet nightshade	<i>Solanum dulcamara</i>	2
Black nightshade	<i>S. nigrum</i>	4
Common lambsquarters	<i>Chenopodium album</i>	2
Downy brome	<i>Bromus tectorum</i>	2
Eastern black nightshade	<i>S. ptycanthum</i>	2
Eggplant (Night Shadow)	<i>S. melongena</i>	3
Green foxtail	<i>Setaria viridis</i>	2
Hairy nightshade	<i>Solanum physalifolium</i>	2
Large crabgrass	<i>Digitaria sanguinalis</i>	2
Litchi tomato	<i>S. sisymbriifolium</i>	2
Pigweed powell	<i>Amaranthus powellii</i>	2
Tumble pigweed	<i>A. albus</i>	1
Potato (Russet Norkotah)	<i>S. tuberosum</i>	2
Rattail fescue	<i>Vulpia myuros</i>	2
Wild oat	<i>Avena fatua</i>	3

Table 2. Isolate characteristics of *Verticillium dahliae* used to determine microsclerotia production when potential host plants were inoculated with *V. dahliae* host-adapted isolates in four greenhouse trials from 2014 to 2016

<i>V. dahliae</i> isolate	VCG ^a	Pathotype	Host ^b
111	2 B	Mint	Mint
155	2 B	Mint	Mint
381	2 A/B	...	Watermelon
461	2	...	Tomato
625	2 B	...	Sugar Beet
653	4 A	Potato	Potato
SF	2 A	...	Sunflower
VMD-4	2 A/B	...	Tomato

^a Vegetative compatibility group.

^b Host of origin for the *V. dahliae* isolate. Isolates originating from potato or mint are the potato or mint pathotypes, respectively.

The number of *V. dahliae* CFU for each dried plant part was determined by placing 1 g of dried plant stem or root on NPX and counting the number of colonies after 10 days using an Olympus SD-ILK dissecting microscope at $\times 7$ to $\times 20$ magnification. *V. dahliae* colonies were identified and differentiated from other fungi using the protocol outlined by Inderbitzin et al. (2011). In brief, colonies were expected to grow on the semiselective medium (NPX), not produce the yellow pigment flavexudan in culture, produce irregularly shaped and melanized microsclerotia, and, finally, produce the characteristic verticillate conidiophores with slimy, terminal ameroconidia.

Data analysis. Differences in the number of observed *V. dahliae* microsclerotia for each *V. dahliae* isolate on a weed or crop host were determined by permutational multivariate analysis of variance (PERMANOVA; Plymouth Routine In Multivariate Ecological Research, PRIMER-E Ltd., v7, Lutton, Ivybridge, UK). PERMANOVA has been employed for testing hypotheses with ecological data sets that do not satisfy parametric assumptions and nonparametric analyses (Anderson 2001). PERMANOVA also has been used recently to test whether both incidence of *V. dahliae*-infected stems and microsclerotia number differed among or within different field crops (Wheeler and Johnson 2016). PERMANOVA was selected for these data because the use of nonparametric permutation techniques do not require parametric assumptions, which is important because not every weedy host interacted with every *V. dahliae* isolate and resulted in zero-rich data. The mean number of microsclerotia recovered from the same plant (experimental unit) was determined by averaging microsclerotia counts from stem and roots in order to remove plant part as a factor in the analysis and reduce the number of factors for PERMANOVA testing. PERMANOVA tests operated under the null hypotheses that the number of microsclerotia was not different within weedy or crop hosts between the eight *V. dahliae* isolates (Tables 1 and 2) and one noninoculated reference control. PERMANOVA conditions are important to note for the purposes of repeatability, and were based upon a $\log(X + 1)$ transformation, followed by the creation of a Bray-Curtis dissimilarity matrix; PERMANOVA was conducted with 9,999 permutations under a reduced model. Monte Carlo tests were employed to calculate *P* values for pairwise comparisons when there were fewer than 100 unique permutations (Anderson et al. 2001). Familywise type I error (false discoveries) can be a problem for numerous pairwise comparisons but this was controlled using a false discovery rate (FDR) (Benjamini and Hochberg 1995; Glickman et al. 2014) to adjust PERMANOVA *P* values to differentiate truly significant differences from false positives, not unlike a Bonferroni correction. The *P* value indicative of significance will vary with each trial in this experiment because the FDR corrects the *P* value for a specific data set.

The *V. dahliae* isolate with the greatest microsclerotia counts within one weed or crop was assigned whenever pairings of one weed and isolate was greater than six of seven comparisons between *V. dahliae* isolates within that same weed. One *V. dahliae* isolate was not expected to produce greater numbers of microsclerotia than every isolate evaluated because multiple isolates came from the same host or were from the same pathotype and, theoretically, could produce similar numbers of microsclerotia within the same type of weedy host. Specific comparisons between microsclerotia counts of any two isolates within a weed were not conducted because the goal was to identify which isolate produced more microsclerotia within a weed compared with a range of isolates.

Results

Quantification of *V. dahliae* CFU in weedy hosts and crops. All weed and crop hosts evaluated were infected by at least one isolate of *V. dahliae*, as made evident by microsclerotia produced in the hosts (Tables 3, 4, 5, and 6). *V. dahliae* infections of weedy hosts often yielded small numbers of observed microsclerotia (< 5 microsclerotia/g of plant) (Tables 3, 4, 5, and 6). Differences in *V. dahliae* microsclerotia production by *V. dahliae* isolate existed for some but not all weedy hosts (Tables 3, 4, 5, and 6).

The FDR-adjusted *P* value for significance was set at $P \leq 0.0149$ for the first trial. A significant host–isolate interaction ($P < 0.0001$) was detected, necessitating comparisons of cell mean microsclerotia counts within the factor of host. The *V. dahliae* potato pathotype (isolate 653; Tables 1 and 2) produced more microsclerotia within litchi tomato than all *V. dahliae* isolates ($P \leq 0.0149$), except when compared with the tomato isolate (isolate 461, $P = 0.0473$; Table 3). A *V. dahliae* isolate from tomato (isolate 461) infected tumble pigweed, resulting in greater numbers of *V. dahliae* microsclerotia than other *V. dahliae* isolates ($P \leq 0.0149$), except when compared with the potato pathotype (isolate 653, $P = 0.0884$, Table 3). No symptoms of Verticillium wilt were observed at any time in any of the weedy hosts evaluated during this trial (data not shown).

The FDR-adjusted *P* value for significance was set at $P \leq 0.0158$ for the second trial. A significant host–isolate interaction ($P < 0.0001$) was detected, necessitating comparisons of cell mean microsclerotia counts within the factor of host. The *V. dahliae* potato pathotype (isolate 653; Tables 1 and 2) produced more microsclerotia within black nightshade than other *V. dahliae* isolates ($P \leq 0.0158$; Table 4). The *V. dahliae* potato pathotype (isolate 653) unexpectedly produced more microsclerotia within eggplant than other *V. dahliae* isolates ($P \leq 0.0158$), except when compared with the isolate SF ($P = 0.1572$). Inoculation of large crabgrass with a *V. dahliae*

Table 3. Mean $\pm$ standard error for the number of microsclerotia from eight isolates obtained from 13 weeds in the greenhouse when inoculated with *Verticillium dahliae* in the first trial

Host	Isolate ^a								Control ^d
	111 ^b	155 ^b	381	461	625	653 ^c	SF	VMD-4	
Annual bluegrass	0.5 $\pm$ 0.3	6.0 $\pm$ 3.2	0.2 $\pm$ 0.2	19.2 $\pm$ 5.4	5.8 $\pm$ 2.3	3.0 $\pm$ 1.1	15.3 $\pm$ 11.6	1.5 $\pm$ 0.9	0.0 $\pm$ 0.0
Annual sowthistle	23.7 $\pm$ 16.2	3.3 $\pm$ 2	2.8 $\pm$ 0.7	2.0 $\pm$ 1.3	11.5 $\pm$ 9.3	2.3 $\pm$ 1.1	11.5 $\pm$ 10.3	0.0 $\pm$ 0.0	0.3 $\pm$ 0.2
Barnyard grass	0.7 $\pm$ 0.4	5.5 $\pm$ 3.3	0.3 $\pm$ 0.2	0.3 $\pm$ 0.3	0.3 $\pm$ 0.3	0.2 $\pm$ 0.2	5.0 $\pm$ 1.8	0.7 $\pm$ 0.4	0.0 $\pm$ 0.0
Black nightshade	3.5 $\pm$ 0.5	9.8 $\pm$ 1.2	8.0 $\pm$ 2.0	6.8 $\pm$ 3.5	5.8 $\pm$ 1.5	16.5 $\pm$ 4.2	8.2 $\pm$ 2.0	12.8 $\pm$ 6.8	0.0 $\pm$ 0.0
Common lambsquarters	1.0 $\pm$ 1.0	6.5 $\pm$ 2.2	12.8 $\pm$ 9.2	44.0 $\pm$ 20.8	16.3 $\pm$ 8.8	0.3 $\pm$ 0.3	7.0 $\pm$ 0.9	5.7 $\pm$ 1.3	0.2 $\pm$ 0.2
Downy brome	2.7 $\pm$ 1.3	0.5 $\pm$ 0.5	100.2 $\pm$ 62.7	22.5 $\pm$ 14.0	7.3 $\pm$ 4.2	48.3 $\pm$ 26.0	34.3 $\pm$ 30.4	26.8 $\pm$ 13.2	0.0 $\pm$ 0.0
Green foxtail	6.5 $\pm$ 3.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	13.7 $\pm$ 9.2	21.5 $\pm$ 13.9	1.2 $\pm$ 0.7	9.3 $\pm$ 4.4	2.7 $\pm$ 1.5	0.0 $\pm$ 0.0
Large crabgrass	0.2 $\pm$ 0.2	2 $\pm$ 0.9	1.2 $\pm$ 0.7	15.5 $\pm$ 11.1	10.2 $\pm$ 4.8	0.8 $\pm$ 0.7	2.8 $\pm$ 1.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Litchi tomato	0.5 $\pm$ 0.3	0.2 $\pm$ 0.2	2.3 $\pm$ 1.7	6.8 $\pm$ 2	1.3 $\pm$ 0.8	5.8 $\pm$ 0.9*	2.3 $\pm$ 0.6	1.3 $\pm$ 0.7	0.0 $\pm$ 0.0
Pigweed powell	1.0 $\pm$ 0.6	0.3 $\pm$ 0.2	1.3 $\pm$ 0.6	1.8 $\pm$ 0.2	3.8 $\pm$ 2.5	23.5 $\pm$ 19.9	9.3 $\pm$ 2.0	1.7 $\pm$ 1.1	0.0 $\pm$ 0.0
Tumble pigweed	5.2 $\pm$ 1.3	2.8 $\pm$ 2.8	0.0 $\pm$ 0.0	12.3 $\pm$ 1.8*	1.2 $\pm$ 0.5	29 $\pm$ 24.3	2.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0
Rattail fescue	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	4.7 $\pm$ 3.0	0.0 $\pm$ 0.0	2.3 $\pm$ 0.7	0.0 $\pm$ 0.0	3.2 $\pm$ 2.5	0.0 $\pm$ 0.0
Wild oat	48.7 $\pm$ 41.5	0.0 $\pm$ 0.0	113.7 $\pm$ 47.2	38.2 $\pm$ 14.8	2.8 $\pm$ 1.8	33.2 $\pm$ 13.2	6.5 $\pm$ 3.4	70 $\pm$ 19.9	0.0 $\pm$ 0.0

^a Data in bold (*) indicate *V. dahliae* pathotype or isolate with greatest microsclerotia counts. Pairwise comparisons were considered significant at corrected $P \leq 0.0149$.

^b Mint pathotype of *V. dahliae*.

^c Potato pathotype of *V. dahliae*.

^d Noninoculated control.

isolate from tomato (isolate 461) caused infections with significantly greater numbers of *V. dahliae* microsclerotia than other *V. dahliae* isolates ($P \leq 0.0158$), except when compared with isolate SF ($P = 0.3766$; Table 4). Similarly, a *V. dahliae* isolate from tomato (isolate 461) caused infections in wild oat with significantly greater numbers

of *V. dahliae* microsclerotia than other *V. dahliae* isolates ($P \leq 0.0158$), except when compared with isolate 381 ($P = 0.0655$; Table 4). No symptoms of Verticillium wilt were observed at any time in any of the weedy hosts evaluated during this trial (data not shown).

Table 4. Mean $\pm$ standard error for the number of microsclerotia from eight isolates obtained from 12 weeds and one crop in the greenhouse when inoculated with *Verticillium dahliae* in the second trial

Host	Isolate ^a								Control ^d
	111 ^b	155 ^b	381	461	625	653 ^c	SF	VMD-4	
Annual bluegrass	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.7 $\pm$ 0.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.5 $\pm$ 0.2	29.5 $\pm$ 9.9	8.8 $\pm$ 5.0	0.0 $\pm$ 0.0
Annual sowthistle	0.2 $\pm$ 0.2	0.8 $\pm$ 0.4	12.0 $\pm$ 6.8	0.0 $\pm$ 0.0	4.2 $\pm$ 1.0	2.2 $\pm$ 0.6	3.5 $\pm$ 1.6	1.0 $\pm$ 0.4	0.0 $\pm$ 0.0
Barnyard grass	1.3 $\pm$ 0.2	0.0 $\pm$ 0.0	0.7 $\pm$ 0.2	1.7 $\pm$ 0.3	0.0 $\pm$ 0.0	17.2 $\pm$ 13.3	3.2 $\pm$ 0.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Black nightshade	3.2 $\pm$ 1.4	2.0 $\pm$ 0.3	0.5 $\pm$ 0.2	3.0 $\pm$ 2.0	0.3 $\pm$ 0.2	25.0 $\pm$ 13.6*	3.3 $\pm$ 1.2	5.7 $\pm$ 0.7	0.0 $\pm$ 0.0
Common lambsquarters	10.7 $\pm$ 3.1	7.8 $\pm$ 5.1	1.8 $\pm$ 0.9	25.8 $\pm$ 15.4	9 $\pm$ 1.9	0.7 $\pm$ 0.3	18.0 $\pm$ 8.7	2.8 $\pm$ 0.8	0.0 $\pm$ 0.0
Downy brome	5.0 $\pm$ 2.0	0.0 $\pm$ 0.0	1.3 $\pm$ 0.7	22.5 $\pm$ 11.8	0.0 $\pm$ 0.0	2.8 $\pm$ 1.3	74.0 $\pm$ 33.4	0.8 $\pm$ 0.5	0.0 $\pm$ 0.0
Eggplant	0.0 $\pm$ 0.0	8.0 $\pm$ 0.9	2.8 $\pm$ 0.8	6.2 $\pm$ 2.5	4.3 $\pm$ 1.5	15.0 $\pm$ 1.2*	29.5 $\pm$ 16.8	2.2 $\pm$ 1.1	0.0 $\pm$ 0.0
Green foxtail	0.2 $\pm$ 0.2	5.2 $\pm$ 5.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	26.8 $\pm$ 17.2	2.7 $\pm$ 0.3	0.0 $\pm$ 0.0
Large crabgrass	0.0 $\pm$ 0.0	0.7 $\pm$ 0.4	0.8 $\pm$ 0.8	6.3 $\pm$ 2.2*	0.0 $\pm$ 0.0	1.0 $\pm$ 0.5	25.7 $\pm$ 20.2	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0
Litchi tomato	2.7 $\pm$ 0.7	3.5 $\pm$ 2.4	3.0 $\pm$ 0.8	0.0 $\pm$ 0.0	8.2 $\pm$ 2.8	10.7 $\pm$ 2.6	5.8 $\pm$ 2.9	5.2 $\pm$ 3.1	0.0 $\pm$ 0.0
Pigweed powell	2.0 $\pm$ 0.6	0.0 $\pm$ 0.0	2.2 $\pm$ 1.0	2.0 $\pm$ 1.5	0.3 $\pm$ 0.3	0.0 $\pm$ 0.0	1.5 $\pm$ 0.3	0.7 $\pm$ 0.4	0.0 $\pm$ 0.0
Rattail fescue	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Wild oat	5.2 $\pm$ 1.7	1.0 $\pm$ 0.6	68.2 $\pm$ 46.7	101.5 $\pm$ 22.5*	6.3 $\pm$ 2.2	58.5 $\pm$ 3.2	2.7 $\pm$ 1.4	0.5 $\pm$ 0.3	0.0 $\pm$ 0.0

^a Data in bold (*) indicate *V. dahliae* pathotype or isolate with greatest microsclerotia counts. Pairwise comparisons were considered significant at corrected $P \leq 0.0158$.

^b Mint pathotype of *V. dahliae*.

^c Potato pathotype of *V. dahliae*.

^d Noninoculated control.

Table 5. Mean $\pm$ standard error for the number of microsclerotia from eight isolates obtained from seven weeds and two crops in the greenhouse when inoculated with *Verticillium dahliae* in the third trial

Host	Isolate ^a								Control ^d
	111 ^b	155 ^b	381	461	625	653 ^c	SF	VMD-4	
Black nightshade	10.3 $\pm$ 1.1	7.2 $\pm$ 0.2	7.1 $\pm$ 0.6	9.8 $\pm$ 1.8	5.1 $\pm$ 0.7	14.0 $\pm$ 0.7*	7.0 $\pm$ 0.5	3.3 $\pm$ 0.3	0.0 $\pm$ 0.0
Bittersweet nightshade	2.1 $\pm$ 0.3	5.3 $\pm$ 0.6	1.3 $\pm$ 0.2	3.6 $\pm$ 0.5	6.0 $\pm$ 0.8	1.7 $\pm$ 0.2	3.6 $\pm$ 0.6	2.6 $\pm$ 0.3	0.0 $\pm$ 0.0
Eastern black nightshade	74.0 $\pm$ 10.2	28.4 $\pm$ 5.4	75.7 $\pm$ 8.2	63.6 $\pm$ 6.5	5.1 $\pm$ 0.6	53.8 $\pm$ 1.8	40.7 $\pm$ 8.8	8.3 $\pm$ 1.6	0.0 $\pm$ 0.0
Eggplant	1.4 $\pm$ 0.2	2.9 $\pm$ 0.4	5.2 $\pm$ 1.0	37.5 $\pm$ 1.7*	17.2 $\pm$ 2.3	10.1 $\pm$ 0.5	11.0 $\pm$ 1.7	2.1 $\pm$ 0.5	0.0 $\pm$ 0.0
Hairy nightshade	183.6 $\pm$ 24.5	15.1 $\pm$ 1.1	27.9 $\pm$ 7.6	210.3 $\pm$ 14.8	169.0 $\pm$ 10.6	102.9 $\pm$ 10.1	38.5 $\pm$ 2.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Henbit	66.5 $\pm$ 10.5	7.4 $\pm$ 0.8	65.1 $\pm$ 8.7	101.4 $\pm$ 4.2*	3.4 $\pm$ 1.7	3.4 $\pm$ 0.8	49.4 $\pm$ 5.8	11.7 $\pm$ 1.7	0.0 $\pm$ 0.0
Litchi tomato	2.0 $\pm$ 0.2	1.3 $\pm$ 0.1	2.9 $\pm$ 0.4	6.3 $\pm$ 0.4	4.1 $\pm$ 0.4	9.5 $\pm$ 1.3	2.0 $\pm$ 0.2	1.9 $\pm$ 4.2	0.0 $\pm$ 0.0
Potato	8.5 $\pm$ 1.5	17.4 $\pm$ 3.2	2.3 $\pm$ 0.7	8.2 $\pm$ 1.5	42.5 $\pm$ 5.9	212.8 $\pm$ 26.9*	51.4 $\pm$ 6.2	7.5 $\pm$ 0.7	0.0 $\pm$ 0.0
Wild oat	1.7 $\pm$ 0.9	2.0 $\pm$ 0.4	0.8 $\pm$ 0.4	185.5 $\pm$ 19.7*	2.4 $\pm$ 0.3	2.7 $\pm$ 0.3	2.1 $\pm$ 0.4	10.7 $\pm$ 2.7	0.0 $\pm$ 0.0

^a Data in bold (*) indicate *V. dahliae* pathotype or isolate with greatest microsclerotia counts. Pairwise comparisons were considered significant at corrected $P \leq 0.0264$.

^b Mint pathotype of *V. dahliae*.

^c Potato pathotype of *V. dahliae*.

^d Noninoculated control.

Table 6. Mean $\pm$ standard error for the number of microsclerotia from eight isolates obtained from 12 weeds and one crop in the greenhouse when inoculated with *Verticillium dahliae* in the fourth trial

Host	Isolate ^a								Control ^d
	111 ^b	155 ^b	381	461	625	653 ^c	SF	VMD-4	
Black nightshade	5.7 $\pm$ 1.7	2.3 $\pm$ 0.8	7.3 $\pm$ 2.0	17.3 $\pm$ 0.5	9.7 $\pm$ 2.9	52.0 $\pm$ 1.2*	8.3 $\pm$ 0.2	3.7 $\pm$ 1.6	0.0 $\pm$ 0.0
Bittersweet nightshade	3.7 $\pm$ 1.3	1.0 $\pm$ 0.3	6.7 $\pm$ 0.4	0.0 $\pm$ 0.0	4.0 $\pm$ 2.0	10.0 $\pm$ 3.0	10.3 $\pm$ 0.5	12.7 $\pm$ 5.2	0.0 $\pm$ 0.0
Eastern black nightshade	51.0 $\pm$ 25.5	10.3 $\pm$ 5.0	0.0 $\pm$ 0.0	43.3 $\pm$ 3.6	10.0 $\pm$ 4.9	24.7 $\pm$ 2.5	0.0 $\pm$ 0.0	13.0 $\pm$ 5.2	0.0 $\pm$ 0.0
Eggplant	5.3 $\pm$ 2.0	18.0 $\pm$ 5.8	14.6 $\pm$ 4.7	39.0 $\pm$ 6.5	31.7 $\pm$ 10.5	17.3 $\pm$ 6.5	32.0 $\pm$ 6.8	1.0 $\pm$ 0.5	0.0 $\pm$ 0.0
Hairy nightshade	153.3 $\pm$ 55.6	20.3 $\pm$ 1.0	44.0 $\pm$ 13.4	294.3 $\pm$ 35.7	149.3 $\pm$ 25.4	55.7 $\pm$ 11.8	36.0 $\pm$ 13.0	37.3 $\pm$ 5.6	0.0 $\pm$ 0.0
Henbit	14.0 $\pm$ 6.9	4.3 $\pm$ 1.3	75.7 $\pm$ 33.4	67.0 $\pm$ 30.1	0.0 $\pm$ 0.0	5.3 $\pm$ 2.1	2.7 $\pm$ 1.5	7.0 $\pm$ 0.6	0.0 $\pm$ 0.0
Litchi tomato	1.3 $\pm$ 0.4	2.7 $\pm$ 0.5	2.3 $\pm$ 0.5	7.3 $\pm$ 2.5	5.0 $\pm$ 1.7	15.0 $\pm$ 1.5	2.3 $\pm$ 0.2	3.0 $\pm$ 0.3	0.0 $\pm$ 0.0
Potato	42.7 $\pm$ 6.2	14.0 $\pm$ 0.3	21.7 $\pm$ 5.6	50.3 $\pm$ 9.9	70.7 $\pm$ 5.3	160.1 $\pm$ 15.8*	34.3 $\pm$ 1.1	35.7 $\pm$ 9.6	0.0 $\pm$ 0.0

^a Data in bold (*) indicate *V. dahliae* pathotype or isolate with greatest microsclerotia counts. Pairwise comparisons were considered significant at corrected $P \leq 0.0193$.

^b Mint pathotype of *V. dahliae*.

^c Potato pathotype of *V. dahliae*.

^d Noninoculated control.

The FDR-adjusted P value for significance was set at $P \leq 0.0264$ for the third trial. A significant host–isolate interaction ($P < 0.0001$) was detected, necessitating comparisons of cell mean microsclerotia counts within the factor of host. The *V. dahliae* potato pathotype (isolate 653; Tables 1 and 2) produced more microsclerotia within black nightshade than other *V. dahliae* isolates ($P \leq 0.0264$), except when compared with isolate 461 ($P = 0.0368$; Table 5). The *V. dahliae* potato pathotype produced more microsclerotia within potato than other *V. dahliae* isolates ($P \leq 0.0264$). Russet Norkotah potato was used as a universally susceptible control in this experiment but it was expected that the potato would also be more susceptible to the potato pathotype of *V. dahliae* (Table 5). A *V. dahliae* isolate from tomato (isolate 461) infected henbit with significantly greater numbers of *V. dahliae* microsclerotia than other *V. dahliae* isolates ($P \leq 0.0264$; Table 5). A *V. dahliae* isolate from tomato (isolate 461; Tables 1 and 2) caused infections in wild oat with significantly greater numbers of *V. dahliae* microsclerotia than other *V. dahliae* isolates ($P \leq 0.0264$; Table 5). No symptoms of Verticillium wilt were observed at any time in any of the weedy hosts evaluated during this trial (data not shown).

The FDR-adjusted P value for significance was set at $P \leq 0.0193$ for the fourth trial. A significant host–isolate interaction ($P < 0.0001$) was detected, necessitating comparisons of cell mean microsclerotia counts within the factor of host. The *V. dahliae* potato pathotype (isolate 653) produced more microsclerotia within black nightshade than other *V. dahliae* isolates ($P \leq 0.0193$; Table 6). The *V. dahliae* potato pathotype produced more microsclerotia within potato than other *V. dahliae* isolates ($P \leq 0.0193$; Table 6). No symptoms of Verticillium wilt were observed at any time in any of the weedy hosts evaluated during this trial (data not shown).

The *V. dahliae* potato pathotype (isolate 653) produced more microsclerotia than other *V. dahliae* isolates within black nightshade in three of four trials (Fig. 1). The *V. dahliae* isolate from tomato (isolate 461) produced more microsclerotia than other *V. dahliae* isolates

within wild oat in two of three trials (Fig. 1). That same *V. dahliae* isolate from tomato (isolate 461) caused infections with significantly greater numbers of *V. dahliae* microsclerotia than other *V. dahliae* isolates in tumble pigweed (first trial), large crabgrass (second trial), and henbit (third trial) (Fig. 1) but only in one trial each. Other nightshade weedy hosts such as bittersweet nightshade did not produce many microsclerotia when infected with any of these *V. dahliae* isolates in either trial (<5 microsclerotia for one of eight in the third trial and four of eight in the fourth trial; Tables 5 and 6). Conversely, weedy hosts such as eastern black nightshade and hairy nightshade were susceptible to infection by six of eight and all eight *V. dahliae* isolates, respectively, although no one isolate produced more microsclerotia than the seven other isolates (Tables 5 and 6). However, both hairy and eastern black nightshade were susceptible to infection by the *V. dahliae* potato pathotype (isolate 653; Tables 5 and 6), as was made evident by numerous microsclerotia recovered from the dried tissues.

Discussion

A broad range of potential weedy hosts was evaluated for *V. dahliae* susceptibility in the first two greenhouse trials. The third and fourth trials focused on nightshades because of the interaction of black nightshade with the potato pathotype of *V. dahliae*. Every weed tested in this study was confirmed (or reconfirmed) as a host for *V. dahliae*, as indicated by the recovery of *V. dahliae* from plants grown in greenhouse settings. Detection of significant crop–isolate interactions in all four trials for mean microsclerotia counts supports the existence of differential aggressiveness for isolates of *V. dahliae*.

Symptoms of Verticillium wilt such as chlorosis, stunting, and premature senescence (Mace et al. 1981) were not observed in any of the weedy hosts evaluated within any of the four trials despite the presence of microsclerotia in infected stems. *V. dahliae* infections that lack symptoms have been described previously (Thanassouloupoulos et al. 1981) and are referred to as asymptomatic infections (Wheeler

Fig. 1. Summary of the number of trials where a *Verticillium dahliae* pathotype or isolate was more aggressive than the other isolates. Aggressiveness (differential effect of pathotype) was determined by a greater number of microsclerotia produced by one isolate than the other isolates for a specific weedy host. The host of origin or the pathotype of the *V. dahliae* isolate is written within or above each bar, and the weedy host evaluated is along the horizontal axis.

and Johnson 2016; Woolliams 1966). Asymptomatic *V. dahliae* infections have been noted previously from the recovery of *V. dahliae* from susceptible crops in rotation with potato (Wheeler and Johnson 2016), some weedy hosts (Woolliams 1966), and even monocot hosts such as barley (Mol 1995).

The CFU observed on semiselective media (NPX) were likely derived from microsclerotia of *V. dahliae* instead of conidia or hyphae because plant samples were dried before grinding. The absence of *V. dahliae* infection in most of the weedy hosts that were not inoculated with any *V. dahliae* isolate (nontreated controls) in each trial suggests that potting medium or seedlings planted were generally free of *V. dahliae* microsclerotia or infected plant material. The 0.2 to 0.3 mean microsclerotia that were observed in the first trial were few in number and unlikely to confound microsclerotia counts from plants inoculated with a particular *V. dahliae* isolate.

The *V. dahliae* potato pathotype produced more microsclerotia than other isolates when infecting black nightshade in three of four trials. Eastern black nightshade and hairy nightshade were generally susceptible to most of the *V. dahliae* isolates, including the potato pathotype, but no particular isolate produced more microsclerotia than the other isolates as a result of infection. Weedy hosts such as black nightshade and hairy nightshade could increase the microsclerotia of potato pathotype isolates of *V. dahliae* present in the field. These potato-aggressive *V. dahliae* isolates are likely to persist between potato rotations provided no *Verticillium* control practice eliminates them or weed management eliminates potential hosts.

This is not the first report of *V. dahliae* infections on the monocotyledonous plant hosts. Previous reports suggest that new inoculum of *V. dahliae* can form in monocot stems and roots such as barley (Mathre 1989; Schnathorst 1981; Wheeler and Johnson 2016). Monocotyledonous weeds such as wild oat have also been reported as hosts for *V. dahliae* (Thanassouloupoulos et al. 1981). Although interesting, these observations of increased microsclerotia as a result of infection with isolate 461 (from tomato) in wild oat and large crabgrass did not occur in every trial that they were included. Microsclerotia from the potato pathotype of *V. dahliae* were observed also in wild oat, indicating that the potato pathotype can infect wild oat; however, these infections did not produce significantly greater numbers of microsclerotia than all the other isolates infecting wild oat. The possibility for potato-aggressive isolates of *V. dahliae* to persist through infections of wild oat exists and control of such weeds should be included in managing *Verticillium* wilt of potato. This effect of monocot susceptibility to *V. dahliae* may be inconsistent in the field and depends on the susceptibility of the individual plant in question, therefore explaining why the result was not consistent across all trials.

Eggplant and potato were planted as positive controls that were susceptible to infection by *V. dahliae*, and some isolates were expected to produce more microsclerotia when infecting these hosts. The potato pathotype consistently produced more microsclerotia than other isolates of *V. dahliae* within potato, as anticipated. Eggplant was susceptible to all *V. dahliae* isolates used in these experiments, although one isolate from tomato (isolate 461) produced more microsclerotia than other isolates in one of three trials, while the *V. dahliae* potato pathotype produced more microsclerotia also in one of three trials. The disparity in which *V. dahliae* isolate was aggressive reflects the overall susceptibility of eggplant to all of these different *V. dahliae* isolates, in that no consistent trend emerged across the three trials that eggplant was included.

The weeds used in this experiment were not expected to be genetically uniform because the seed used to produce them were from open-pollinated plants. The difference in *V. dahliae* susceptibility in these weedy hosts, especially to different pathotypes, may have varied between years due to the genetic variability of the seed. Each plant evaluated in each trial was genetically distinct and possibly more or less susceptible to the given *V. dahliae* isolate. Even though the weeds were genetically diverse, consistent observations of increased microsclerotia production by aggressive isolates of *V. dahliae* within a weedy host means that these weeds could pose a potential threat to potato production.

The recurring observation of black nightshade being susceptible to the potato pathotype of *V. dahliae* is important in managing *Verticillium* wilt of potato because locations where black nightshade are prevalent in the potato field may have greater inoculum pressure from *V. dahliae* aggressive to potato in subsequent years. Linde et al. (2016) highlighted that management of disease where pathogen aggressiveness can be maintained from a crop to a weedy host must include an understanding of two important factors: (i) the maintenance of pathogen aggressiveness and (ii) ultimately, control of the weed host. These greenhouse trials have highlighted how black nightshade could be an important source of microsclerotia from the potato pathotype of *V. dahliae*, which is the first factor Linde et al. (2016) described. Further research can link the field location of weedy hosts such as black nightshade and the distribution of *V. dahliae* microsclerotia in potato fields. The goal of improving *Verticillium* wilt disease management by simultaneously decreasing weedy competitors and eliminating alternative hosts for potato-aggressive isolates of *V. dahliae* could then be better achieved.

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