

Susceptibility of Litchi Tomato and Weedy Hosts to Crop-aggressive Isolates of *Verticillium dahliae* and *Colletotrichum coccodes*

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Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae*, is one of the most important diseases of potato in North America (Agrios 2005; Omer et al. 2008). Development of Verticillium wilt outbreaks depends on the number of microsclerotia in potato field soil prior to planting, the presence of a susceptible host, and an environment conducive to infection (Schnathorst 1981). Reducing the number of *V. dahliae* microsclerotia, or limiting their ability to germinate, is an important consideration prior to planting potatoes (Mace, Bell, and Beckman 1981).

Successful Verticillium wilt management hinges on the combined effects of chemical treatments (soil fumigants), suppressive soils, the use of resistant or moderately resistant cultivars, and irrigation management (where applicable) (Johnson and Dung 2010; Pegg and Brady 2002). *Verticillium 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 viable 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, Bell, and Beckman 1981; Schnathorst 1981).

Certain *V. dahliae* isolates are considered more aggressive than other isolates when more severe symptoms or greater numbers of microsclerotia are produced within one host compared to other plants (Douhan and Johnson 2001). Repeated infection of the same host by the same *V. dahliae* isolate is thought to accentuate aggressiveness (Bhat and Subbarao 1999). Individual *V. dahliae* isolates that are aggressive to a particular host, such as potato, are called host-adapted pathotypes (Dung et al. 2013; Pegg and Brady 2002), and is sometimes abbreviated to pathotype for ease of explanation.

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 and space (Alvarez and Hutchinson 2005). These weedy hosts are also known hosts for *V. dahliae* (Woolliams 1966). An understanding of the interaction of aggressive isolates of *V. dahliae* with weedy hosts is unknown, yet important to successful long-term management of Verticillium wilt of potato because locations where *Verticillium*-susceptible weedy hosts grow in the potato field could pose greater risk of increased initial inoculum from *V. dahliae* aggressive to potato in subsequent years. Knowledge of the location of microsclerotia from aggressive *V. dahliae* isolates are likely to be distributed in a field could be used to direct management strategies to provide greater Verticillium wilt control.

The Pale Cyst Nematode (PCN, *Globodera pallida*) is also an important soilborne potato pathogen (Dandurand 2013; Timmermans et al. 2007). PCN is a regulated pathogen under a Federal Domestic Quarantine Order from USDA Animal and Plant Health Inspection Service and the Idaho State Department of Agriculture (Dandurand 2013, https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/SA_Nematode/sa_potato/ct_pcn_home). Litchi tomato (*Solanum sisymbriifolium*) is a trap crop for PCN (Dandurand 2013; Scholte and Vos 2000; Timmermans et al. 2007). Trap crops are defined as plants that release root exudates that stimulate nematode egg hatch but are not a host to the nematode (Dandurand 2013; Timmermans et al. 2007). Understanding the

interaction of litchi tomato and soilborne potato diseases such as *V. dahliae* and *C. coccodes* is important to potato crop rotation sequences if litchi tomato is going to be as a PCN trap crop to avoid increasing soilborne potato pathogen inoculum.

The objectives of this study were to: (i) identify the susceptibility of 16 weedy hosts from the Columbia basin to eight *V. dahliae* isolates and (ii) identify weedy hosts where the potato or mint pathotype produce greater numbers of microsclerotia compared to the other *V. dahliae* isolates. The objectives of this study were to also (i) quantify inoculum production density of two pathotypes of *V. dahliae* and an isolate of *C. coccodes* in stems and roots of greenhouse-grown litchi tomato plants to potato cvs. that differ in susceptibility to *V. dahliae*, and (ii) evaluate litchi tomato grown in tandem with potato in field soils infested with endemic *C. coccodes* and *V. dahliae* populations to determine if microsclerotia are formed within stems and roots.

Susceptibility of weedy hosts to crop-aggressive isolates of *V. dahliae* under greenhouse conditions.

Methods. Four greenhouse trials were established in 2014 to 2016 to determine the susceptibility of weedy hosts to crop-aggressive isolates of *V. dahliae*. Sixteen weedy hosts were evaluated for susceptibility to *V. dahliae* and number of microsclerotia produced when infected with one of eight *V. dahliae* isolates (Table 1). The eight *V. dahliae* isolates were from six different crop hosts and included isolates that were aggressive on mint or potato, called the mint and potato pathotypes, respectively. Two crop hosts were also employed: eggplant as a universally susceptible control to ensure isolate infectivity was maintained and potato to confirm the aggressiveness of the potato pathotype was maintained. Weedy hosts and potato seedlings no greater than 8 cm in length were inoculated by submerging the hypocotyl and primary root in agitated conidial suspension of one *V. dahliae* isolate at a concentration of approximately 1.0×10^6 colony forming units (CFU)/ mL for 2 to 3 seconds. Weedy and crop host seedlings were then directly transplanted into moistened soilless potting medium in 3.79 liter pots and arranged in a randomized complete block design with three blocks. The plants were allowed to grow for four months before stems and roots were harvested separately and were dried to facilitate the formation of microsclerotia. Dried stems and roots were ground separately and one gram of dried, ground plant parts were placed on a semiselective medium designed for *V. dahliae*. Colony Forming Units derived from microsclerotia of both pathogens were counted.

Results. 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 oats in two of three trials (Fig. 1). Greater numbers of microsclerotia from the *V. dahliae* isolate from tomato (isolate 461) were observed than other *V. dahliae* isolates in pigweed tumble, large crabgrass, and henbit (Fig. 1) but only in one trial each. Other nightshade weedy hosts, such as bittersweet nightshade, did not produce many microsclerotia (<5 microsclerotia) when infected with any of these *V. dahliae* isolates in either trial (data not shown). 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 (data not shown).

Susceptibility of Litchi Tomato to Crop-aggressive Isolates of *Verticillium dahliae* and *Colletotrichum coccodes* under greenhouse conditions.

Methods. Greenhouse trials were established in 2013 and 2014 to determine the response of litchi tomato to *C. coccodes* and aggressive pathotypes of *V. dahliae* from the Columbia Basin. Two isolates of *V. dahliae* (potato and mint pathotypes) and an isolate of *C. coccodes* were selected for experimentation, as well as the potato cultivars Russet Alturas, Ranger Russet, and Russet Norkotah as resistant, moderately resistant, and susceptible hosts, respectively, to *V. dahliae*. Microsclerotia (30 microsclerotia/g) of *V. dahliae* or *C. coccodes* were mixed with soilless potting mix and litchi tomato seedlings and sprouted potato tubers were planted into the infested potting mix. Plants were arranged in the greenhouse in a completely randomized design and allowed to grow for four months before plants were dried to facilitate the formation of microsclerotia. Dried plants were ground and one gram of dried, ground plant parts were placed evenly on the surface of a semiselective medium designed for *V. dahliae*. Colony forming units derived from microsclerotia of both pathogens were counted.

Results. Greater numbers of microsclerotia were observed for the *V. dahliae* potato pathotype than the mint pathotype for all potato cultivars (Ranger Russet, Russet Alturas, and Russet Norkotah) in the greenhouse the in 2013 ($P \leq 0.05$, Fig. 2). The number of *V. dahliae* microsclerotia of the potato pathotype was less in litchi tomato than each of the potato cultivars Ranger Russet, Russet Alturas, and Russet Norkotah the in 2013 ($P \leq 0.05$, Fig. 2). Litchi tomato planted in soilless mix infested with either pathotype of *V. dahliae* was infected, but the number of microsclerotia did not differ between either *V. dahliae* pathotype in litchi tomato (Fig. 2).

Greater numbers of microsclerotia for the potato pathotype of *V. dahliae* than mint pathotype were observed from Russet Norkotah and Ranger Russet roots in 2014 ($P \leq 0.05$, Fig. 3). Otherwise, there were no differences in the number of *V. dahliae* microsclerotia in Russet Alturas, Russet Norkotah, and Ranger Russet stems, regardless of pathotype, which is inconsistent with results in 2013 (Fig. 2). Greater numbers of *V. dahliae* potato pathotype microsclerotia were observed in stems and roots of Russet Norkotah than litchi tomato ($P < 0.0001$, Fig. 3). Otherwise, the amount of *V. dahliae* microsclerotia did not differ between any potato cultivar and litchi tomato, regardless of *V. dahliae* pathotype. This is in contrast to Litchi tomato having fewer *V. dahliae* CFU of either pathotype than all potato cultivars in 2013 (Fig. 2). Symptoms of Verticillium wilt on litchi tomato were not observed in either greenhouse trial (data not shown).

The number of microsclerotia of *C. coccodes* from stems was significantly lower in litchi tomato than Ranger Russet, Russet Alturas, and Russet Norkotah in 2013 ($P \leq 0.05$, Fig. 4). No differences were noted between the *C. coccodes* microsclerotia from stems or roots of any of the potato cultivars and litchi tomato in 2014 (Data not shown). No symptoms of black dot were observed on litchi tomato grown in the greenhouse (data not shown).

Evaluation of litchi tomato susceptibility to *V. dahliae* and *C. coccodes* under field conditions.

Methods. A field trial was conducted to confirm the susceptibility of litchi tomato to *V. dahliae* and *C. coccodes*, and the relative amounts of microsclerotia produced from infection in litchi tomato compared to potato cultivars. Field soil was naturally infested (5 to 15 *V. dahliae* or *C. coccodes* microsclerotia/g). Litchi tomato transplants were planted in a randomized complete block design in Prosser, WA with potato cultivar Russet Burbank in 2015. Litchi tomato and potato plants were allowed to grow from April to August, and were harvested in August and

dried. Dried plants were ground and the number of microsclerotia of both *V. dahliae* and *C. coccodes* were determined by plating dried, ground plant matter on a semiselective medium as previously described.

Results. Greater numbers of *V. dahliae* microsclerotia were observed in stems and roots of Russet Burbank than litchi tomato at Prosser, WA in 2015 ($P < 0.05$, Fig. 5). The number of *C. coccodes* microsclerotia did not differ between stems of either plant at Prosser, WA. Greater numbers of *C. coccodes* microsclerotia were observed in roots of Russet Burbank than litchi tomato at Prosser, WA in 2015 ($P < 0.05$, Fig. 6). No symptoms of Verticillium wilt and black dot were observed on litchi tomato grown in the field (data not shown).

Discussion:

Litchi tomato was confirmed as a host for both *V. dahliae* and *C. coccodes*, as indicated by the presence of both pathogens in stems and roots of test plants. Microsclerotia production of *V. dahliae* in litchi tomato was consistently less than in Russet Norkotah and equivalent to less than the production in Ranger Russet. There was no difference in the number of microsclerotia between the mint and potato pathotypes of *V. dahliae* when infecting litchi tomato in the greenhouse. Infected litchi tomato contained fewer *V. dahliae* microsclerotia than Russet Burbank potatoes planted next to them in the field in Prosser, WA. Consequently, if litchi tomato is used in rotation with potato production, more microsclerotia of the potato pathotype would not be produced than the mint pathotype. Widespread planting of litchi tomato will likely return some microsclerotia of *V. dahliae* to soil, but less than susceptible potato cultivars and not necessarily of a pathotype that is aggressive on potato.

The observation of few *C. coccodes* microsclerotia generated in infected litchi tomato and absence of black dot symptoms on inoculated plants in the greenhouse was consistent with the same observations of few *C. coccodes* microsclerotia and no black dot symptoms in field-grown litchi tomato. Widespread planting of litchi tomato will likely return some microsclerotia of *C. coccodes* to soil, but less than susceptible potato cultivars.

Different sets of litchi tomato plants were evaluated in the experiments in 2013 and 2014, and they likely varied in resistance to the two pathotypes of *V. dahliae*. The difference in litchi tomato susceptibility to the *V. dahliae* potato pathotype could be attributed to the lack of genetic uniformity in seed. Each litchi tomato plant is unlikely to be genetically uniform because the litchi tomato seeds used for the experiment were from open pollinated plants grown in the field.

A broad range of weedy hosts was evaluated for *V. dahliae* susceptibility in the first two trials. The third and fourth trial focused on nightshades because of the interesting result of the interaction of black nightshade with the potato pathotype of *V. dahliae*, and questions were raised if that observation was consistent with other nightshades. Every weed tested in this study was confirmed (or re-confirmed) as a host for *V. dahliae* as indicated by the recovery of *V. dahliae* from plants grown in greenhouse settings.

The weedy hosts evaluated for *V. dahliae* susceptibility in this experiment were not expected to be genetically uniform because the seeds used to produce them were from open pollinated plants. The difference in *V. dahliae* susceptibility, especially to different pathotypes, in these weedy hosts may have varied between years due to the genetic variability in seed. Each plant evaluated in each trial was genetically distinct and possibly more or less susceptible to this *V. dahliae* isolate. Even though the weedy hosts are genetically diverse, consistent observations

of increased microsclerotia production by aggressive isolates of *V. dahliae* within one weedy hosts means this weedy hosts could pose a potential threat to potato production.

The consistent 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 weedy host and then back to the original crop 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 the potato pathotype of *V. dahliae*, which is the first factor for what 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 with the goal of improving Verticillium wilt disease management by simultaneously decreasing weedy competitors and eliminating alternative hosts for potato-aggressive isolates of *V. dahliae*.

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Table 1. Weedy and crop hosts, number of trials, and isolate characteristics of *Verticillium dahliae* used to determine microsclerotia production when potential host plants were inoculated with *V. dahliae* host-adapted isolates in four trials in 2014-2016.

Common Name	Latin Binomial	No. 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>Solanum nigrum</i>	4
Common Lambsquarters	<i>Chenopodium album</i>	2
Downy Brome	<i>Bromus tectorum</i>	2
Eastern Black Nightshade	<i>Solanum ptycanthum</i>	2
Eggplant (cv. 'Night Shadow')	<i>Solanum 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>Solanum sisymbriifolium</i>	2
Pigweed Powell	<i>Amaranthus powellii</i>	2
Pigweed Tumble	<i>Amaranthus albus</i>	1
Potato (cv. 'Russet Norkotah')	<i>Solanum tuberosum</i>	2
Rattail Fescue	<i>Vulpia myuros</i>	2
Wild Oat	<i>Avena fatua</i>	3

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

^a VCG: vegetative compatibility group

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

Fig. 1. Summary of the number of trials where a *V. dahliae* isolate had a differential effect on inoculum production because of greater number of microsclerotia than the other seven isolates for a specific weedy or crop host. The host of origin or the pathotype of the *V. dahliae* isolate is written within or above each bar, and the weedy host is along the horizontal axis.

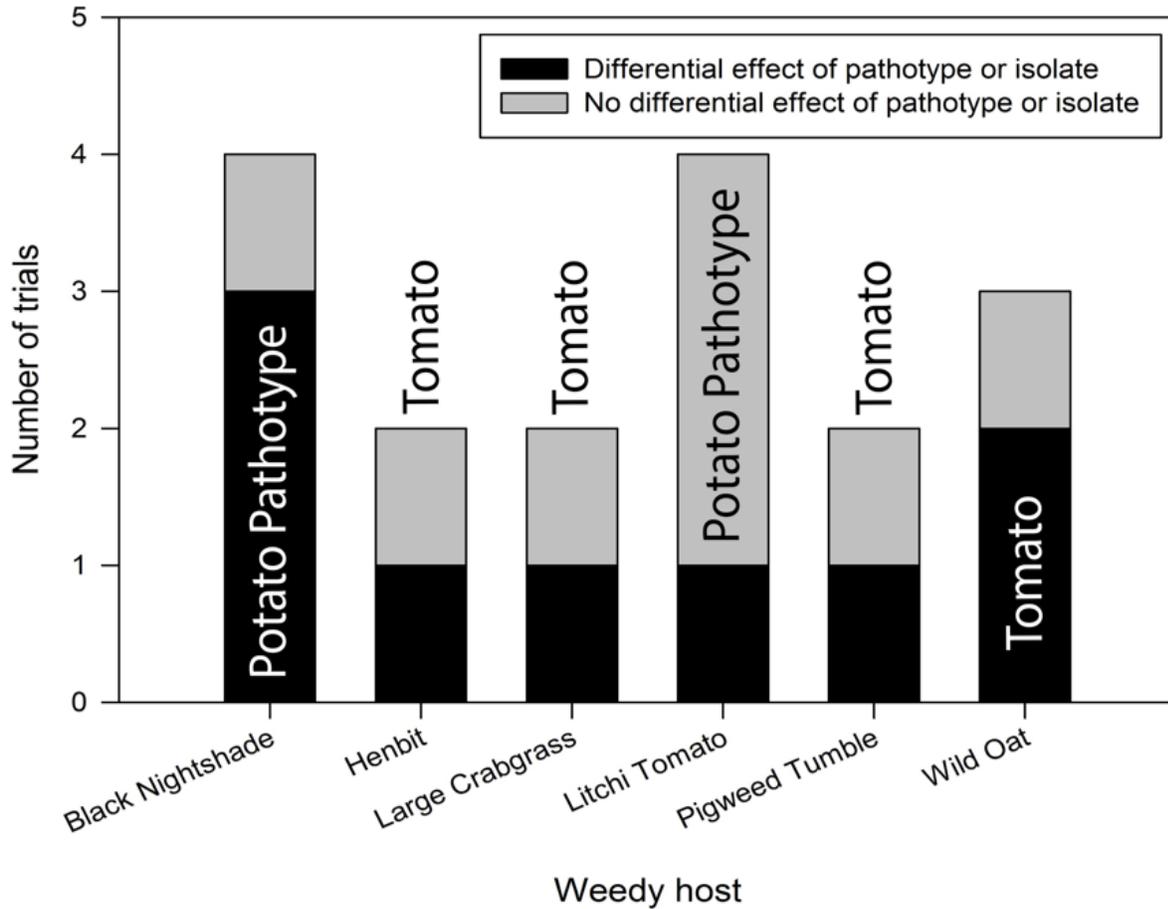


Fig. 2. Mean number of *Verticillium dahliae* microsclerotia from stems of three potato cultivars Alturas, Russet Norkotah, and Ranger Russet, and litchi tomato in a greenhouse experiment in 2013.

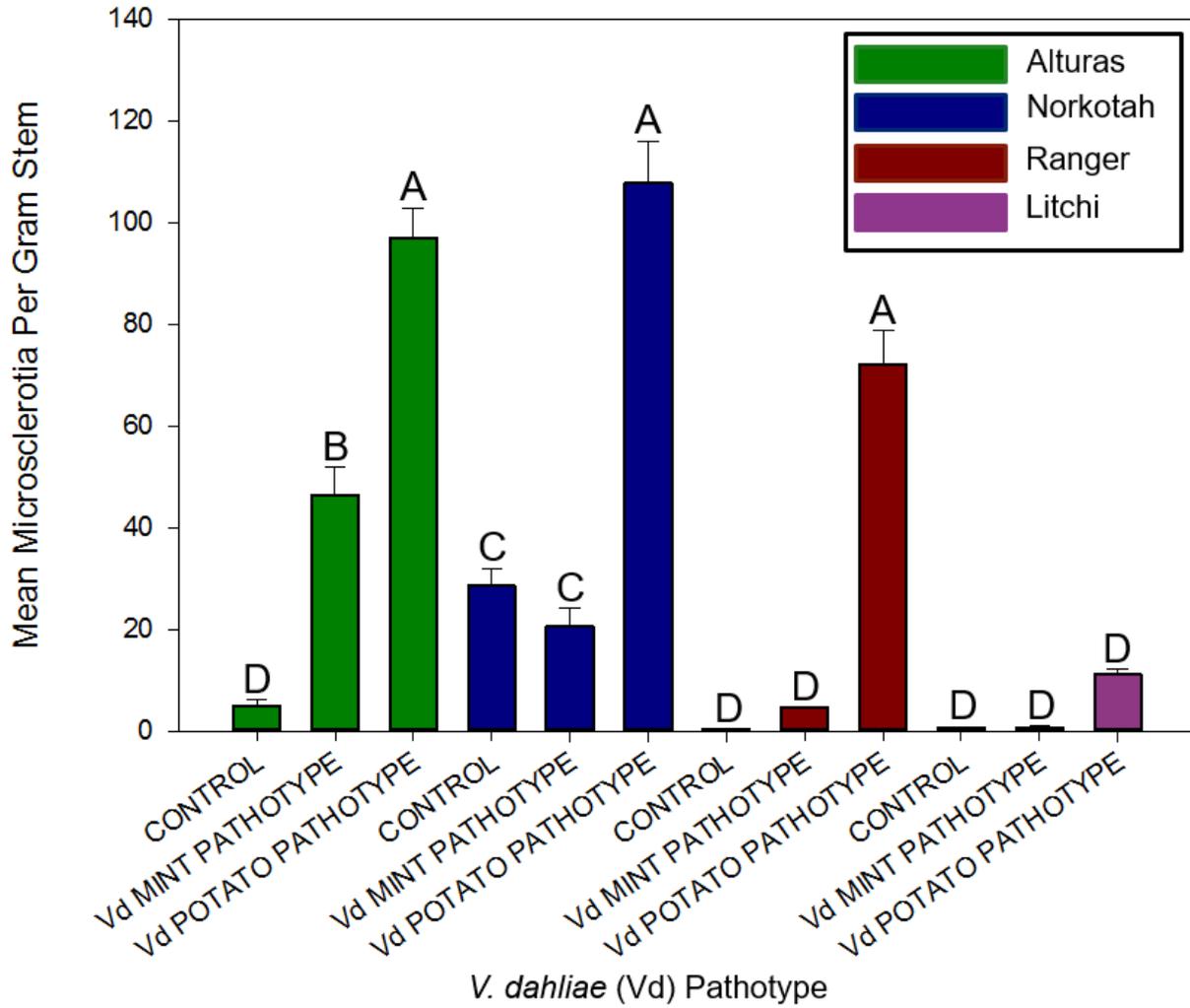


Fig. 3. Mean number of *Verticillium dahliae* CFU from roots of three potato cultivars Alturas, Russet Norkotah Ranger Russet, and litchi tomato (*Solanum sisymbriifolium*) in a greenhouse in 2014.

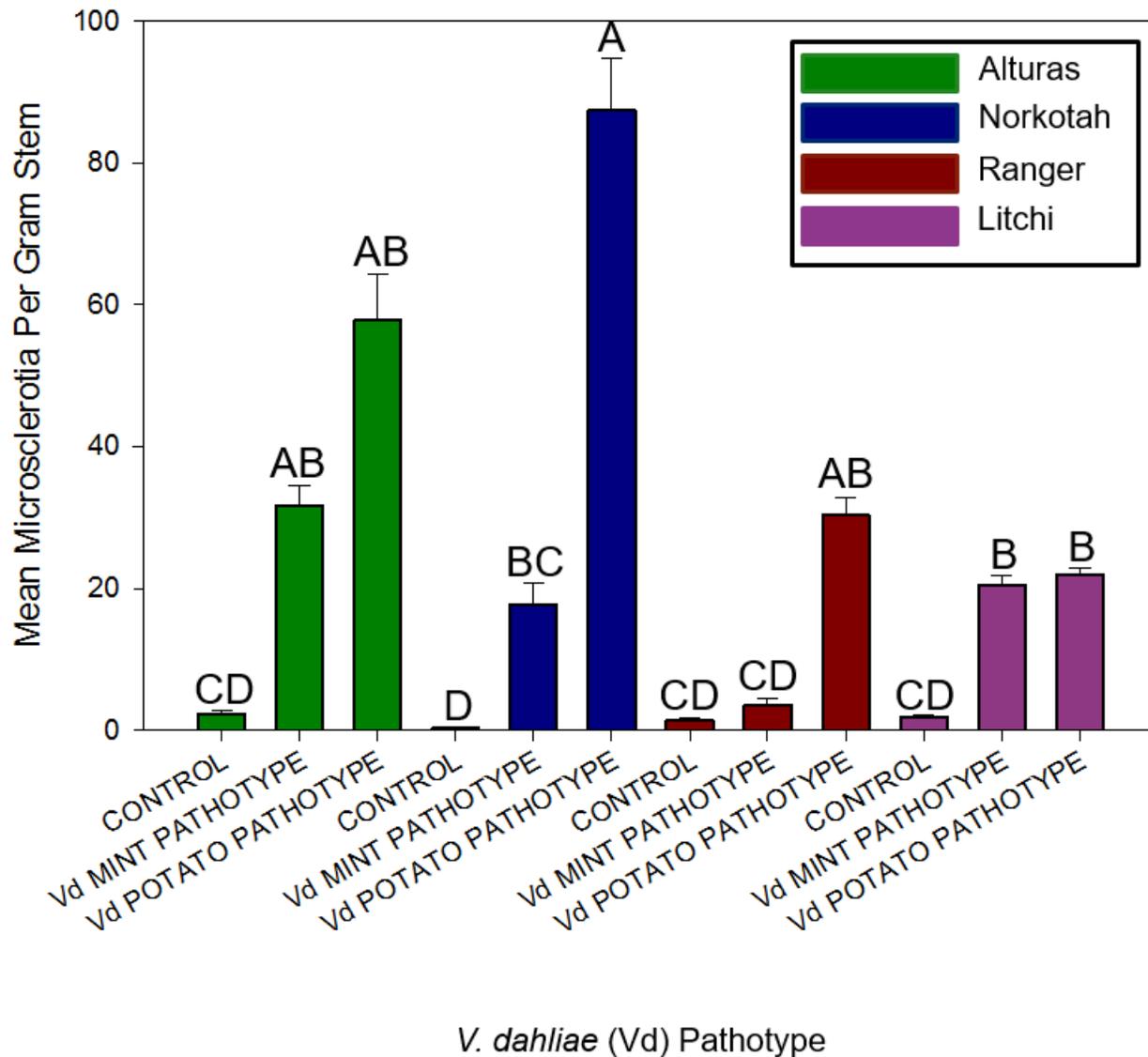


Fig. 4. Mean number of *Colletotrichum coccodes* microsclerotia from stems of three potato cultivars Alturas, Russet Norkotah, and Ranger Russet, and litchi tomato in a greenhouse experiment in 2013.

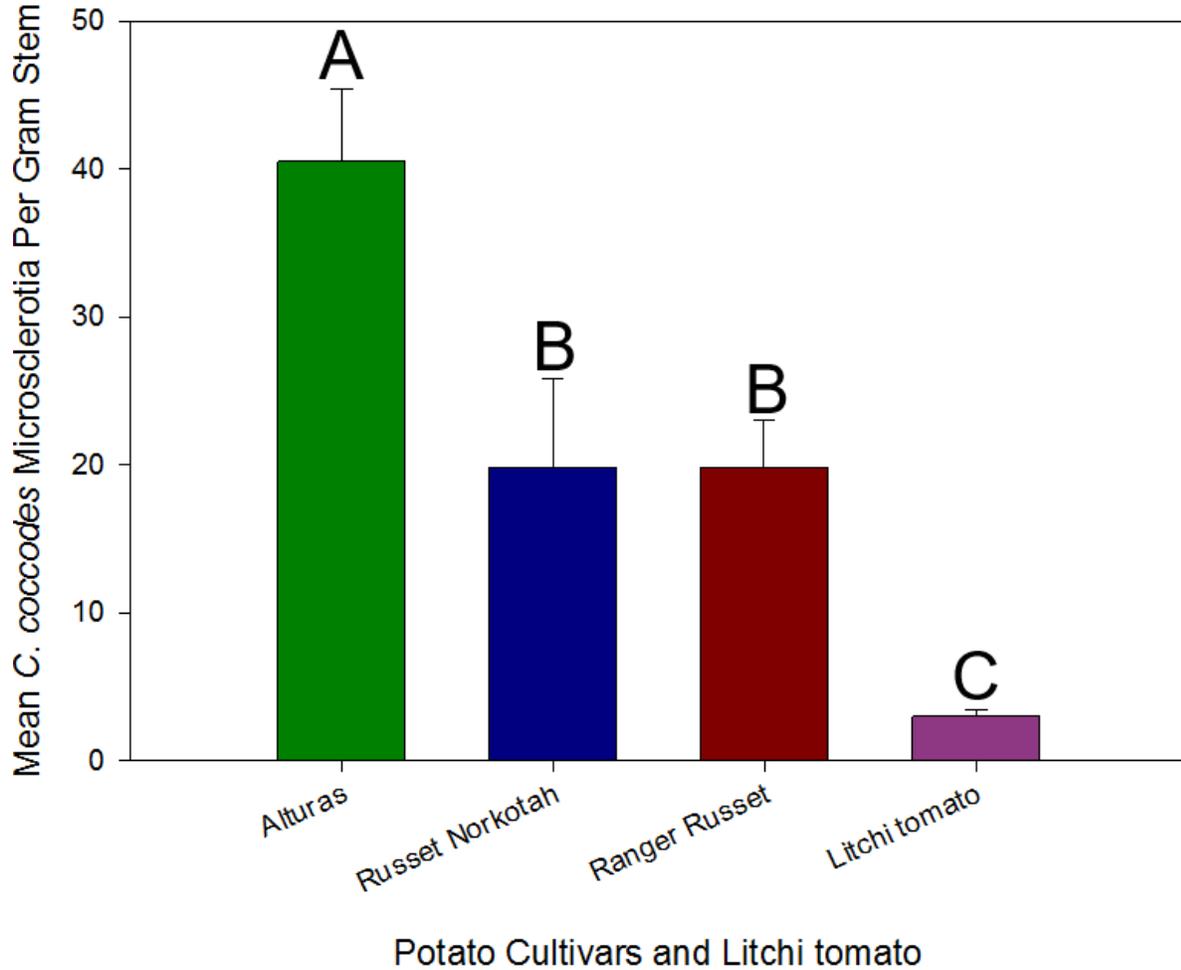


Fig. 5. Mean number of *Verticillium dahliae* microsclerotia from stems and roots of potato and litchi tomato (*Solanum sisymbriifolium*) in the 2015 field trial in Prosser, WA.

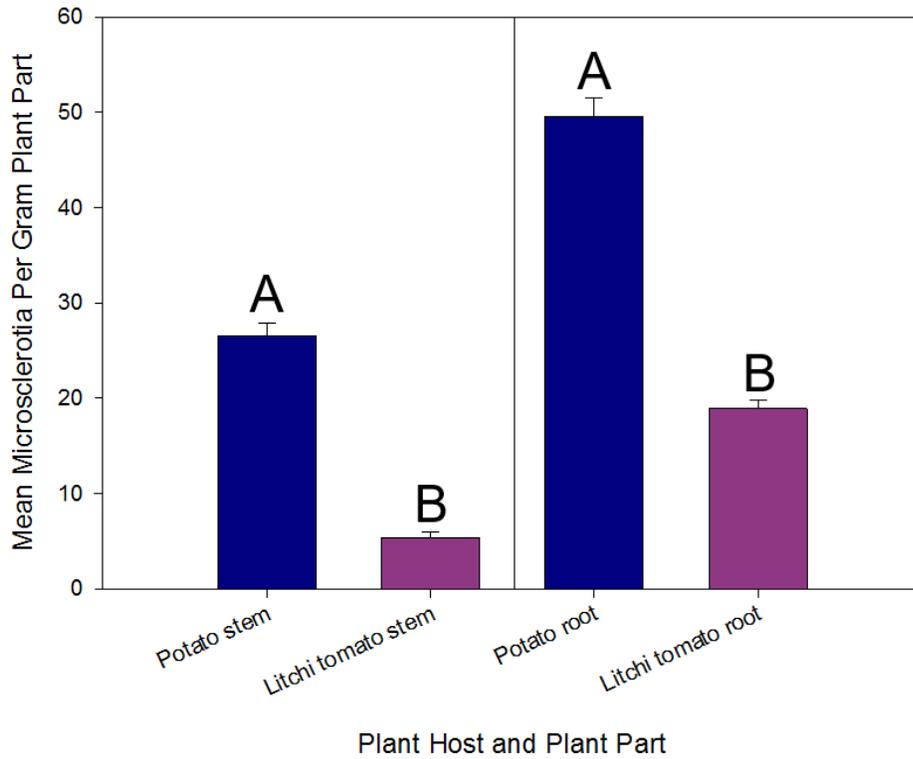


Fig. 6. Mean number of *Colletotrichum coccodes* microsclerotia from stems and roots of potato and litchi tomato (*Solanum sisymbriifolium*) in the 2015 field trial in Prosser, WA.

