Evaluation of *Solanum sisymbriifolium* as a Potential Inoculum Source of *Verticillium dahliae* and *Colletotrichum coccodes*

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Abstract

Solanum sisymbriifolium, the litchi tomato, is a perennial herbaceous plant from South America that is used as a trap crop to reduce soilborne populations of the pale cyst nematode *Globodera pallida*, an important potato pathogen. Possible interactions of soilborne potato pathogens *Verticillium dahliae* and *Colletotrichum coccodes* with litchi tomato are unknown, yet important for potato production if litchi tomato is to be planted as a trap crop. The goal of this research was to quantitatively assess if litchi tomato is a potential inoculum source for *C. coccodes* and *V. dahliae* by comparing colony forming units (CFU) observed in litchi tomato to susceptible and resistant potato cultivars. The potato cvs. Alturas ($P = 0.0003$), Ranger Russet ($P = 0.0193$), and Russet Norkotah

($P = 0.0022$) produced more CFUs of the potato pathotype of *V. dahliae* than litchi tomato the first of two years of greenhouse trials. Significantly more CFUs of the potato pathotype of *V. dahliae* were quantified from stems and roots of only cv. Russet Norkotah compared with litchi tomato ($P = 0.0001$) in the second year. The CFUs for *C. coccodes* varied between litchi tomato and the potato cvs., perhaps due to varying levels of resistance since litchi tomato is from a selected intermated seed source. Based on these data, the effect of litchi tomato in rotation with potato is likely to have limited effect on the proliferation of *V. dahliae* or *C. coccodes* populations in the soil when compared with a susceptible potato cultivar.

Two important diseases of potato in the northwestern United States are Verticillium wilt and black dot, caused by the soilborne fungi *Verticillium dahliae* Kleb. and *Colletotrichum coccodes* Wallr., respectively. *V. dahliae* infects a wide range of plants, making it one of the most important pathogens of dicotyledonous crop plants in North America (Agrios 2005; Bhat and Subbarao 1999; Dung et al. 2013). Despite the wide host range of *V. dahliae*, individual isolates vary in aggressiveness when introduced to different plant hosts under controlled conditions, which has also been noted from field isolations (Bhat and Subbarao 1999; Dung et al. 2013; Pegg and Brady 2002). Host adaptation of *V. dahliae* may occur over a relatively short period of time within both annual and perennial crop hosts such as pepper and mint, respectively (Bhat et al. 2003; Fordyce and Green 1960). Individual *V. dahliae* isolates that are aggressive to a particular host have been called host-adapted pathotypes (Dung et al. 2013; Pegg and Brady 2002). Certain *V. dahliae* isolates that are grouped into host-adapted pathotypes often fall into certain vegetative compatibility groups (VCGs; Katan 2000), although this is not true for all isolates (Bhat and Subbarao 1999; Dung et al. 2013). The VCGs of *V. dahliae* are scored by complementation of nitrate-nonutilizing (*nir*) mutants that are generated by culture on 1% potassium chlorate-amended medium and able to fuse and grow on media with defined nitrogen sources (Joaquim and Rowe 1990, 1991).

The complete host range of another important soilborne plant pathogen, *C. coccodes*, is not yet fully understood (Lees and Hilton 2003). Solanaceous crops such as tomato (Alkan et al. 2012; Lees and Hilton 2003) and weeds from the Cucurbitaceae, Fabaceae, and Solanaceae (Chesters and Hornby 1965) plant families are other known hosts. *C. coccodes* is the causal agent of black dot on potato (Agrios 2005; Johnson and Miliczky 1993). Damage caused by *C. coccodes* infection of potato was considered to be a minor problem on potato until 1990, when observations of yield losses began (Johnson and Miliczky 1993; Tsror et al. 1999).

The pale cyst nematode (PCN, *Globodera pallida*) is also an important 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). The nematode was identified in southeastern Idaho in 2006 and has become the focus of quarantine and eradication efforts (Dandurand 2013). One possible approach to PCN eradication is the use of trap crops. Trap crops are defined as plants that release root exudates that stimulate nematode egg hatch but are not a host to the nematode (Agrios 2005; Dandurand 2013; Timmermans et al. 2007). Litchi tomato (*Solanum sisymbriifolium*) is a trap crop for PCN (Dandurand 2013; Scholte and Vos 2000; Timmermans et al. 2007) as well as other nematodes such as *Meloidogyne* spp. (Dias et al. 2012) and *Pratylenchus* spp. (Evans and Kerry 2007).

Employing a trap crop such as the litchi tomato as part of PCN eradication may have unintended effects on populations of other soilborne potato pathogens. Litchi tomato was found to be resistant to *V. dahliae* and consequently used as a rootstock for eggplant in Greece (Bletsos et al. 2003). However, the work of Bletsos et al. (2003) should be expanded to include information on the interaction of litchi tomato with *V. dahliae* in other crop production regions, such as North America. It is also important to understand the response of litchi tomato to different host adapted pathotypes of *V. dahliae* compared with that of susceptible potato cultivars in order to determine whether or not the use of litchi tomato as a trap crop for PCN could exacerbate potential Verticillium wilt outbreaks on potato.

The goal of this research was to evaluate the ability of *V. dahliae* host-adapted pathotypes and *C. coccodes* to infect and produce microsclerotia within the stems and roots of litchi tomato, and to quantify microsclerotia production in potato cultivars that are susceptible and moderately resistant to *V. dahliae* relative to litchi tomato. Understanding the potential role of litchi tomato in *V. dahliae* and *C. coccodes* microsclerotia production is important to potato crop rotation sequences if litchi tomato is going to be a PCN trap crop to avoid increasing soilborne potato pathogen inoculum. The objectives of this study were to (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 cultivars that differ in susceptibility to *V. dahliae*, and (ii) evaluate litchi

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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.

Materials and Methods

Origin and preparation of isolates. *V. dahliae* and *C. coccodes* isolates were identified and previously characterized for aggressiveness to different hosts and VCG identity by Dung et al. (2013) and Nitzan et al. (2002), respectively (Table 1). Inoculum for *V. dahliae* was prepared by placing a single CFU arising from single microsclerotium on semiselective media (NPX) for *V. dahliae* (Butterfield and DeVay 1977) for 10 days. *C. coccodes* inoculum was prepared in the same way except that potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) was used instead of NPX. One subsection of the actively growing border of the colony was excised with a cork borer (7 mm diameter) and one agar disc was placed in Czapek-dox broth (Sigma-Aldrich, St. Louis, MO) for *V. dahliae* and potato dextrose broth (Difco Laboratories) for *C. coccodes*. Liquid cultures were shaken at 150 RPM at room temperature (21 to 23°C) for 10 days. Microsclerotia were generated from this conidial suspension by mixing the suspension with autoclaved sand (0.0197 to 0.0234 mm diameter) and placing the sand/conidia mixture on sterilized mesh in a Pyrex 4,543 ml oblong baking dish (Corning Inc., Corning, NY). The sand/conidia mixture was then placed inside a sterilized enclosure and allowed to grow for 7 days at room temperature (21 to 23°C) before drying for approximately 3 weeks to facilitate the formation of microsclerotia, stirring every 48 h to prevent clumping.

Determining the minimum inoculum density of *V. dahliae* (CFU/g) in potting medium for litchi tomato infection. The *V. dahliae* inoculum density to be used in subsequent greenhouse inoculations was determined experimentally. The experiment consisted of three plant species (litchi tomato, potato cv. Ranger Russet, and eggplant cv. Night Shadow) by four inoculum levels (5, 30, 50, and 100 CFU/g) and one *V. dahliae* isolate (potato pathotype, Table 1) and replicated four times in a completely randomized design. Plants were directly seeded in soilless potting medium (Sunshine Advanced Growing Mix #4, Sun Gro, Agawam, MA) infested with one isolate of the *V. dahliae* potato pathotype for 4 months before drying for 1 month to allow the formation of microsclerotia. Dried stem sections from the crown to 16 cm up the plant were collected for *V. dahliae* and *C. coccodes* CFU enumeration. Roots were not collected in this experiment. Dried stems 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, was surface sterilized with a mixture of 70% ethanol and 30% deionized water between plant samples. One gram of ground stem was placed evenly on the surface of a Petri plate containing NPX without serial dilution and the number of *V. dahliae* and *C. coccodes* CFU was counted under an Olympus SD-ILK dissecting microscope (Olympus Optical Ltd, Tokyo, Japan) after incubating for 10 days.

Quantification of *V. dahliae* and *C. coccodes* CFU in greenhouse grown potato and litchi tomato. Seed of litchi tomato was germinated on moistened filter paper to determine if planted seed was free of *V. dahliae* and *C. coccodes*. Seed free of *V. dahliae* and *C. coccodes* was determined by visually assessing germinated seed for the presence or absence of disease symptoms, sporulation, and microsclerotia associated with either pathogen. Potato tubers from cvs. Alturas, Russet Norkotah, and Ranger Russet were assayed

for the absence of both fungal pathogens by slicing an approximately 0.5 cm thick segment from the stolon end of the tuber, surface sterilized by submerging in 10% sodium hypochlorite (NaOCl, 6.0%, Independent Marketing Alliance, Houston, TX) for 3 min, rinsing in deionized water, and blotting dry. The segment from the stolon end of the tuber was then placed on NPX and incubated at room temperature (21 to 23°C) for 10 days, after which the presence or absence of *C. coccodes* and *V. dahliae* microsclerotia was confirmed through visual observation of microsclerotia and sporulation using an Olympus SD-ILK dissecting microscope (Olympus Optical Ltd). Potato tubers that did not test positive for microsclerotial growth and development from either fungal pathogen were planted in 3.79 liter pots with soilless potting medium and allowed to grow for 1 month before the potting medium was infested with *V. dahliae* or *C. coccodes* to ensure emergence. Soils for each litchi tomato and potato plant (experimental unit) were infested with either *V. dahliae* VCG 2B (mint pathotype, Table 1), *V. dahliae* VCG 4A (potato pathotype, Table 1), or a *C. coccodes* isolate (Table 1) so that each pot contained 30 microsclerotia/g potting medium. Litchi tomato and potato seedlings were then directly transplanted into infested potting medium. The greenhouse was maintained at a daytime average of approximately 23°C and a nighttime temperature of 15.5°C.

The experiment had four levels for host (litchi tomato, potato cvs. Russet Norkotah, Alturas, and Ranger Russet) and three levels for pathogen (*V. dahliae* potato pathotype, *V. dahliae* mint pathotype, *C. coccodes*) as well as noninoculated controls arranged in a completely randomized design with four replicates. Cultivar Russet Norkotah is susceptible to *V. dahliae* (Bae et al. 2007), Ranger Russet is moderately resistant to *V. dahliae* (Bae et al. 2007), and Alturas is resistant to *V. dahliae* in the field (Novy et al. 2003). Soilless potting medium from each experimental unit was infested with one pathogen. Plants were visually inspected for the presence or absence of Verticillium wilt or black dot symptoms once a week for 5 months post inoculation. Plants were harvested in both 2014 and 2015 after 5 months of growth in the greenhouse 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 16-cm dried stem section from each experimental unit was excised starting from the base of the crown, ground as previously described until particle size was approximately less than 4 mm², and 1 g was placed on NPX to enumerate the number of *V. dahliae* or *C. coccodes* CFU that were present after incubation for 10 days as previously described. The greenhouse trial was conducted in the same manner in both 2013 and 2014. *V. dahliae* or *C. coccodes* CFU were enumerated in the stems of all plants in both 2013 and 2014. Root tissue consisting of the combined primary and secondary roots from the crown down 16 cm was also collected in 2014 for *V. dahliae* or *C. coccodes* CFU enumeration. Root samples were dried, ground, and plated as previously described but separately from stems.

Evaluation of litchi tomato susceptibility to *V. dahliae* and *C. coccodes* under field conditions. Field trials were established to evaluate inoculum production density of *V. dahliae* and *C. coccodes* in infected litchi tomato under field conditions. Research fields were selected based on having a previous history of potato cultivation as well as Verticillium wilt and black dot, with 5 to 15 *V. dahliae* or *C. coccodes* microsclerotia per gram of soil based on soil samples. Soil samples were collected from a depth of 10 cm with a ⅝" × 21" plated soil probe (AMS, American Falls, ID), dried for 1 month

Table 1. *Verticillium dahliae* and *Colletotrichum coccodes* isolates used to confirm host status of litchi tomato (*Solanum sisymbriifolium*) for either *V. dahliae* or *C. coccodes*

Pathogen	Host-adapted pathotype	State of origin	Original plant host	VCG ^y	Source citation
<i>V. dahliae</i>	Potato	Idaho	Potato seed tuber	4A	Dung et al. 2013
<i>V. dahliae</i>	Mint	Washington	Peppermint	2B	Dung et al. 2013
<i>C. coccodes</i>	- ^z	Montana	Potato seed tuber	3	Nitzan et al. 2002

^y Vegetative compatibility group.

^z Not considered for this study.

at ambient temperatures of 21 to 24°C, and 1 g of dry soil was placed on NPX, incubated for 10 days, and *V. dahliae* or *C. coccodes* microsclerotia were quantified as previously described for dry plant matter.

An observational study to evaluate inoculum production density of *V. dahliae* and *C. coccodes* in infected litchi tomato under field conditions was established in Othello, WA, in 2014 and Powell Butte, OR, in 2015. Natural levels of inoculum (>5 *V. dahliae* or *C. coccodes* microsclerotia) already present in the soil were used in both field trials. A one-row plot was established in Othello, WA, with five replicates containing four potato plants (cv. Ranger Russet) and three litchi tomato plants. The row was 9 m long row with 0.9 m alleys, with plant spacing of 25 cm for both potato and litchi tomato. Potato seed tubers were planted on 28 April and litchi tomato was planted on 28 May 2014 in Othello, WA. Flowers of litchi tomato were removed in July and August to prevent seed dispersal. In September 2014, all plants in Othello, WA, were individually harvested and dried slowly for 3 weeks in the dark at temperatures of 21 to 24°C to facilitate the formation of microsclerotia. Thirty litchi tomato plants were planted on 15 May 2015 in Powell Butte, OR, approximately 45 cm apart. Plants were harvested after 4 months in the field on 23 September, dried as previously described. Stem and root sections 16 cm in length were excised from dried potato or litchi tomato and ground as in previously described greenhouse experiments. Dried plant matter was placed evenly on the surface of NPX to determine the number of *V. dahliae* and *C. coccodes* CFU from the stem and roots of each experimental unit after 10 days' incubation as previously described.

Field trials with potato cv. Russet Burbank and litchi tomato were established in Prosser, WA, in 2015 to evaluate and compare inoculum production density of *V. dahliae* and *C. coccodes* in infected litchi tomato to potato under field conditions. Russet Burbank and litchi tomato were planted in the field at Prosser, WA, in a randomized complete block design. This experiment consisted of six litchi tomato or six potato plants per replicate with six replicates for a total of 72 experimental units. Potato and litchi tomato transplants approximately 15 to 18 cm tall were planted approximately 25 cm apart in June and harvested in September 2015. Stem and root sections 16 cm in length were excised from dried potato or litchi tomato and ground as previously described for the greenhouse experiments. Dried plant matter was placed evenly on the surface of NPX to determine the number of *V. dahliae* and *C. coccodes* CFU from the stem and roots of each experimental unit after 10 days' incubation as previously described.

Data analysis. Analysis of variance (ANOVA) was performed in SAS University Edition (SAS Inc., Cary, NC) followed by

Table 2. *Verticillium dahliae* potato pathotype microsclerotia counts (CFU/g) from eggplant, potato, or litchi tomato (*Solanum sisymbriifolium*) stems at senescence by initial *V. dahliae* colony forming units (CFU)/g potting medium

	<i>V. dahliae</i> CFU/g potting medium			
	5	30	50	100
Potato cv. Ranger Russet	49.5 bc ^z	55.6 ab	72.9 ab	114.5 a
Eggplant cv. Night Shadow	6.4 c	27.0 bc	16.6 bc	39.9 bc
Litchi tomato	2.7 c	4.1 c	8.1 c	10.6 bc

^z Letters denote mean separation by Tukey's honestly significant difference test for all pairwise comparisons across columns and rows ($P \leq 0.05$).

Tukey's honestly significant difference test for all pairwise comparisons to determine differences in the number of CFU observed between plants subjected to the same fungal pathogen, separated by plant part (stem or root). Greenhouse trial data were not pooled prior to analysis. Fields from Prosser, WA, were pooled prior to analysis (with ANOVA), with each field serving as the replicates. *V. dahliae* and *C. coccodes* data were analyzed separately because the experiment was designed to compare inoculum production density of a single pathogen between the four plant hosts. ANOVA assumptions were verified using the same program. Data sets that did not initially meet ANOVA assumptions were log transformed to allow continued use of parametric analysis, except for values of zero.

Results

Determining the minimum inoculum density of *V. dahliae* (CFU/g) in potting soil for litchi tomato infection. The number of *V. dahliae* CFU in litchi tomato and eggplant did not differ regardless of the inoculum level in the potting medium (5 CFU/g [$P = 0.9972$], 30 CFU/g [$P = 0.84$], 50 CFU/g [$P = 0.9967$], 100 CFU/g [$P = 0.53$], Table 2). Eggplant was not used to compare differences in *V. dahliae* inoculum production density because of nonsignificant differences in the number of observed *V. dahliae* CFU between eggplant and litchi tomato at any evaluated inoculum level (Table 2). *V. dahliae* infection of litchi tomato was observed at all levels of *V. dahliae* inoculum but litchi tomato had significantly fewer observed *V. dahliae* CFU than potato cv. Ranger Russet at inoculation levels of 30 CFU/g ($P < 0.0001$), 50 CFU/g ($P < 0.0001$), and 100 CFU/g ($P < 0.0001$) (Table 2). Therefore, in subsequent experiments, plants were inoculated with 30 CFU/g, which was the minimum *V. dahliae* inoculum density that consistently infected litchi tomato and had significantly fewer observed CFU than potato.

Quantification of *V. dahliae* and *C. coccodes* CFU in greenhouse grown potato and litchi tomato. The two-factor ANOVA test for differences in the number of microsclerotia between plant hosts was significant ($P < 0.0001$), and the interaction effect of plant host and fungal isolate was not significant ($P = 0.3115$). Greater numbers of CFUs were recorded for the *V. dahliae* potato pathotype than the mint pathotype on cv. Ranger Russet ($P = 0.0331$), Alturas ($P < 0.0001$), and Russet Norkotah ($P < 0.0001$) in 2013 (Table 3). Cultivars Alturas ($P = 0.0003$), Ranger Russet ($P = 0.0193$), and Russet Norkotah ($P = 0.0022$) produced greater numbers of *V. dahliae* potato pathotype CFUs than litchi tomato (Table 3). Litchi tomato planted in soilless mix infested with either pathotype of *V. dahliae* was infected, based on the production of microsclerotia within the plants, but no difference in CFUs was observed between the potato and mint pathotypes of *V. dahliae* ($P = 0.1044$, Table 3). No Verticillium wilt symptoms were observed on litchi tomato inoculated with either the *V. dahliae* mint or potato pathotype (data not shown) in 2013.

The two-factor ANOVA test for differences in the number of microsclerotia between plant hosts was significant ($P < 0.0001$ for both stems and roots), and the interaction effect of plant host and fungal isolate was not significant ($P = 0.1058$ in stems, $P = 0.27$ for roots). Greater numbers of CFU of the *V. dahliae* potato pathotype were observed than the mint pathotype in cvs. Russet Norkotah ($P = 0.0432$) and Ranger Russet ($P = 0.0029$) roots in 2014 (Table 4). Significantly greater numbers of *V. dahliae* potato pathotype CFU were found in stems and roots of cv. Russet Norkotah than litchi tomato

Table 3. 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

Isolate	Mean CFU/g stem ^z			
	Alturas	Russet Norkotah	Ranger Russet	Litchi tomato
Potato pathotype	97.0 a	107.8 a	72.0 a	11.1 d
Mint pathotype	46.3 b	20.5 c	4.5 d	0.8 d
Noninoculated control	5.0 d	18.5 c	0.2 d	0.6 d

^z Letters denote mean separation by ANOVA followed by Tukey's honestly significant difference test for all pairwise comparisons for *V. dahliae* CFU counts across columns and rows ($P \leq 0.05$).

($P = 0.0001$, Table 4). This is in contrast to litchi tomato having fewer *V. dahliae* CFU of either pathotype than all potato cultivars in 2013 (Table 3). The number of *V. dahliae* potato and mint pathotype CFUs did not differ in litchi tomato stems ($P = 0.3962$) or roots ($P = 1.0$) in 2014 (Table 4). No Verticillium wilt symptoms were observed on litchi tomato inoculated with either the *V. dahliae* mint or potato pathotype (data not shown) in 2014.

The number of observed CFUs of *C. coccodes* from stems was significantly lower in litchi tomato than potato cv. Alturas ($P = 0.0057$), Russet Norkotah ($P = 0.0041$), and Ranger Russet ($P = 0.0041$) in 2013 (Table 5). No differences were noted between the *C. coccodes* CFUs from stems or roots of any of the potato cvs. as well as litchi tomato roots compared with potato roots in 2014 ($P > 0.05$, Table 5). No black dot symptoms were observed on litchi tomato inoculated in the greenhouse experiments (data not shown) in either 2013 or 2014.

Evaluation of litchi tomato susceptibility to *V. dahliae* and *C. coccodes* under field conditions. Greater numbers of CFUs of *V. dahliae* were observed in stems ($P = 0.0307$) and roots ($P = 0.0465$) of cv. Russet Burbank than the litchi tomato in the Prosser, WA, field trial (Table 6). The numbers of CFUs of *C. coccodes* did not differ in stems ($P = 0.6296$) and roots ($P = 0.0576$) of cv. Russet Burbank when compared with litchi tomato at this site (Table 6). Both pathogens infected and produced microsclerotia in litchi tomato in the Othello, WA, field trial in 2014 and Powell Butte, OR, field trial in 2015 (Table 6). Comparisons could not be made between *C. coccodes* or *V. dahliae* CFU between potato or litchi tomato because of insufficient field replication in Othello, WA, and no potato plants were planted in Powell Butte, OR. No symptoms of either pathogen were observed on litchi tomato at harvest in the field (data not shown).

Discussion

Litchi tomato was confirmed as a host for *V. dahliae* and *C. coccodes* as indicated by the isolation of *V. dahliae* and *C. coccodes* from the stems and roots of plants grown in both greenhouse and field settings. Inoculum production density of the *V. dahliae* potato and mint pathotypes from litchi tomato did not significantly differ, which indicates the potato pathotype was not consistently more aggressive on litchi tomato. The difference in aggressiveness between the *V. dahliae* potato and mint pathotypes was replicated on the potato cultivars in the greenhouse as evidenced by the increased numbers of observed CFU produced on the stems or roots of the Verticillium-susceptible cv. Russet Norkotah. In 2014, Russet Norkotah seed pieces were unexpectedly infected with *V. dahliae* and produced

microsclerotia; however, further improvements and diligence in checking seed pieces for infection reduced the number of microsclerotia observed in the subsequent trials. Because plant samples were dried before grinding, the CFU observed on the semiselective media (NPX) were likely derived from *V. dahliae* or *C. coccodes* microsclerotia instead of conidia or hyphae.

The potato cv. Russet Norkotah is highly susceptible to *V. dahliae* (Bae et al. 2007), which explains why this cultivar consistently had the greatest numbers of *V. dahliae* CFU/g tissue. Observations of decreased inoculum production densities in litchi tomato stems in 2013 and roots in 2014 compared with cv. Russet Norkotah led to the initial conclusion that litchi tomato was less susceptible to *V. dahliae* than a susceptible potato cultivar. However, litchi tomato did not consistently have significantly greater *V. dahliae* inoculum production density than cv. Ranger Russet, a cultivar that is moderately resistant to *V. dahliae* infection (Bae et al. 2007). The lack of a difference in inoculum production densities of *V. dahliae* potato pathotype between Ranger Russet and litchi tomato in 2014 was probably due to partial resistance on the part of both Ranger Russet and litchi tomato.

The litchi tomato plants used in this experiment were not expected to be genetically uniform because the seeds used to produce them were from selected intermated plants. The difference in *V. dahliae* resistance in litchi tomato likely varied between years due to the genetic

Table 6. Mean number of *Verticillium dahliae* or *Colletotrichum coccodes* CFU from stems of potato and litchi tomato (*Solanum sisymbriifolium*) in the 2014 field trial in Othello, WA, and the 2015 field trials in Prosser, WA, and Powell Butte, OR

Year	Location	Plant	<i>V. dahliae</i>		<i>C. coccodes</i>	
			Stem ^w	Root ^w	Stem ^w	Root ^w
2015	Prosser, WA	Potato ^x	26.6 a	49.6 a	7.6 a	19.6 a
	Prosser, WA	Litchi tomato	5.4 b	18.9 b	2.3 a	1.6 a
2014	Othello, WA ^y	Potato ^z	22.4	16.8	18.9	23.3
	Othello, WA ^y	Litchi tomato	1	1.1	0	0
2015	Powell Butte, OR ^y	Litchi tomato	20	3.3	43.3	30

^w Letters denote mean separation by ANOVA followed by a Tukey's honestly significant difference test for all pairwise comparisons within columns only ($P \leq 0.05$). Each pathogen and each plant part were analyzed separately.

^x Potato cv. Russet Burbank.

^y No statistical test conducted (insufficient replication).

^z Potato cv. Ranger Russet.

Table 4. Mean number of *Verticillium dahliae* CFU from stems and roots of three potato cultivars Alturas, Russet Norkotah Ranger Russet, and litchi tomato (*Solanum sisymbriifolium*) in a greenhouse in 2014

Mean CFU/g plant part ^z					
Plant part	<i>V. dahliae</i> isolate	Alturas	Russet Norkotah	Ranger Russet	Litchi tomato
Stem	Potato pathotype	40.8 abc	88.4 a	25.4 abcd	6.1 cdef
	Mint pathotype	22.5 abcd	19.5 abcde	6.2 def	12.4 ef
	Noninoculated control	2.3 f	2.0 f	0.6 f	1.1 f
Root	Potato pathotype	57.8 ab	87.4 a	30.4 ab	20.5 b
	Mint pathotype	31.7 ab	17.8 bc	3.6 cd	22.0 b
	Noninoculated control	2.3 cd	0.3 d	1.4 cd	1.9 cd

^z Letters denote mean separation by ANOVA followed by a Tukey's honestly significant difference test for all pairwise comparisons for *V. dahliae* CFU counts across columns and rows ($P \leq 0.05$).

Table 5. Mean number of *Colletotrichum coccodes* CFU from stems and roots of three potato cultivars Alturas, Russet Norkotah Ranger Russet, and litchi tomato (*Solanum sisymbriifolium*) in a greenhouse in 2013 and 2014

			Mean CFU/g plant part ^z			
Year	Plant part	Pathogen	Alturas	Russet Norkotah	Ranger Russet	Litchi tomato
2013	Stem	<i>C. coccodes</i>	40.5 a	19.8 b	19.8 b	3.0 c
2014	Stem	<i>C. coccodes</i>	56.0 a	77.0 a	28.8 a	14.8 a
	Root	<i>C. coccodes</i>	56.0 a	79.3 a	33.0 a	46.1 a

^z Letters denote mean separation by ANOVA followed by a Tukey's honestly significant difference test for all pairwise comparisons for *C. coccodes* CFU counts across the 2013 row and for all columns and rows in 2014 ($P \leq 0.05$). Each year was analyzed separately.

variability in seed. Different sets of litchi tomato plants that were evaluated in 2013 and 2014 varied in *V. dahliae* resistance, and these plants also varied in resistance to different pathotypes of *V. dahliae*. For example, comparisons of the mint pathotype of *V. dahliae* on roots in 2014 revealed that lower inoculum production density within Ranger Russet than litchi tomato but the same trend was not observed when inoculum was the mint pathotype and litchi tomato plants used in 2013.

The inconsistencies in litchi tomato resistance to the two specific *V. dahliae* pathotypes leaves no clear trend that has emerged across both years. Resistance on the part of litchi tomato could be imparted on certain seedlings, and not others, by genetic variability in resistance genes. The case can be made for partial resistance since litchi tomato plants were infected but resulted in quantitatively less inoculum production than susceptible potato cultivars. Additionally, symptoms of black dot did not develop on inoculated litchi tomato plants. Litchi tomato could be a source of resistance that would be useful in breeding programs for solanaceous crops where *Verticillium* wilt is a limiting factor to potato production. Only populations of litchi tomato selected for resistance to *V. dahliae* should be used as a trap crop for nematodes.

Greater *C. coccodes* inoculum production was observed in potato cvs. Alturas, Russet Norkotah, and Ranger Russet than litchi tomato in 2013. However, no differences between any of the three potato cultivars and litchi tomato were observed in 2014 due to high variability in the number of microsclerotia observed in all plant hosts between replicates. The inconsistency in less *C. coccodes* inoculum production density in litchi tomato stems compared with Alturas and Russet Norkotah is a possible indicator of partial resistance in some individual litchi tomato plants to *C. coccodes* as plants were infected in 2013 but with quantitatively less inoculum than susceptible potato. As with litchi tomato resistance to *V. dahliae*, possible litchi tomato resistance to *C. coccodes* could be imparted on certain seedlings, and not others, by genetic variability in resistance genes.

Litchi tomato was infected with *V. dahliae* in all three field sites in Othello and Prosser, WA, and Powell Butte, OR. In field trials in Prosser, WA, litchi tomato produced fewer *V. dahliae* CFUs as a result of natural infection than potato cv. Ranger Russet. Litchi tomato infection with *C. coccodes* was observed in Prosser, WA, and Powell Butte, OR, but not Othello, WA, which could support litchi tomato was partially resistant to infection by *C. coccodes* or a disease escape occurred. However, litchi tomato planted at the Prosser, WA, site did not produce fewer *C. coccodes* CFUs as a result of natural infection than potato cv. Russet Burbank, suggesting not all litchi tomato plants are resistant to infection by *C. coccodes*. Variation in severity of infection by *C. coccodes* and *V. dahliae* between field sites is likely due to differences in inoculum density, but could also be influenced by weather and irrigation events. Consistent trends in susceptibility to either pathogen between field sites is important for identifying resistance in litchi tomato. Numerically, greater numbers of *V. dahliae* and *C. coccodes* inoculum production densities were sometimes observed in litchi tomato grown in the greenhouse than in the field, and perhaps this is due to greater levels of inoculum (30 CFU/g) or an environment more conducive for infection by one or both pathogens in the greenhouse.

Litchi tomato was confirmed as a host for *V. dahliae* and *C. coccodes*, and the variation in inoculum production densities between the potato and mint pathotypes of *V. dahliae* indicate that neither pathotype is consistently more aggressive on litchi tomato. Observations of decreased *V. dahliae* inoculum densities in litchi tomato compared with potato cv. Russet Norkotah but not Ranger Russet suggests that litchi tomato was partially resistant to *V. dahliae* like Ranger Russet. The inconsistency in decreased *C. coccodes* inoculum production density in litchi tomato stems compared with potato cvs. Alturas and Russet Norkotah is evidence supporting some individual plants of litchi tomato could also be partially resistant to *C. coccodes*. Variation in resistance within the litchi tomato population is expected given that the litchi tomato seeds were from an intermated selected population that was genetically variable. Further supporting evidence of litchi tomato partial resistance to both pathogens is the lack of

visible disease symptoms for either pathogen in greenhouse trials when plants were artificially inoculated.

The use of litchi tomato as a PCN trap crop is likely to have limited effect on the proliferation of *V. dahliae* or *C. coccodes* populations in the soil. Slattery (1981) documented an increase of microsclerotia in soil over several seasons in the Red River Valley of Minnesota and North Dakota with the use of the potato cv. Kennebec that returned relatively high numbers of microsclerotia to the soil. The conclusions from Slattery (1981) indicate that the amount of *V. dahliae* inoculum produced on preceding crops and potato cultivars has influence on the severity of *Verticillium* wilt in following potato crops (Slattery 1981). Care would certainly be needed to manage both *V. dahliae* and *C. coccodes* in a field planting of litchi tomato since litchi tomato can be infected by both pathogens and possibly return microsclerotia inoculum to the soil, albeit less than a susceptible potato cultivar such as Russet Norkotah. Only populations of litchi tomato selected for resistance to *V. dahliae* and *C. coccodes* should be used as a trap crop for nematodes.

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