Cryptic Infection and Systemic Colonization of Leguminous Crops by *Verticillium dahliae*, the Cause of Verticillium Wilt

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Abstract

Verticillium dahliae, the cause of Verticillium wilt, is a widespread pathogen that affects many crops in California and throughout the world. Cover cropping with leguminous species is often integrated into a rotation scheme for its contribution to soil nitrogen, and can contribute to management of Verticillium wilt provided the chosen crop does not support development of *V. dahliae*. Seven cool season legumes (faba bean, bell bean, field pea, hairy vetch, common vetch, purple vetch, and woollypod vetch), and three warm season legumes (sesbania, sunn hemp, and black-eyed pea) were evaluated as hosts for reproductive growth of *V. dahliae*. All 10 legumes were colonized by *V. dahliae*, while remaining symptomless, when subjected to a root-dip inoculation. Similar results

were obtained when plants were grown in infested potting soil, albeit with a lower frequency of infection than in root-dip assays. All tested legumes were also infected in field trials, with the exception of bell bean. Overall, warm season legumes sustained higher rates of infection than cool season legumes. Common vetch was the most extensively colonized of the cool season legumes. Based on the results of this study, legumes may not be an appropriate rotation crop in fields where Verticillium wilt is a problem.

Keywords: fungi, fruit, small fruits, disease management, cultural and biological practices, epidemiology, pathogen survival

Verticillium wilt is caused by *Verticillium dahliae*, a soilborne fungus that infects roots and colonizes the xylem of susceptible plants, causing economic damage to many crops. Management of Verticillium wilt is challenged by persistence of the pathogen, in part due to production of microsclerotia, which can survive several years in soil (Wilhelm 1955). Also problematic is a wide host range, which, in California, includes many high value commodities such as lettuce (Bhat and Subbarao 1999), tomato (Tjamos 1981), cotton (Ashworth 1983), and strawberry (Gordon et al. 2006). Effective control of Verticillium wilt in strawberry has been achieved through preplant soil fumigation with a combination of methyl bromide and chloropicrin. Before the adoption of this practice in the 1960s, Verticillium wilt caused devastating losses to the strawberry industry (Paulus 1990).

Presently, Verticillium wilt represents a major limitation in organic strawberry production and is increasingly a concern for conventional strawberry growers, because of changes in the composition of fumigants that are used and the manner in which they are delivered (Gordon et al. 2016). Even where fumigation reduces soil populations of *V. dahliae* below damaging levels, the pathogen likely remains present at low levels. Consequently, once fields are no longer fumigated, Verticillium wilt problems tend to return over time. Where fumigation is not used, or is less than fully effective, management of *V. dahliae* relies on disease-resistant cultivars (Shaw et al. 2010b), soil amendments, crop rotation, and various other cultural practices (Subbarao et al. 2007). Crop rotation can provide a host-free interval, during which soil populations of the pathogen decline by attrition. However, this practice will not be effective if rotation crops support significant development of the pathogen.

The wide host range of *V. dahliae* makes it challenging to identify suitable crops to be grown in rotation with strawberries. Legumes (crops in the family Fabaceae) are appealing in this respect because

with a few exceptions (e.g., Berbegal and Armengol 2009; Ligoixakis and Vakalounakis 1994; Ligoixakis et al. 2002), they are not known to be susceptible to Verticillium wilt. However, the absence of symptoms does not ensure that a crop will not support growth of *V. dahliae*. For example, Mol (1995) reported that *V. dahliae* colonized faba bean (*Vicia faba* ‘Victor’) and field pea (*Pisum sativum* ‘Finale’) and formed microsclerotia in shoot tissue in plants that retained a healthy appearance. Findings such as these indicate that legumes could compromise or entirely negate the benefits of crop rotation in disease suppression.

Little is known about the extent to which legumes used as cover or rotation crops in California can serve as cryptic reproductive hosts to *V. dahliae*. For this reason, the present study was undertaken to evaluate 10 leguminous crops for their potential to support growth and reproduction of *V. dahliae*.

Materials and Methods

Crops. Ten leguminous cover crops commonly grown in California (hereafter referred to as legumes or crops) were evaluated to assess their suitability as reproductive hosts for *V. dahliae* (Table 1). This included seven species cultivated during cool seasons (bell bean [BB], broad ‘Windsor’ bean (aka faba bean [FB]), common vetch [CV], field pea [FP], hairy vetch [HV], purple vetch [PV], and woollypod vetch [WV]), and three species cultivated during warm seasons (black-eyed pea [BEP], sesbania [SES], and sunn hemp [SH]). All seeds were obtained from Peaceful Valley Farm Supply, Grass Valley, CA.

Root-dip inoculations. For each of the 10 legumes to be tested, seeds were sown in 3.79-liter containers of steam-sterilized U.C. Davis potting soil (50% washed sand and 50% sphagnum peat moss, amended with each of the following in grams per liter: Potassium nitrate [0.15], Potassium sulfate [0.15], single superphosphate [1.5], dolomite lime [4.5], and calcium carbonate [1.5]). Pots were maintained in a growth chamber set for day/night temperatures of 15.6/12.8°C, with a 12-h photoperiod and 70% relative humidity. After 10–15 days, seedlings were gently up-rooted and dipped into tap water to remove soil adhering to roots. Thereafter, roots were submerged in a suspension of *V. dahliae* spores for 2 min and repotted into moistened U.C. Davis potting soil, four seedlings per 3.79-liter pot.

Spore suspensions were prepared from an isolate of *V. dahliae* (Vd002) known to be pathogenic to strawberries (Gordon et al.

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Funding: Walmart Foundation, grant no. 28931-03/28932-19.

The author(s) declare no conflict of interest.

Accepted for publication 28 May 2019.

2006), which was grown on plates of potato dextrose agar (PDA) for 2 weeks under fluorescent light at room temperature ($24 \pm 2^\circ\text{C}$). Fully colonized plates of PDA were flooded with 0.5% KCl in sterile water, and a sterile glass slide was used to disrupt mycelium and spores, which were passed through sterile cheesecloth to remove hyphal fragments. Spore density was estimated using a hemacytometer, and adjusted to 1.0×10^6 spores per ml by the addition of sterile 0.5% KCl. To serve as controls, seedlings of each legume were treated in the same manner as inoculated plants but were submerged in tap water for 2 min instead of a spore suspension. Beginning 48 h after transplanting, plants were irrigated as needed with dilute fertilizer containing N:P:K (2:1:2). A replication consisted of one pot with four seedlings, and a trial included four replications of each leguminous species inoculated with *V. dahliae*. For the purpose of visual comparisons to inoculated plants, a single pot of four noninoculated plants was included for each legume in each experiment, which was conducted twice.

Thirty days after inoculation, the dominant (i.e., tallest) stem of each plant was removed and divided into seven equally sized segments. A 2–4 mm length of stem, corresponding to a leaf node, was taken from each segment. Samples from every plant included the first (lowest) true leaf node, the leaf node just below the stem apex, and five intervening nodes. Fresh tissue segments were immersed briefly in sterile water with 0.1% Tween 10, followed by 10 s in 70% ethanol and 2 min in 1% sodium hypochlorite. Owing to succulence of the tissue, time of exposure to sodium hypochlorite was reduced from 2 to 1.5 min for stem samples from four crops (CV, WV, PV, and HV). Surface-treated tissue samples were placed on NP-10 semiselective medium (Kabir et al. 2004) in 90-mm-diameter petri plates and maintained in darkness at room temperature for 3–4 weeks. Tissue pieces were examined under a dissecting microscope and scored as positive or negative for recovery of *V. dahliae*, which was identified by formation of microsclerotia in the agar (Fig. 1) and in tissue (Fig. 2). Mean recovery rates were calculated for each replication of four plants.

Infested potting soil assays. To evaluate the potential for systemic colonization following exposure of roots to soilborne inoculum, plants were grown in artificially infested U.C. Davis potting soil. To produce inoculum, 1 ml of an aqueous suspension of approximately 10^3 *V. dahliae* spores (isolate = Vd002) was spread over the surface of a 125-mm-diameter sterilized filter paper disc (Whatman 1001-125) overlaid on NP-10 medium in a 136-mm-diameter petri plate. After incubating in darkness at room temperature for 3–4 weeks, each filter paper was blended (Waring 700 commercial blender model 31BL46) with 150 ml of sterile, deionized water for approximately 30 s, 3–4 times in succession. The resulting slurry was added to U.C. Davis potting soil and mixed thoroughly to obtain the high inoculum density treatment. Low inoculum density was obtained by mixing a portion of the infested potting soil with fresh, noninfested potting soil. Based on dilution plating, as described by Gordon et al. (2002), low and high inoculum densities were

$1,484 \pm 221$ and $49,225 \pm 16,882$ CFU per gram of soil, respectively, in trial one, and 512 ± 92 and $13,073 \pm 4,660$ CFU per gram of soil, respectively, in trial two. Five seeds of each legume were sown in 3.79-liter pots containing potting soil with either low or high inoculum density, or with no inoculum (controls). Pots were thinned as needed to obtain a total of four seedlings per pot. An experiment consisted of two pots of each treatment for each legume, with four plants per pot (= one replication). The experiment was conducted twice.

Plants were maintained in a growth chamber under conditions described above. After 6–8 weeks, the dominant stem from each plant was divided into five equally sized segments. A 2–4 mm length of stem, corresponding to a leaf node, was taken from each segment. Samples from every plant included the first (lowest) true leaf node, the leaf node just below the stem apex, and three intervening nodes. Samples were surface-treated before culturing on NP-10, as described above. After incubation for 3–4 weeks in darkness at room temperature, cultured plant tissue was examined under a dissecting microscope and scored as positive or negative for the presence of *V. dahliae*, as described above.

Field trial evaluations. Trials were conducted at two locations. One was the University of California Kearney Agricultural Research and Extension Center (KARE) in Fresno County (latitude $36^\circ 36' \text{N}$, longitude $119^\circ 30' \text{W}$, elevation 102 m), where soil is a Hanford soil series, sandy loam. The other site was the University of California at Davis, Plant Pathology research farm (UCD) in Solano County (latitude $38^\circ 31' \text{N}$, longitude $121^\circ 45' \text{W}$, elevation 16 m) with a Yolo soil series, silty clay loam. Seven cool season legumes (BB, FB, CV, FP, HV, PV, and WV) were tested at UCD in 2012 and again in 2013. Three warm season legumes (BEP, SH, and SES) were tested in trials at UCD and KARE in 2013.

Two isolates of *V. dahliae* (SB1442 and Vd9602), used in all trials, were recovered from symptomatic strawberry plants in commercial

Table 1. Ten leguminous crops tested for suitability as hosts to *Verticillium dahliae*

Scientific name	Common name ^y	Abbreviation	Season ^z
<i>Vicia faba</i>	Bell bean	BB	Cool
<i>Vicia faba</i> var. <i>faba</i>	Faba bean	FB	Cool
<i>Vicia sativa</i> spp. <i>sativa</i>	Common vetch	CV	Cool
<i>Pisum arvense</i>	Field pea	FP	Cool
<i>Vicia sativa</i> spp. <i>sativa</i>	Hairy vetch	HV	Cool
<i>Vicia benghalensis</i>	Purple vetch	PV	Cool
<i>Vicia dasycarpa</i>	Woollypod vetch	WV	Cool
<i>Vigna unguiculata</i>	Black-eyed pea	BEP	Warm
<i>Crotalaria juncea</i>	Sunn hemp	SH	Warm
<i>Sesbania marocarpa</i>	Sesbania	SES	Warm

^y Three of the tested legumes had cultivar designations: Field pea = ‘Biomaster’, Faba bean = ‘Broad Windsor’ and Woollypod vetch = ‘Lana’.

^z Season during which crop is typically grown.

Fig. 1. Colony of *Verticillium dahliae* on NP-10 semiselective medium.

Fig. 2. Microsclerotia in stem tissue of purple vetch (A) and sesbania (B).

production fields in California, and demonstrated to be pathogenic to strawberries (Bhat and Subbarao 1999; Lloyd 2015). These isolates were used instead of Vd9602 because a pathogenicity test conducted after the growth chamber experiments showed the latter isolate to have lost virulence to strawberry. SB1442 and Vd9602 were grown separately, as described below, and combined to obtain approximately equal inoculum densities of the two isolates. Microsclerotia were produced by spreading an aqueous suspension of approximately 50 *V. dahliae* spores on NP-10 (Kabir et al. 2004) in 90-mm-diameter petri plates. After incubating in the dark at room temperature ($22 \pm 2^\circ\text{C}$) for 3–4 weeks, colonized agar was placed in a blender (Waring 700 commercial blender model 31BL46) with 150 ml of sterile, deionized water and blended for approximately 30 s, 3–4 times. The resulting homogenate was added to steam-pasteurized sand and left to air dry at room temperature for 4 weeks. Samples of inoculated sand were assayed by soil dilution plating (Gordon et al. 2002) to estimate the number of CFU per gram of sand. Inoculated sand was stored at 4°C and reassayed before use. For the cool season legumes tested at UCD in 2012 and 2013, inoculum densities were 4.2×10^5 and 1.2×10^5 CFU per gram of sand, respectively. For warm season legume trials at KARE and UCD in 2013, inoculum density was 1.5×10^5 CFU per gram of sand at both locations.

All trials were set up as randomized complete block designs with four replications. Each bed included a single plant line, with 30 cm spacing between plants and 30 plants per replicate plot (9.1 m length $\times$ 0.30 m width). Into each planting hole, 150 ml of *V. dahliae*-infested sand was mixed with the top 12 cm of soil. Two seeds of each species were placed in inoculated soil within each planting hole, in each of four replicate plots. Plants were established using sprinkler irrigation, and maintained by furrow irrigation thereafter. The experimental planting was bordered by mixed legumes, two rows on each side and an additional 1.5 m at the beginning and end of each row to serve as a buffer. Fields were managed by the respective research facilities. Weeds were removed by hoeing or suppressed by herbicide application as needed. No preplant or in-season fertilizer was applied.

At 10 weeks after planting, seven plants per plot were removed using a shovel. The first true leaf node was taken from the dominant stem of each plant. Fresh stem segments were treated by brief immersion in sterile water with 0.1% Tween 10 to remove soil, followed by a 10 s dip in 70% ethanol and 2 min in 1% sodium hypochlorite. Surface-treated stem samples were placed on NP-10 medium and maintained in the dark at room temperature for 3–4 weeks. Stem pieces were scored as positive or negative for recovery of *V. dahliae*, as described above.

Data analysis. Data were analyzed using SAS version 9.1. Analysis of variance (ANOVA), as implemented in proc mixed was used to test for significant treatment effects. Where significant F values were obtained, the significance of mean differences was assessed

using Tukey all pairwise comparison test, with the Bonferroni correction for multiple comparisons. The significance of random effects was evaluated with a χ^2 test to compare residual log likelihood values obtained with and without the random term included in the model. Unless otherwise indicated, only random effects that improved the fit of the model were retained. Proportion data were transformed as the arcsine square root of mean recovery rates prior to analysis. Regression analyses were performed using proc reg.

Results

Root-dip inoculations. All control and inoculated plants remained healthy throughout both experiments. No visible symptoms typical of Verticillium wilt were observed. For the analysis of variance, crop, node, and the crop $\times$ node interaction were coded as fixed effects, and all other terms as random effects. An overall ANOVA detected significant main effects of crop ($P = 0.0039$) and node ($P = 0.0002$) on the frequency of colonization by *V. dahliae*. No random terms were significant ($P \geq 0.1386$), with the exception of the crop $\times$ block interaction ($P = 0.0185$), indicating that data could not be pooled across experiments.

In an analysis of data separately by node, the crop $\times$ block interaction was not significant in any case ($P \geq 0.7791$), and the effect of crop was significant for frequency of colonization at each of the seven nodes assayed ($P < 0.0001$). *V. dahliae* was recovered from all seven nodes of every crop, with the exception of hairy vetch and field pea, for which node seven was not colonized (Table 2). Black-eyed pea, sesbania, sunn hemp, and common vetch all sustained a high frequency of colonization, and no significant differences in mean recovery rates were detected among these legumes at any of the seven nodes ($P > 0.05$). Overall, the lowest frequency of recovery was from field pea (Table 2).

Infested potting soil assay. All plants grown in infested soil retained a healthy appearance for the duration of the experiment, with no symptoms of Verticillium wilt at either the low or high inoculum density. At the high inoculum density, all crops except bell bean were colonized, at least at node one. ANOVA showed the effect of crop on frequency of recovery of *V. dahliae* to be significant ($P = 0.0002$) and the effect of node not to be significant ($P = 0.1181$). After removal of random terms from the model, all of which were not significant ($P \geq 0.2367$), data were analyzed separately by node. The effect of crop on recovery of *V. dahliae* was significant at every node ($P < 0.0001$). Sesbania was the most extensively colonized at all five nodes (Table 3), and the frequency of recovery was significantly higher than for BB, FB, FP, HV, PV, and WV at every node ($P < 0.05$). Black-eyed pea was also extensively colonized, with a frequency of recovery at nodes one and two that did not differ significantly from sesbania ($P < 0.05$). At the low end, bell bean was not colonized at any of the five nodes sampled, and woollypod vetch was colonized only at the first node (Table 3). Continuity of colonization across

Table 2. Recovery of *Verticillium dahliae* from seven stem nodes of 10 legumes subjected to root-dip inoculations

Crop ^y	Percentage colonized by <i>Verticillium dahliae</i> ^x						
	Node 1 ^z	Node 2	Node 3	Node 4	Node 5	Node 6	Node 7
BEP	98.3 $\pm$ 0.6 a	75.0 $\pm$ 1.3 a	83.0 $\pm$ 1.5 a	62.9 $\pm$ 3.2 a	61.1 $\pm$ 3.4 a	50.0 $\pm$ 4.0 ab	10.3 $\pm$ 1.4 abc
SES	93.3 $\pm$ 0.8 ab	87.6 $\pm$ 1.1 a	77.8 $\pm$ 1.4 a	75.0 $\pm$ 0.6 a	62.9 $\pm$ 1.1 a	75.0 $\pm$ 1.3 a	33.9 $\pm$ 3.0 ab
SH	91.6 $\pm$ 1.2 ab	85.4 $\pm$ 1.3 a	77.8 $\pm$ 1.1 a	46.7 $\pm$ 1.4 ab	59.8 $\pm$ 0.4 a	53.3 $\pm$ 1.4 ab	49.7 $\pm$ 1.6 a
CV	87.6 $\pm$ 1.0 ab	77.8 $\pm$ 1.2 a	66.1 $\pm$ 1.1 ab	69.1 $\pm$ 0.8 a	62.9 $\pm$ 1.1 a	43.5 $\pm$ 1.4 abc	8.4 $\pm$ 1.2 abc
BB	56.5 $\pm$ 1.4 abc	40.2 $\pm$ 2.3 ab	56.5 $\pm$ 2.3 abc	43.5 $\pm$ 0.6 abc	30.9 $\pm$ 1.9 ab	10.4 $\pm$ 1.4 bcd	6.7 $\pm$ 0.9 abc
HV	50.0 $\pm$ 3.2 abc	37.1 $\pm$ 2.8 ab	8.4 $\pm$ 1.2 bc	5.2 $\pm$ 1.2 bc	1.7 $\pm$ 0.6 b	0.4 $\pm$ 0.4 d	0 c
PV	50.0 $\pm$ 2.7 abc	31.7 $\pm$ 2.4 ab	35.3 $\pm$ 1.4 abc	18.8 $\pm$ 1.1 abc	16.0 $\pm$ 0.9 ab	6.7 $\pm$ 1.4 bcd	2.2 $\pm$ 0.8 bc
WV	46.7 $\pm$ 4.9 abc	40.2 $\pm$ 4.0 ab	40.2 $\pm$ 4.0 abc	34.0 $\pm$ 3.0 abc	22.2 $\pm$ 2.0 ab	12.4 $\pm$ 1.1 bcd	0.4 $\pm$ 0.4 c
FB	43.5 $\pm$ 2.2 bc	30.9 $\pm$ 2.8 ab	30.9 $\pm$ 2.7 ab	22.2 $\pm$ 2.2 abc	12.4 $\pm$ 1.2 ab	10.3 $\pm$ 1.7 bcd	2.7 $\pm$ 1.1 bc
FP	6.7 $\pm$ 1.5 c	3.8 $\pm$ 1.4 b	2.7 $\pm$ 1.2 c	3.8 $\pm$ 1.4 c	2.7 $\pm$ 1.1 b	2.7 $\pm$ 1.1 cd	0 c

^x Percentage of nodes from which *Verticillium dahliae* was isolated ($n = 32$, except for PV for which $n = 28$). Means ($\pm$ standard error) in the same column followed by a common letter are not significantly different at $P = 0.05$, according to Tukey's all-pairwise comparison test with correction for multiple comparisons.

^y Crops tested for colonization by *Verticillium dahliae*: BEP = black-eyed pea, SES = sesbania, SH = sunn hemp, CV = common vetch, BB = bell bean, HV = hairy vetch, PV = purple vetch, WV = woollypod vetch, FB = faba bean, and FP = field pea.

^z Node 1 was the lowest true leaf node and all others were progressively higher on the stem. Node 7 was just below the stem apex.

nodes was evident in all legumes except field pea, in which *V. dahliae* was recovered at a low rate from node five but not from nodes three and four. The pathogen presumably passed through these lower nodes but may not have remained there in a viable form.

Much lower levels of infection were observed in the low inoculum density treatment, and few differences between crops were evident. Analysis of data at this inoculum level showed the effect of crop on frequency of recovery of *V. dahliae* to be significant ($P = 0.0271$), whereas the effect of node was not ($P = 0.4580$). Sesbania and sunn hemp were consistently infected at all five nodes, common vetch and purple vetch were infected at all but node five, whereas field pea was colonized at nodes one to three, and not higher. Woollypod vetch and bell bean were colonized only at node one, and black-eyed pea, faba bean, and hairy vetch were free of infection at all five tested nodes (data not shown).

Field trials. Seven cool season legumes were tested at UCD in 2012 and 2013. Ambient temperatures ranged from 7 to 22°C, with a daily mean of 14°C during the 2012 trial, whereas temperature ranged from 3 to 15°C, with a daily mean of 9°C in 2013. In 2012, *V. dahliae* was recovered from the stem of every crop except bell bean, which was entirely free of infection. In 2013, only hairy vetch and woollypod vetch were colonized, and each of them in only one replication. Lower infection levels in 2013 may have been due to a lower density of inoculum (1.2×10^5 versus 4.2×10^5 CFU per gram of sand in 2013 and 2012, respectively) and lower temperatures during the trial (daily mean of 9°C versus 14°C in 2013 and 2012, respectively).

Owing to the absence of infection in 5 of the 7 legumes in the 2013 trial, analysis was limited to data from the 2012 trial. Based on that data set, the effect of crop on frequency of recovery from the stem was significant ($P = 0.0030$), whereas the effect of replication was not ($P > 0.9999$). The highest rates of recovery were from common vetch and field pea, and the lowest from bell bean (no recovery), faba bean, and purple vetch. Woollypod vetch was intermediate and the mean rate of recovery was not separable at $P < 0.05$ from any of the other legumes (Fig. 3).

Three warm season legumes (BEP, CRT, and SES) were tested in field trials at UCD and KARE in 2013. Ambient temperatures ranged from 14 to 32°C, with a daily mean of 22°C at UCD. At KARE, ambient temperature ranged from 17 to 35°C, with a daily mean of 26°C. Data were evaluated using a two-way ANOVA, with replication nested within location, location, and location $\times$ crop coded as random effects, all of which were not significant ($P \geq 0.2733$). In this overall ANOVA, the effect of crop was not significant ($P = 0.2094$). However, using the model with the best fit, based on the Akaike information criterion (AIC) and the Bayesian Information Criterion (BIC), which excludes the interaction term but retains other random terms,

the effect of crop was significant ($P = 0.0159$). Under the model of best fit, mean frequency of recovery of *V. dahliae* from sesbania ($11.4 \pm 1.1\%$) was significantly greater than from black-eyed pea ($0.2 \pm 0.2\%$) ($P < 0.05$), whereas mean frequency of recovery from sunn hemp ($7.0 \pm 1.1\%$) was not significantly different than either of the other two legumes ($P > 0.05$).

Regression analyses. To assess the value of controlled inoculations in predicting the risk of infection under field conditions, frequency of infection of seven cool season legumes in the 2012 UCD field trial was regressed on the frequency of infection, by node, in both root-dip and infested soil assays conducted in a growth chamber. Results of root-dip assays explained little of the variation observed in the field test, with adjusted R^2 values ranging from -0.0178 based on node seven to 0.221 at node two (Table 4). None of these regression coefficients was significant ($P \geq 0.1090$). Frequency of infection of plants grown in infested soil was more predictive of infection in the field. In this case, adjusted R^2 values ranged from 0.317 at node four to 0.637 at node five, with P values ranging from 0.0042 at node five to 0.0641 at node four (Table 4).

Discussion

Management of diseases caused by soilborne fungi is challenged by persistence of a resident pathogen population. It is usually not possible to eradicate a pathogen that has become established in soil, so management relies on maintaining the population below a damaging threshold. In principle, this can be achieved by avoiding the susceptible crop for one or more years and instead growing crops that are not susceptible to the disease of concern. This practice is predicated on the assumption that rotation crops do not support development of the pathogen. In general, this assumption is justified by the absence of symptoms but without any determination of the extent to which the pathogen may colonize a crop asymptotically. Where such cryptic colonization occurs, the potential benefit of crop rotation may not be realized. This issue is of particular concern for vascular wilt pathogens, such as *V. dahliae*, because crops may sustain systemic infections that do not result in expression of symptoms. This has been shown to occur in both wheat (Krikun and Bernier 1987) and barley (Mathre 1986; 1989). *V. dahliae* has also been shown to colonize stems of several crops grown in rotation with mint and potato (Wheeler and Johnson 2016). In addition, some crops that are susceptible to Verticillium wilt can sustain systemic infections and support production of microsclerotia without developing visible symptoms (Jansky 2009; Shaw et al. 2010a).

Our evaluation of 10 legumes was based on frequency of recovery of the pathogen from stems of plants subjected to root-dip inoculations, and grown in infested soil in a growth chamber and in the field. All 10 legumes supported colonization by *V. dahliae* up through

Table 3. Recovery of *Verticillium dahliae* from stem nodes of 10 legumes grown in infested potting soil^w

Crop ^y	Percentage colonized by <i>Verticillium dahliae</i> ^x				
	Node 1 ^z	Node 2	Node 3	Node 4	Node 5
SES	99.9 $\pm$ 1.9 a	95.0 $\pm$ 3.9 a	95.0 $\pm$ 0.9 a	98.3 $\pm$ 3.7 a	37.7 $\pm$ 2.4 a
BEP	82.1 $\pm$ 1.9 a	89.0 $\pm$ 1.9 ab	53.7 $\pm$ 2.6 b	8.7 $\pm$ 2.3 bc	1.3 $\pm$ 1.1 b
SH	45.5 $\pm$ 2.4 ab	53.1 $\pm$ 3.4 bc	62.6 $\pm$ 4.7 ab	62.7 $\pm$ 4.1 ab	8.9 $\pm$ 1.1 ab
CV	34.0 $\pm$ 1.9 abc	23.9 $\pm$ 1.3 cd	15.4 $\pm$ 1.5 bc	10.7 $\pm$ 1.7 bc	6.9 $\pm$ 1.7 b
HV	9.3 $\pm$ 1.4 bcd	1.7 $\pm$ 0.7 d	1.7 $\pm$ 0.7 c	0.40 $\pm$ 0.40 c	0 $\pm$ 0 b
WV	3.1 $\pm$ 1.3 bcd	0 $\pm$ 0 d	0 $\pm$ 0 c	0 $\pm$ 0 c	0 $\pm$ 0 b
PV	2.7 $\pm$ 1.2 bcd	1.0 $\pm$ 1.0 d	0.40 $\pm$ 0.40 c	0.40 $\pm$ 0.40 c	0.40 $\pm$ 0.40 b
FP	0.56 $\pm$ 0.56 cd	3.6 $\pm$ 1.4 cd	0 $\pm$ 0 c	0 $\pm$ 0 c	3.0 $\pm$ 1.2 b
FB	0.40 $\pm$ 0.40 cd	1.0 $\pm$ 1.0 d	0.40 $\pm$ 0.40 c	1.0 $\pm$ 1.0 c	0 $\pm$ 0 b
BB	0 $\pm$ 0 d	0 $\pm$ 0 d	0 $\pm$ 0 c	0 $\pm$ 0 c	0 $\pm$ 0 b

^w Data are averaged across two experiments at the high inoculum density, which was estimated to be $49,225 \pm 16,882$ per gram of soil, and $13,073 \pm 4,660$ CFU per gram of soil, in experiments one and two, respectively.

^x Percentage of nodes from which *Verticillium dahliae* was isolated ($n = 16$). Means ($\pm$ standard error) followed by a common letter are not significantly different at $P = 0.05$, according to Tukey's all-pairwise comparison test with correction for multiple comparisons.

^y Crops tested for colonization by *Verticillium dahliae*: SES = sesbania, BEP = black-eyed pea, SH = sunn hemp, CV = common vetch, HV = hairy vetch, WV = woollypod vetch, PV = purple vetch, FP = field pea, FB = faba bean, and BB = bell bean.

^z Node 1 was the lowest true leaf node and all others were progressively higher on the stem. Node 5 was just below the stem apex.

node six when plants were root-dip inoculated. Typical symptoms of *Verticillium* wilt, including stunting, foliar chlorosis, vascular discoloration, and wilting, were not observed on any of the infected plants. In contrast, Krikun and Bernier (1987) found an isolate from faba bean to be pathogenic on that host when grown in infested substrate. The contrast with our findings is suggestive of significant variation in virulence among isolates of *V. dahliae*. In addition, Berbegal and Armengol (2009) reported that an isolate of *V. dahliae* from a field regularly rotated between broad beans (aka faba bean) and artichoke (susceptible to *Verticillium* wilt) caused stunting in faba bean, which may indicate that cropping patterns can select for enhanced virulence to this crop. More generally, these findings suggest that strains now seen as benign may become damaging to legumes that presently support extensive colonization without symptoms.

In our study, root-dip inoculations verified the capacity for *V. dahliae* to grow within stems of all 10 legumes that were tested. Growth of the same crops in infested soil revealed less extensive colonization. Under these conditions, which are more in line with how infection would occur in the field, only five of 10 legumes were colonized at all five nodes, and at a lower frequency at higher stem nodes than in root-dipped inoculated plants. In addition, although *V. dahliae* was recovered from the lowest node of a single bell bean plant at low inoculum density, it was not recovered from any plants at the high ID. That bell bean presents a low risk of colonization by *V. dahliae* is reinforced by the lack of recovery from this crop in both field trials in which it was included. Across all seven cool season legumes, regression analysis shows results of infested soil assays to be better than root-dip assays in predicting infection frequency in field tests.

Aside from bell bean, all cool season legumes became infected in the 2012 field trial at UCD. A high frequency of colonization in common vetch (Fig. 3) is consistent with results of both root-dip and infested soil assays conducted in a growth chamber (Tables 2 and 3). On the other hand, whereas field pea was not significantly different from common vetch in the field trial, it was infected at significantly lower frequency in both growth chamber tests. The reason(s) for this discrepancy is not known but could indicate that

colonization of field pea is particularly sensitive to environmental conditions.

For warm season legumes, both growth chamber tests and field trials confirmed that sesbania, black-eyed pea, and sunn hemp can be colonized by *V. dahliae*. Sesbania supported the highest frequency of colonization in growth chamber tests in infested soil, though it was not significantly different than the other two at the first node. Sesbania also supported the highest frequency of stem colonization

Table 4. Regression analysis to assess the predictive value of controlled inoculations for infection of legumes by *Verticillium dahliae* under field conditions

Inoculation method ^w	Node ^x	Regression coefficient ^y	P value ^z
Root-dip	1	0.181	0.1226
Root-dip	2	0.221	0.1090
Root-dip	3	0.079	0.2209
Root-dip	4	0.169	0.1422
Root-dip	5	0.215	0.1123
Root-dip	6	0.179	0.3441
Root-dip	7	-0.178	0.7747
Infested soil	1	0.393	0.0396
Infested soil	2	0.465	0.0237
Infested soil	3	0.341	0.0552
Infested soil	4	0.317	0.0641
Infested soil	5	0.637	0.0042

^w Plants were inoculated either by submersing roots in a spore suspension (= root-dip) or by growing plants in potting soil into which inoculum of *V. dahliae* was incorporated (= infested soil). In both cases, plants were maintained in a growth chamber.

^x The mean frequency of recovery of *V. dahliae* in root-dip inoculated plants at the node indicated served as the "X" value in regression analysis, and mean frequency of recovery from the base of the stem in the 2012 UCD field trial was the "Y" value.

^y Adjusted R² value returned by regression analysis.

^z Significance of the regression coefficient.

Fig. 3. Mean percentage ($n = 28$) of stems from which *Verticillium dahliae* was recovered. Plants were grown in infested soil in a field trial at UC Davis in 2012. Error bars correspond to $2 \times$ the standard error of the mean. Bars denoted by a common letter are not significantly different at $P < 0.05$.

in field trials. Overall, warm season legumes appear to be more prone to colonization by *V. dahliae* than cool season legumes.

The loss of methyl bromide for use in preplant fumigation and expanding production of organically grown strawberries argue for greater attention to the role of cover crops in maintaining populations of *V. dahliae* in soil. Our findings indicate that legumes can pose a risk in this regard. Bell bean appears to represent the lowest risk, but the possibility remains that extant isolates of *V. dahliae* differ in their capacity to infect this crop. It is also possible that regular cropping of legumes where *V. dahliae* is present will select for strains that are more aggressive colonizers of these crops, possibly becoming pathogens.

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