Incidence and Impact of *Verticillium dahliae*-Infested Tare Dirt Associated with Certified Potato Seed Lots

Jeremiah K.S. Dung[1], Philip B. Hamm[2], Jordan E. Eggers[2], and Dennis A. Johnson[1]
[1]Department of Plant Pathology, Washington State University, Pullman, WA
[2]Department of Botany & Plant Pathology, Hermiston Agricultural Research & Extension Center, Oregon State University, Hermiston, OR

Introduction

Verticillium wilt caused by *Verticillium dahliae* is an economically important disease of potato. The fungus causes wilt, chlorosis, and premature senescenceo. *Verticillium dahliae* has a wide host range but can be classified according to vegetative compatibility group (VCG). Most isolates that are collected from potato in the Columbia Basin belong to VCG 4A and are highly aggressive on potato. Initial inoculum of *V. dahliae* for the current growing season is primarily soilborne microsclerotia, which can survive in soils for years. Although *V. dahliae* can be found in the vascular system of infected certified seed tubers (5), vascular infection of seed pieces appears to have little effect on Verticillium wilt symptoms and potato yields (2,3). However, infected seed tubers may be an important way to introduce inoculum to soils not previously used to grow potato, or where a management practice such as fumigation with metam sodium has been applied to reduce soilborne inoculum.

Another potential source of *V. dahliae* inoculum is soil associated with seed tubers, either in the form of soil adhering to the surface of seed tubers ("seed tuber soil") or as loose soil associated with the handling and transport of seed lots ("seed lot soil"). If infested with *Verticillium*, these soils could be additional sources of inoculum in commercial potato fields following fumigation. The purpose of this research was to quantify the amount of inoculum in seed tubers, in soil adhering to the surface of seed tubers, and in loose soil associated with seed lot transport. Isolates were obtained in pure culture when possible and were characterized using VCG tests. Seed lots of Russet Norkotah and Russet Burbank, each with varying amounts of *V. dahliae* infection and soil infestation, were planted in a replicated field trial to assess the impacts on yield and resulting *Verticillium* levels in plants and field soil.

Materials and Methods

Seed Lot Soil Assays

A total of 55, 64 and 108 seed lots intended for production fields in the Columbia Basin of Washington and Oregon were obtained in 2009, 2010 and 2011 respectively. Seed tuber soil (soil adhering to and scraped from the surface of seed potatoes) and seed lot soil (free soil associated with seed lot handling) were assayed for *V. dahliae* infestation. Soils were bulked by seed lot, and in both cases (seed tuber soil and seed lot soil) a 70 to 100 mg sample from each seed lot was weighed and dry-plated onto semi-selective NP-10 medium. Plates were incubated at room temperature for two weeks and visually inspected for *V. dahliae* colonies using a dissecting microscope. The numbers of colony forming units (CFU) per gram of soil were calculated based on the amount of soil plated and the number of colonies observed.

Vegetative Compatibility Group (VCG) Assays

Pure cultures of *V. dahliae* isolates were obtained, when possible, for VCG analysis. The VCG of isolates was determined using nitrate-nonutilizing (*nit*) mutants. Each *nit* mutant was paired with known VCG tester strains at least twice. Plates were checked for VCG complementation at 2-3 weeks and all reactions were recorded, but only tests resulting in strong complementation were considered to be a positive result.

Field experiments

Twelve certified seed lots, six each of cultivars 'Russet Norkotah' (very susceptible) and 'Russet Burbank' (moderately susceptible) were planted in 2010 and 2011. Treatments were arranged as single 50' rows in a randomized complete block design with four replications. Plot areas were fumigated prior to planting. Seed lots were selected because assays indicated they either had low (0-20 CFU/g) or high (> 20 CFU/g) levels of *V. dahliae* in seed tuber surface soil associated with each seed lot. Mid-season sampling of randomly selected stems from each replication and cultivar was performed to measure the level of *V. dahliae* in plant sap. Plots were harvested, graded, and yields were determined. Tare soil associated with tuber harvesting and grading was assayed for *V. dahliae*. Field plot soil was assayed prior to planting and at the end of the season to assess *V. dahliae* levels. Analysis of covariance (ANCOVA) was performed to test for significant interactions between levels of *V. dahliae* in preplant seed tuber soil (the independent variable) and CFUs in field plot soil (the covariate) on stem sap colonization at midseason, pathogen levels in progeny tuber tare soils and field plot soils after harvest, and yield.

Results and Discussion

Soil was not available from the surface of seed tubers in some lots. As a result, a total of 39, 59 and 41 lots were sampled for infested soil on the surface of seed tubers in 2009, 2010, and 2011 respectively. *Verticillium dahliae* was found in 25 out of 39 seed tuber soil samples in 2009, 41 out of 59 seed tuber soil samples in 2010, and 28 out of 41 seed tuber soil samples in 2011 (Fig. 1a). The amount of *V. dahliae* propagules in seed tuber soil varied greatly, ranging from 0 to approximately 2000 CFU/g scraped soil. Approximately 23% of lots yielded between 1 and 20 CFU/g seed tuber soil over the three year period. However, high levels of *V. dahliae* (101-500 CFU/g seed tuber surface soil) were found in several samples in all years, and six samples exhibiting > 501 CFU/g seed tuber soil in 2010. The pathogen was recovered from loose seed lot soil in 46 out of 55 samples in 2009, 51 out of 64 samples in 2010, and 90 out of 108 samples in 2011 (Fig. 1b). Most seed lots exhibited *V. dahliae* levels less than 20 CFU/g loose seed lot soil. However, 15% of loose seed lot soil samples contained levels of *V. dahliae* greater than 50 CFU/g soil. Pre-plant levels of *V. dahliae* in seed tuber soil and seed lot soil were correlated for Russet Burbank ($r = 0.87$; $P < 0.001$) and Russet Norkotah ($r = 0.66$; $P = 0.02$). These results demonstrate the capacity for long-distance movement of *V. dahliae* in soil adhering to the surface of seed tubers and in loose seed lot soil associated with the handling and transport of seed lots.

Vegetative compatibility group analysis indicated that all 16 *V. dahliae* isolates collected from infested seed tuber soil were VCG 4, with 14 isolates belonging to the VCG 4A subgroup and 1 isolate each belonging to VCG 4B and VCG4A/B. All 16 isolates obtained from loose seed lot soil were VCG4A. Some VCG 4A isolates exhibited weak complementation with the VCG 4B tester strains, and vice-versa, but the reactions were not strong enough to warrant a VCG

4A/B designation. The prevalence of VCG4A among isolates collected from seed tuber and loose seed lot soil is consistent with the predominance of this VCG in Columbia Basin potato production. The transport of infested soil associated with seed tubers provides a means for VCG 4A strains to become introduced into areas not previously cropped to potato and, once established, can increase after the cropping of susceptible hosts or cultivars (1).

Significant interactions between *V. dahliae* levels in seed tuber soil and inoculum levels in field plot soils on pathogen populations in stem sap were observed for both cultivars (Fig. 2). *V. dahliae* populations in stem sap increased as inoculum densities in field plot soils increased only when *V. dahliae* levels in seed tuber soil were low. The same relationship was not observed when *V. dahliae* levels in seed tuber soils were high. However, high levels of *V. dahliae* in seed tuber soils resulted in increased stem sap colonization when levels of *V. dahliae* in field soil were low. Increased colonization of potato stems by *V. dahliae* due to infested seed tuber soil may increase the potential for microsclerotia production during plant senescence and increase the amount primary inoculum available for the next season.

Figure 2 also illustrates one effect of cultivar resistance and susceptibility on the impact of *V. dahliae*-infested tare dirt with regards to stem colonization by the pathogen. An infection threshold is represented where the two lines intersect in each graph. The moderately susceptible cultivar Russet Burbank exhibited an infection threshold value of approximately 18 CFU/g soil, which is similar to previously reported values (4). Russet Norkotah, which is very susceptible to Verticillium wilt, exhibited a lower infection threshold than Russet Burbank (approximately 5 CFU/g soil). The lower infection threshold for Russet Norkotah means that this cultivar will be infected more severely and at lower inoculum levels than Russet Burbank. Replacing highly susceptible potato cultivars with more resistant cultivars has the potential to reduce the impact of Verticillium wilt in the future.

Post-harvest levels of *V. dahliae* increased in all plots compared to pre-plant levels (Fig. 3). Field plots planted to seed lots with high levels of *V. dahliae* in seed tuber soil contained significantly greater levels of *V. dahliae* in field plot soils after harvest compared to field plots planted with seed lots containing low levels of the pathogen (P = 0.04) (Fig. 4). Increased inoculum levels immediately following a potato crop may contribute to more severe symptoms if potatoes are planted in succession or planted without the use of disease management tactics such as soil fumigation. Greater levels of *V. dahliae* in field soil were also significantly related to increased levels of *V. dahliae* in post-harvest tare soils collected during tuber harvest and grading (R^2 = 0.50, P < 0.0001). Since *V. dahliae* can be moved from field to field via infested soil associated with harvested tubers, it is important to clean all equipment following the harvest and grading of tubers. This is especially important when harvesting seed tubers intended for distribution to commercial production areas or when equipment is shared among multiple fields.

Significant differences were not observed between high and low levels of *V. dahliae* in seed tuber soil with regards to postharvest levels of *V. dahliae* in progeny tuber soil or yield characteristics using ANCOVA. However, infected tubers and infested soils in certified seed lots may contribute to the spread and establishment of the pathogen in commercial production areas. This is important because microsclerotia of *V. dahliae* can survive for up to 10 years or more, and the low inoculum threshold (as few as 3 to 15 CFU/g of soil) required to reduce yields enough to justify treatment with fumigation in moderately susceptible cultivars like Russet Burbank (4). The long-term benefits of reducing *V. dahliae* in certified seed lots by chemical or other means may outweigh the short-term costs by reducing the introduction of additional inoculum, increasing or extending the effectiveness of soil fumigation, and improving sustainability in commercial potato production. Research is currently underway to reduce the impact of *V. dahliae* contamination on seed.

REFERENCES

1. Davis, J.R., Pavek, J.J., Corsini, D.L., Sorensen, L. H., Schneider, A.T., Everson, D.O., Westermann, D.T., and Huisman, O.C. 1994. Influence of continuous cropping of several potato clones on the epidemiology of Verticillium wilt of potato. Phytopathology 84:207-214.
2. Dung, J. K. S., Ingram, J. T., Cummings, T. F., and Johnson, D. J. 2012. Impact of seed lot infection on the development of black dot and Verticillium wilt of potato in Washington. Plant Dis. 96:1179-1184.
3. Dung, J. K. S., and Johnson, D. A. 2012. Roles of infected seed tubers and soilborne inoculum on Verticillium wilt of 'Russet Burbank' potato. Plant Dis. 96:379-383.
4. Hamm, P. B., Ingham, R. E., Jaeger, J. R., Swanson, W. H., and Volker, K. C. 2003. Soil fumigant effects on three genera of potential soilborne pathogenic fungi and their effect on potato yield in the Columbia Basin of Oregon. Plant Dis. 87:1449-1456.
5. Omer, M. A., Johnson, D. A., and Rowe, R. C. 2000. Recovery of *Verticillium dahliae* from North American certified seed potatoes and characterization of strains by vegetative compatibility and aggressiveness. Am. J. Potato Res. 77:325-331.

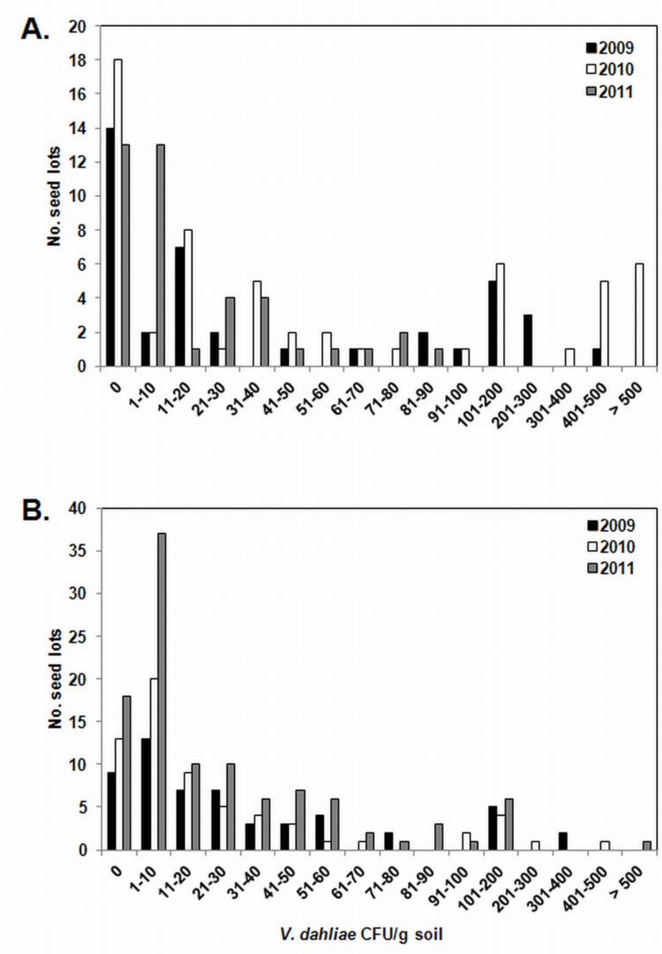

Figure 1. CFUs of *Verticillium dahliae* in (A) seed tuber soil adhered to certified seed tubers and (B) loose seed lot soil collected from bags used to transport certified seed lots intended for Columbia Basin production fields.

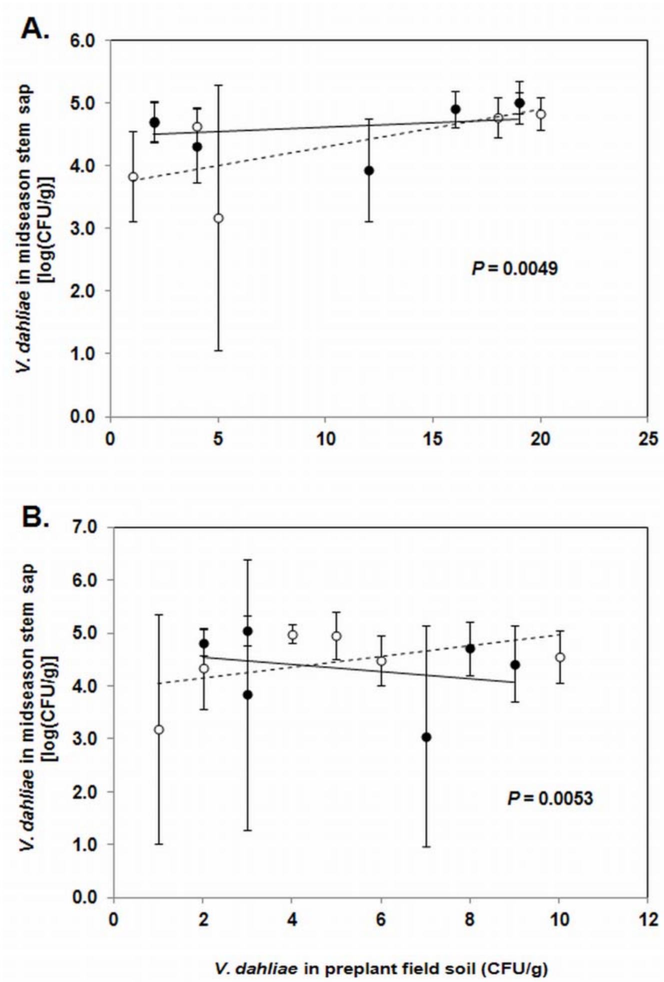

Figure 2. Relationships between CFUs of *Verticillium dahliae* in preplant field plot soil and in stem sap of plants from seed lots of (A) 'Russet Burbank' and (B) 'Russet Norkotah' with high (filled circles) and low (open circles) amounts of *V. dahliae* in seed tuber soil (soil attached to seed tubers). The solid and dotted regression lines represent regressions for high and low concentrations, respectively, and *P*-values indicate significant differences between slopes. Error bars represent standard deviations of means. Data are combined from field plots planted in 2010 and 2011.

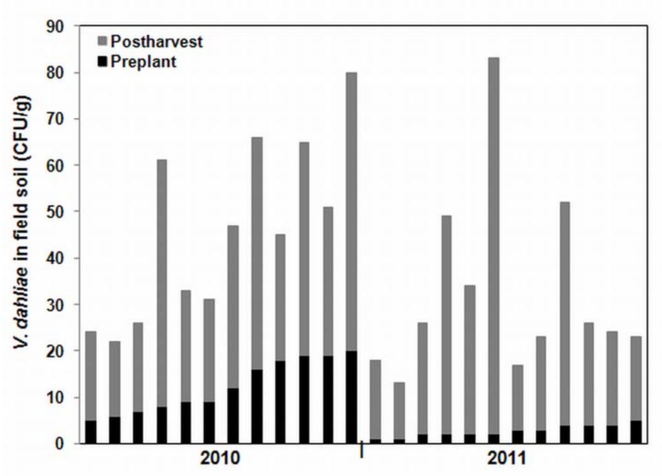

Figure 3. CFUs of *Verticillium dahliae* in field plot soils sampled at preplant and postharvest.

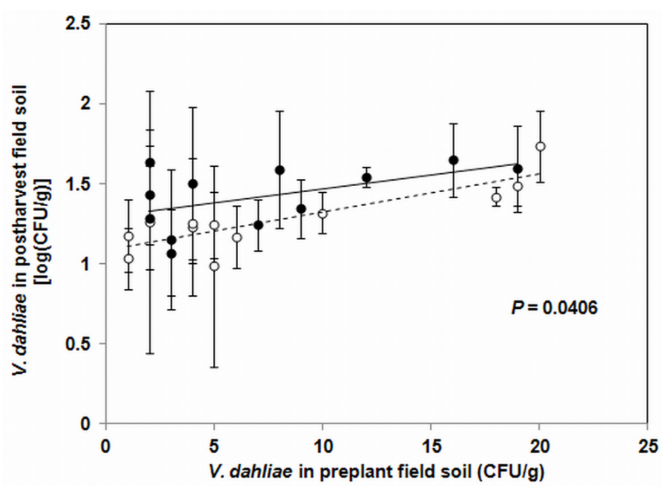

Figure 4. Relationship between CFUs of *Verticillium dahliae* in preplant field plot soil and in postharvest field plot soil after planting seed lots of ‘Russet Burbank’ and ‘Russet Norkotah’ with high (filled circles) and low (open circles) concentrations of *V. dahliae* in seed tuber soil (soil attached to seed tubers). The solid and dotted regression lines represent regressions for high and low concentrations, respectively, and the *P*-value indicates a significant difference between y-intercepts. Error bars represent standard deviations of means. Data are combined from field plots planted in 2010 and 2011.