Relative roles of tuber and soilborne inoculum in the development of Verticillium wilt in the potato cultivar "Russet Burbank"

Jeremiah Dung and Dennis A. Johnson

Department of Plant Pathology, Washington State University, Pullman 99164-6430

INTRODUCTION

Verticillium wilt of potato is a disease of major economic importance to potato growing regions in North America. The primary causal agent of Verticillium wilt in the Pacific Northwest (PNW) is *Verticillium dahliae*, a soilborne fungal pathogen with an extensive host range and widespread distribution. Although no sexual stage is known to occur, genetic diversity does exist in the form of several distinct vegetative compatibility groups (VCG). Isolates from potatoes and PNW potato fields are predominantly VCG 4A and VCG 4B, and VCG 4A isolates have been found to be more aggressive on potato than other VCGs (4, 7, 8). Symptoms of Verticillium wilt of potato include leaf epinasty, wilting and chlorosis, which progress acropetally and often occur unilaterally. Entire stems eventually become necrotic and senesce prematurely, but remain upright. Vascular discoloration in stems and tubers is often associated with VW infection, but may also result from physiological factors or other pathogens and thus is not diagnostic. Reports on the effects of Verticillium wilt on yield are variable, ranging from 12% to 30% or more, and reductions in potato yields are not necessarily correlated with above ground symptoms. Yield reductions and symptom expression are influenced by soil and environmental conditions (9) and can be more pronounced during periods of heat stress and high rates of evapotranspiration. Synergistic interactions between *Pratylenchus penetrans*, the root lesion nematode, and VCG 4A isolates of *V. dahliae* can increase disease severity, lower the disease thresholds of both pathogens and severely reduce yields.

Primary inoculum in soil consists of microsclerotia, which form during plant senescence and can persist in soil for long periods of time. Reported disease thresholds for Verticillium wilt in potato range between 5-30 cfu/cm^3 of soil for *V. dahliae* alone and 2-13 cfu/cm^3 soil when *P. penetrans* is present (9). Microsclerotia are stimulated to germinate in response to host root exudates and hyphae invade the cortex and xylem, where it produces abundant conidia which are systemically translocated through the host vascular system. Root colonization of resistant and non-hosts has also been shown to occur, however the fungus appears to be prevented from extensively colonizing the cortex or xylem (1). A short saprophytic phase occurs at host senescence, during which *V. dahliae* produces conidia and microsclerotia in colonized tissue. Conidia are short-lived and not thought to be significant in disease progress or development. Microsclerotia production is most abundant on aerial stems and colonized host debris can increase inoculum levels if incorporated into the soil. Furthermore, infested soil carried on seed tubers has been shown to contribute to Verticillium wilt symptoms (10, 11).

In addition to soilborne inoculum, intratuber inoculum of *V. dahliae* can be found in the vascular system of certified seed tubers. A 1968-1969 survey detected *Verticillium albo-atrum* and *V. dahliae* in 39% of 244 certified seed lots, with incidence in lots typically between 1-2% and rarely greater than 5% (2). A more recent study of seed lots imported from northern Europe

to Israel between 1995 and 1998 detected *V. dahliae* in 20 to 30% of seed lots at incidences < 5% and found up to 16% of seed lots with *V. dahliae* infection in >5% of seed tubers (12). Surveys of 224 seed lots intended for North American production fields in 1995 and 1996 detected *V. dahliae* in 29% of the lots and 3.6% of the seed tubers, of which 64% of isolates were VCG 4A, 33% were VCG 4B and 3% were VCG 4AB (8). The detection of *V. dahliae* in certified potato seed lots and prevalence of the more aggressive VCG 4A in both seed tubers and PNW fields (7) may have important implications in both seed and production systems as well as the epidemiology and distribution of the disease.

Despite the prevalence of *V. dahliae* in certified commercial seed lots, the contributions of intratuber inoculum in the development and epidemiology of Verticillium wilt are not fully understood. Robinson et al. (10) determined that vascular infection of seed tubers by *V. albo-atrum* did not cause symptomatic plants in several potato cultivars. A 1979 field study found no effect of intratuber infection by *V. dahliae* on plant growth, disease symptoms, yield or quality in the potato cultivar "Norgold Russet" (3), however, severity of wilt resulting from intratuber infection can vary among cultivars (10) and the effects of vascular infection by *V. dahliae* in seed tubers has not been evaluated in "Russet Burbank", the major cultivar under production in the PNW. The objectives of this study were to: (i) determine the relative roles of intratuber and soilborne inoculum in the development of Verticillium wilt symptoms in the potato cultivar "Russet Burbank"; (ii) compare plants grown from apical and basal-end seed pieces cut from infected seed tubers, since vascular infection of progeny tubers presumably occurs via underground stolons; (iii) quantify incidence of vascular host colonization, microsclerotial production on senescent stems and the incidence of infected progeny tubers to assess the potential contributions of seed tuber infection to overwintering inoculum.

MATERIALS AND METHODS

Seed tuber assays. Seventeen seed lots of potato cultivar "Russet Burbank", which is moderately susceptible to Verticillium wilt, were obtained in 2007 from Washington (WA), Idaho (ID), Montana (MT), and Alberta, Canada certified seed sources and assayed for natural *V. dahliae* infection. A total of ten WA, ID and MT certified seed lots were assayed in 2008. A random sample of thirty-five tubers from each lot were thoroughly scrubbed with a sponge under running distilled water and allowed to air dry. A ~15 mm round disk which included vascular tissue was aseptically cut from the stem-end of the tuber and plated onto either modified potato dextrose agar (5), NP-10 medium, or both. Plates were incubated at 23° C for 14 days. Positive identification of *V. dahliae* colonies was verified by sub-plating onto potato dextrose agar when necessary and eleven tubers were selected for use as naturally infected seed piece treatments. Eleven tubers were chosen as noninfected seed piece treatments based on negative results in the plate assays. Care was taken to use tubers in which other fungal pathogens (e.g. *Colletotrichum coccodes, Fusarium* spp.) were not detected.

Disease evaluation. Soil inoculum consisted of rye berries colonized with *V. dahliae* isolate 653 and was prepared. Isolate 653 was isolated from potato and identified as VCG 4A and pathogenic on potato. Berries were ground in a mill and the ground inoculum was quantified via serial dilution. Infested soil treatments were prepared by adding ground inoculum to 5.0 L of Sunshine L2 greenhouse potting mix to achieve a concentration of approximately 10 CFU/cm^3. Ground noninoculated rye was added to the noninfested soil treatments. Approximately 32 g of granular 16-16-16 N-P-K fertilizer was added to each pot prior to planting the seed pieces. The

22 tubers selected in the assays previously described were cut aseptically crosswise and then lengthwise into four equal sized pieces (approximately 60 g) with at least two eyes each. Blocks consisted of four seed pieces derived from one infected tuber and four seed pieces derived from one disease-free tuber; both seed tubers were from the same lot. Apical and basal-end seed pieces were equally divided among seed and soil treatments. Pots were arranged in the greenhouse as a randomized complete block (RCB) design with 11 replicates. The trial was performed once in 2007 and repeated in 2008.

Disease symptoms were assessed at 65 days after planting and approximately weekly thereafter until crop senescence (142 days for the first trial (2007) and 133 days for the second trial (2008)). Plants were evaluated for total percentage of chlorosis and necrosis over the entire plant as well as on a 1-6 scale where 1= no symptoms, 2 = slight chlorosis, 3 = extensive chlorosis (≥ 50% of plant), 4 = extensive chlorosis and necrosis ≥ 25% of plant, 5 = extensive chlorosis and necrosis ≥50% plant, and 6 = dead/nearly dead plant. Disease ratings and total chlorosis and necrosis over time were converted to area under disease progress curves (AUDPC), area under chlorosis progress curves (AUCPC) and area under necrosis progress curves (AUNPC).

Stem sampling and progeny tuber assays. A single aboveground stem from each plant was destructively sampled when plants appeared to be within a week of senescence (after the plant was >80% necrotic but before desiccation of the stem). A one cm section, taken 30 cm above the soil-line, was plated onto NP-10 medium and incubated for one week to detect vertical stem colonization of *V. dahliae.* The remaining stems were left to dry for 3 weeks in their containers and visually assayed for *V. dahliae* microsclerotial colonization using a dissecting microscope. Microsclerotial colonization was recorded as the percentage of stem colonization in relation to total stem length; all remaining stems were assayed and results combined to calculate the mean microsclerotial colonization per plant. A total of seven randomly selected progeny tubers from each plant were assayed for *V. dahliae* infection as described above.

RESULTS

Seed tuber assays. Assays of certified seed lots intended for Washington State production fields detected *V. dahliae* in 35% of lots with 2.0% of tubers infected in 2007 and in 70% of lots with 6.9% of tubers infected in 2008. Incidence of infected tubers within lots ranged from 0 to 11.4% in 2007 and 0 to17.1% in 2008.

Disease evaluation. Mean AUDPC and AUNPC values were significantly ($p \leq 0.05$) higher in potato plants grown in infested soil than in noninfested soil in both trials (Table 1). Significant differences in AUDPC and AUNPC were not found ($p > 0.05$) between plants grown from infected and noninfected seed tubers in noninfested soil. AUCPC was significantly higher for the infected tuber treatment compared to the control treatment in the 2007 trial and mean AUCPC was significantly higher for both infested soil treatments in the 2008 trial, however these differences were not consistent over both years. Significant interactions or additive effects were not detected between intratuber infection and soilborne inoculum in either 2007 or 2008. Analysis of disease progress curves showed that necrosis and chlorosis began earlier in plants grown in infested soil compared to those grown in noninfested soil (Figs. 1 and 2). Differences in disease development were not found in plants grown from apical and basal-end seed pieces cut from infected tubers, however mean AUNPC was significantly higher in plants grown from

basal-end seed pieces during the 2007 trial ($p < 0.03$) and a significant seed piece x soil inoculum interaction was also detected ($p < 0.04$).

A number of experimental units were lost due to bacterial soft rot during the 2008 trial. Plants which failed to emerge were included in the ANOVA as missing data points. An entire block became heavily infested with aphids approximately 100 days after planting and was not included in the analysis, bringing the total number of experimental units down from 88 to 55. Despite the reduction in degrees of freedom, ANOVA results from the 2008 trial were consistent with those from the 2007 trial with regards to AUDPC, AUNPC and re-isolation data.

Stem sampling and progeny tuber assays. Vascular infection of seed tubers by *V. dahliae* did not significantly ($p > 0.05$) contribute to aboveground vascular colonization, progeny tuber infection or microsclerotia production in senescent stems compared to noninoculated controls (Table 2). Plants grown in infested soil exhibited significantly ($p \leq 0.05$) more vascular colonization by *V. dahliae* and produced significantly more *V. dahliae*-infected progeny tubers than infected tubers grown in noninfested soil. Mean microsclerotial colonization was significantly higher in plants grown in infested soil compared to plants grown from infected seed pieces in noninfested soil. Mean microsclerotia colonization of stems originating from infected tubers ranged from 0 to7% while stems obtained from plants grown in infested soil exhibited 0 to 91% mean colonization. *V. dahliae* was not detected in or on any stems or progeny tubers from control treatments. Based on the combined stem assays, incidence of aboveground stem infection in potato plants grown from infected tubers was 21% in the 2007 trial and 13% in the 2008 trial and 100% for plants grown in infested soil in both trials.

DISCUSSION

The role of soilborne microsclerotia as primary inoculum of *V. dahliae* has long been understood, however, the pathogen can also be found in the vascular tissue of certified seed tubers. Several field experiments have demonstrated that, despite the presence of *Verticillium* spp. in certified seed lots (2, 8, 12), intratuber infection has little effect on Verticillium wilt symptoms or potato yields in various potato cultivars. These studies, however, have either used cultivars under limited current cultivation (3), artificially inoculated tubers (11) or *V. albo-atrum* (10) and focused on the effects of tuber infection on aboveground symptoms, yield, quality and vascular discoloration of progeny tubers. In addition, the potential contribution of seed tuber infection to the formation of future inoculum, i.e. microsclerotia, was not previously quantified. The results of this study show that intratuber infection exhibited a negligible effect on the development of Verticillium wilt in the commonly grown but moderately susceptible potato cultivar "Russet Burbank" and does not significantly contribute to aboveground stem infection or the formation of microsclerotia in debris.

Assays of certified seed lots detected higher levels of *V. dahliae* in 2008 compared to 2007. Incidence of *V. dahliae* among and within certified seed lots sampled in 2007 was comparable to previous survey of N. American seed lots (8). The higher incidence of the *V. dahliae* in 2008 may be due to the lack of seed sources from Canada, where the prevalent *Verticillium* species is *V. albo-atrum* (9). Assays performed in 2007 did not detect *V. dahliae* in the four seed lots obtained from Canada, however *V. dahliae* has previously been reported in Canadian-grown seed (8).

Previous studies of *V. dahliae* isolates collected from potatoes and PNW potato fields found the majority of isolates to belong to VCG 4A and VCG 4B, with VCG 4A being highly aggressiveness on potato compared to other VCGs (3, 7, 8). Although infected seed tubers used in this study were not tested for infection by the more aggressive VCG 4A, the results of this study are still of practical importance since naturally-infected tubers were used and the negligible effect of intratuber infection on necrosis and disease progression was definitive for both trials ($p > 0.73$). In addition, the density of soilborne inoculum used (10 cfu/cm^3 soil) was low, especially considering a recent study which found that 37% of PNW fields intended for potato production contained ≥ 10 cfu/g soil and 6% had inoculum densities > 30 cfu/g soil (7). Artificially inoculated tubers were not used since artificial inoculation does not simulate the natural infection process and it is difficult to obtain a sufficient number of tubers for study. Omer et al. (8) demonstrated that nearly two-thirds of infected seed tubers intended for Washington production contained isolates of the more aggressive VCG 4A and approximately one-third contained VCG 4B. It is reasonable to assume a roughly similar frequency of VCG distribution was present in seed tubers used in this study.

A second, unrepeated experiment was performed to provide additional confirmation of the results of the repeated trials. Tubers were taken from control and infested soil treatments in the 2007 trial and assayed for vascular infection as previously described. Infected tubers were taken from artificially inoculated soil and presumed to be infected with *V. dahliae* isolate 653 (VCG 4A). Plants were grown from single-drop seed and soil inoculum administered as previously described. Treatments consisted of infected tubers grown in infested soil and noninfected tubers grown in infested and noninfested soil. Plants were arranged as a RCB in the greenhouse with eight replications of each treatment. Mean AUDPC and AUNPC values, vascular and microsclerotia colonization and progeny tuber infection were consistent with results obtained from the 2007 and 2008 trials using naturally-infected seed tubers obtained from commercial seed lots (data not shown).

The development of necrosis in *V. dahliae*-infected tubers and control plants was similar in both trials, indicating that the senescence observed in infected tubers was natural (Fig. 1). AUDPCs and AUNPCs of treatments grown in infested soil were similar regardless of intratuber infection (Figs. 1 and 2). No interactive effects, either additive or synergistic, were detected between soilborne and intratuber inoculum ($p > 0.05$). Treatment comparisons of mean AUNPC and AUDPC were consistent between trials. Treatment comparisons of mean chlorosis yield different results between trials, indicating that necrosis and/or disease ratings which incorporate both necrosis and chlorosis may provide a more consistent evaluation of Verticillium wilt symptoms. Comparisons of AUNPC values indicate that premature necrosis began between 95 and 105 days after planting in plants grown in infested soil, approximately one to two weeks earlier than plants grown in noninfested soils regardless of intratuber infection.

The importance of soilborne inoculum in Verticillium wilt of potato, both for disease development and long-term survival of the pathogen, has been recognized for quite some time. Nitzan et al. (6) suggested that the distribution of soilborne *C. coccodes* inoculum in the root zone provides more potential points of infection. Although not significant, apical-end seed pieces planted in infested soil resulted in higher AUDPC and AUNPC values than basal-end seed pieces in both trials (data not shown); the likely presence of more eyes on apical-end seed pieces, which can sprout into infested soil and provide more opportunities for infection, provides one

possible explanation. In addition to their distribution in the root zone, microsclerotia in soil are capable of repeated germination and can essentially function as several CFU over time, increasing their infection potential in comparison to conidia and reducing the number of propagules required to cause disease.

Since vascular colonization is thought to be required for symptom development (1) it is not completely understood why infection of *V. dahliae* in potato seed tubers does not result in significant Verticillium wilt symptoms. Vascular colonization of aboveground stems was only detected in a few plants grown from infected tubers, indicating that the pathogen does not readily translocate from tuber vascular tissue to aboveground vascular tissue. Pathogen populations in the vascular system of the tuber may be below the threshold required to colonize growing stems and cause disease. The pathogen may also be compartmentalized in progeny tubers, either during infection, storage or growth, preventing complete colonization of the seed tuber and providing opportunities for sprouting eyes to escape infection. Prior research suggests that vascular infection of seed tubers by *V. dahliae* is often unilateral (1), indicating that if occlusion of the pathogen does occur it is likely during infection or storage. Previous studies on potato have shown varietal differences in the progression and density of vascular colonization by *V. dahliae*, with Verticillium wilt-resistant plants showing less vascular colonization than susceptible ones (1).

The results of this study indicate that intratuber infection of seed tubers of potato cultivar "Russet Burbank" does not significantly contribute to symptoms, progeny tuber infection or inoculum production in plant debris, hence management strategies should focus on soilborne inoculum. Since vascular infection of seed tubers does not appear to significantly result in vascular colonization or the production of microsclerotia in plant debris, infected seed tubers probably do not contribute to soilborne inoculum. The possibility exists that soilborne *V. dahliae* inoculum can be introduced into a field solely from infected seed pieces, which could be important if the fungus, or novel strains of the fungus, are introduced into soils not previously used to grow potatoes or where a management practice such as fumigation has been applied to reduce soilborne inoculum. Since the use of soil fumigants is both costly and subject to future restrictions, other methods of reducing *V. dahliae* propagules in field soils need to be utilized. The use of partial or completely resistant cultivars, which can restrict vascular colonization and subsequent microsclerotia formation by *V. dahliae*, have the potential to both reduce symptoms and limit the amount of inoculum in field soils. Molecular detection methods, such as quantitative polymerase chain reaction, can be utilized to help develop resistant cultivars and monitor pathogen populations in the soil (1). A combination of control methods, including resistance, pre-plant monitoring, crop rotation, green manures, proper sanitation and other cultural practices will likely be necessary to sustainably manage potato production fields affected by Verticillium wilt in the future.

LITERATURE CITED

1. Atallah, Z. K., Bae, J., Jansky, S. H., Rouse, D. I., and Stevenson, W. R. 2007. Multiplex real-time quantitative PCR to detect and quantify *Verticillium dahliae* colonization in potato lines that differ in response to Verticillium wilt. Phytopathology 97 (7):865-872.
2. Easton, G. D., Nagle, M. E., and Bailey, D. L. 1972. *Verticillium albo-atrum* carried by certified seed potatoes into Washington and control by chemicals. Amer Potato J 49 (10):397-402.
3. Hoyman, W. G. 1974. Consequence of planting Norgold Russet seed infected with *Verticillium albo-atrum*. American Potato Journal 51 (1):22-25.
4. Joaquim, T. R., and Rowe, R. C. 1991. Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. Phytopathology 81 (5):552-558.
5. Johnson, D. A., Rowe, R. C., and Cummings, T. F. 1997. Incidence of *Colletotrichum coccodes* in certified potato seed tubers planted in Washington State. Plant Disease 81 (10):1199-1202.
6. Nitzan, N., Cummings, T. F., and Johnson, D. A. 2008. Disease potential of soil- and tuberborne inocula of *Colletotrichum coccodes* and black dot severity on potato. Plant Disease 92 (11):1497-1502.
7. Omer, M. A., Johnson, D. A., Douhan, L. I., Hamm, P. B., and Rowe, R. C. 2008. Detection, quantification, and vegetative compatibility of *Verticillium dahliae* in potato and mint production soils in the Columbia Basin of Oregon and Washington. Plant Disease 92 (7):1127-1131.
8. 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. American Journal of Potato Research 77 (5):325-331.
9. Powelson, M. L., and Rowe, R. C. 1993. Biology and management of early dying of potatoes. Annual Review of Phytopathology 31 (1):111-126.
10. Robinson, D. B., and Ayers, G. W. 1961. Verticillium wilt of potato in relation to vascular infection of the tuber. Canadian Journal of Plant Science 41:703-708.
11. Thanassoulopoulos, C. C., and Hooker, W. J. 1968. Factors influencing infection of field grown potato by *Verticillium albo-atrum*. American Journal of Potato Research 45 (6):203-216.
12. Tsror (Lahkim), L., Aharon, M., and Erlich, O. 1999. Survey of bacterial and fungal seedborne diseases in imported and domestic potato seed tubers. Phytoparasitica 27 (3):215-226.

TABLE 1. Mean AUDPC, AUCPC and AUNPC values for potato cultivar "Russet Burbank" grown from *V. dahliae*-infected and noninfected tubers in the presence and absence of soilborne inoculum.

Inoculum Source(s)		AUDPC		AUCPC		AUNPC	
Soil	Tuber	2007 Trial[a]	2008 Trial	2007 Trial	2008 Trial	2007 Trial	2008 Trial
None	None	217 a	169 a	1165 a	814 a	1749 a	1385 a
None	Infected	219 a	169 a	1544 b	832 ab	1781 a	1475 a
Infested	None	274 b	216 b	1372 b	1048 c	3022 b	1975 b
Infested	Infected	272 b	216 b	1529 b	992 bc	2905 b	1916 b

[a] Teatment means compared with Fischer's protected LSD; values with the same letter indicate no significant difference within the trial ($p > 0.05$).

TABLE 2. Differences in vascular colonization, microsclerotia production and progeny tuber infection between "Russet Burbank" potato plants grown from infected and noninfected seed tubers.

Inoculum Source(s)		Stem Colonization (% isolated at 30 cm above soil-line)		Microsclerotia Colonization (% total length)		Infected Progeny Tubers (% isolated)	
Soil	Tuber	2007 Trial[a]	2008 Trial	2007 Trial	2008 Trial	2007 Trial	2008 Trial
None	None	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
None	Infected	8.3 a	6.7 a	0.5 a	0.3 a	0.0 a	0.0 a
Infested	None	95.8 b	92.3 b	52.5 b	46.1 b	14.3 b	15.4 b
Infested	Infested	95.8 b	92.9 b	47.8 b	41.8 c	13.7 b	13.3 b

[a] Treatment means compared with Fischer's protected LSD; values with the same letter indicate no significant difference within the trial ($p > 0.05$).

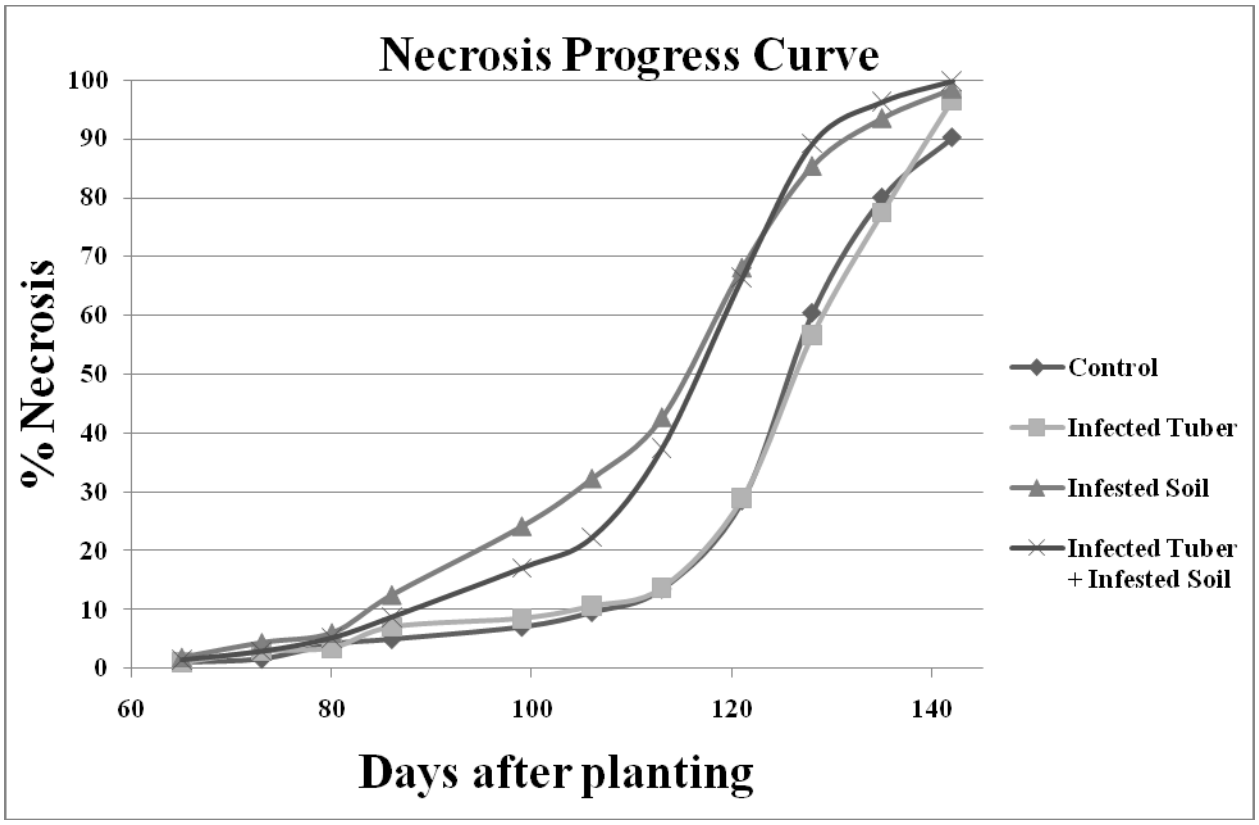

Fig 1. Necrosis progress curves for potato plants grown from *V. dahliae*-infected and noninfected tubers in infested and noninfested soil in 2007.

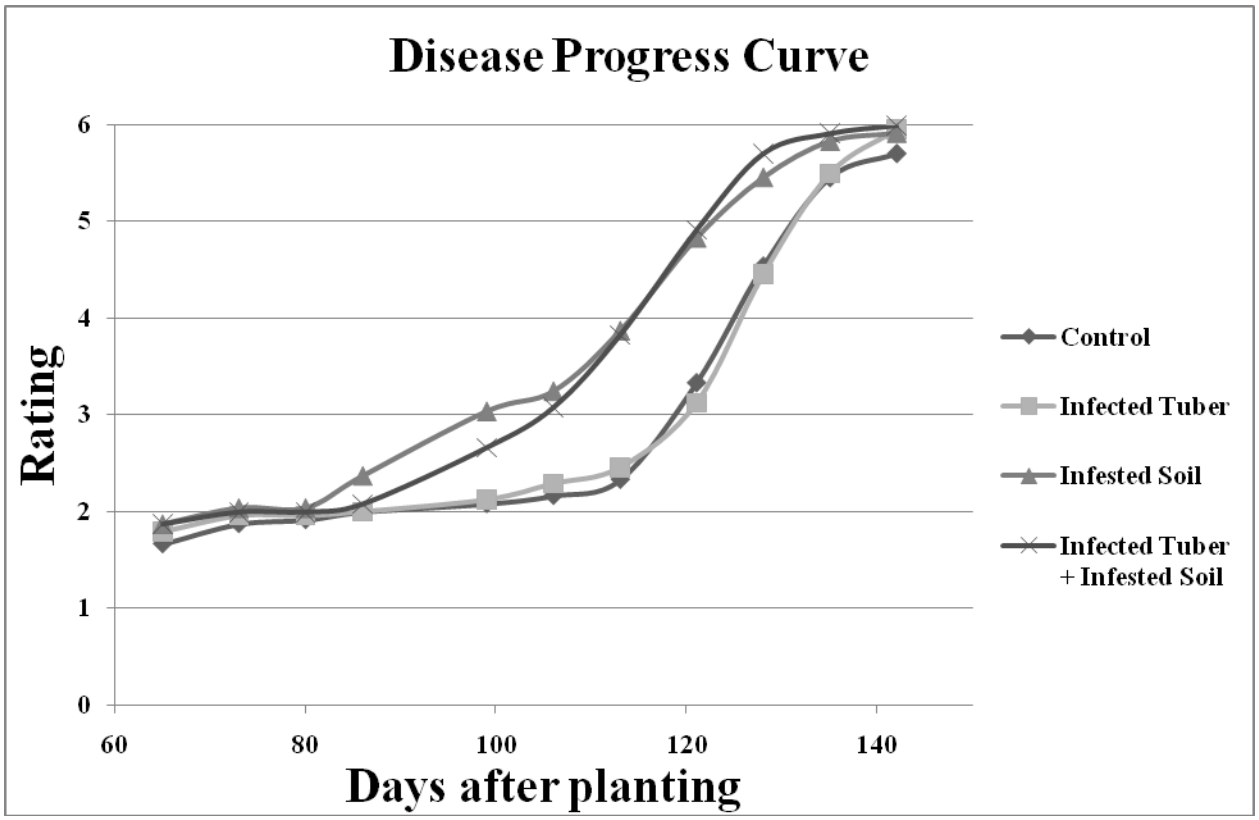

Fig 2. Disease progress curves for potato plants grown from *V. dahliae*-infected and noninfected tubers in infested and noninfested soil in 2007.