Interrelations Between Potato Virus X, *Verticillium dahliae*, and *Colletotrichum atramentarium* in Potato

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ABSTRACT

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The association between potato virus X (PVX), *Verticillium dahliae*, and *Colletotrichum atramentarium* in potato was examined in two center-pivot-irrigated fields in the Columbia River Basin of north central Oregon. One field had been cropped to potatoes 4 of the past 5 yr and the other field had not been previously cropped to potatoes. Plant infection levels by PVX, *V. dahliae*, and *C. atramentarium* were monitored. PVX had no effect on the incidence of infection by *V. dahliae*, but in two of the three cultivar Russett Burbank seed sources tested, infection by PVX was associated with high populations of *V. dahliae* in the potato stems. This association was particularly pronounced in the field previously cropped to potatoes where the early dying disease was severe. Infection and stem colonization by *C. atramentarium* were inversely correlated with PVX infection.

Verticillium dahliae Kleb. can be a major cause of yield reduction, particularly in fields that have been cropped to potatoes for several years (4,14). The importance of seedborne inoculum of *V. dahliae* in potato-growing areas of the Pacific Northwest has not been clearly established. Cases of interactions between *V. dahliae* and other pathogens have been reported (1,6,13,15,19) and a tendency for *Colletotrichum atramentarium* (Berk. & Br.) Taub. and *V. dahliae* to occur together in potatoes suffering from early dying disease has been reported (7,22).

The observations that Verticillium wilt is more severe in nutritionally stressed plants and that potato virus X (PVX) can influence plant vigor (8,18,21) suggest that PVX-infected plants might be more susceptible to colonization by *V. dahliae*. Interactions between fungal and viral diseases have been reported (2,3,11,20). For example, symptom expression in tomato plants infected by *V. dahliae* or *Fusarium* spp. may be modified by the presence of tobacco mosaic virus (23), and PVX decreases the susceptibility of potato tubers to *Fusarium roseum* (16). This report presents the results of a study to identify the association between *V. dahliae*, *C. atramentarium*, and PVX in potatoes.

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MATERIALS AND METHODS

Field plot design. Forty-eight plots ([two treatments] × [two locations] × [three seed sources] × [four replications]) were established in each of two center-pivot-irrigated potato fields in the Columbia River Basin of north central Oregon in 1978. One of these fields, subsequently referred to as the new field, was new to potato production and was selected to serve as a control because soilborne fungal diseases are generally not a yield-limiting factor in first-year potato fields. The second field, subsequently referred to as the old field, had been in potato production for 4 of the past 5 yr with winter wheat as a rotation crop in 1976. In 1977, 1 yr before the initiation of this study, early dying disease had been apparent in 95% of the potato plants in this field 110 days after planting.

Three potato seed lots (A, B, and C) (*Solanum tuberosum* L. 'Russet Burbank'), each from a different source, were planted in the two field locations. Prior to planting, each seed tuber was washed in tap water and surface sterilized for 3 min in a 0.263% sodium hypochlorite solution to eliminate any surfaceborne *V. dahliae*.

The stem end was removed and the remaining portion was cut in half longitudinally to obtain two somewhat bilaterally symmetrical seed pieces that weighed 42–57 g. Sister seed pieces were kept separate for planting at each location. Seed pieces were suberized at 13 C for 6 days prior to planting.

Because the importance of seedborne inoculum of *V. dahliae* in causing disease symptoms is unclear, a comparison was made between *V. dahliae* inoculated and uninoculated seed. Czapek-Dox agar plates were seeded with conidia of *V. dahliae* and incubated at 22 C for 1 mo. Conidia and microsclerotia were harvested by grinding the cultures in a 0.1% methylcellulose solution for 1 min. The inoculum level was adjusted with water to 1,400 microsclerotia per milliliter of suspension (conidia were not counted). Inoculum was applied with a paint sprayer to all surfaces of one half of the sister seed pieces to be planted at each location. The other half of the sister seed pieces were sprayed with 0.1% methylcellulose solution as a control.

The inoculum densities of *V. dahliae* in the fields were determined with an Anderson sampler by using the technique of Butterfield and DeVay (5). Twelve soil samples were taken at a depth of 5–15 cm from each plot immediately prior to planting. The samples were air-dried for 2 wk, subsampled five times and analyzed to determine the number of *V. dahliae* propagules per gram of air-dried soil.

Plots were planted (14 April 1978) in a randomized block design with four replicated plantings at each of the two locations. Sister seed pieces were planted at different locations at 2,150 kg/ha. Each planting of a seed lot consisted of two 6-m rows spaced 86.4 cm apart. Fertilizer and pesticides were applied by commercial production standards by the growers. The new field received (per ha): 457.0 kg N; 280.2 kg P; 336.3 kg K; 11.2 kg Zn; 112.1 kg S; 2.2 kg B; and (for insect control) 4.4 kg Dithane (two applications), and (for weed control) 2.3 L Monitor, 22.4 kg Temik, and 22.4 kg Difonate. The old field received (per ha): 855.29 kg N; 304.9 kg P; 280.2 kg K; 5.6 kg Zn; 44.8 kg S; 1.1 kg B, and (for weed control) 3.5 L Monitor; 22.4 kg Temik; and 4.7 L Bravo (four applications).

Two weeks prior to plot harvest, 25 plants from each of the 48 plots were selected at random and brought back to the laboratory. Fresh stem segments about 6 cm long were cut from the soil line region of the stem. The segments were washed in running tap water and then surface sterilized in a 0.263% sodium hypochlorite solution for 3 min. The epidermis was removed and a 2-cm segment was removed from the middle of the 6-cm stem segment. Each segment was chopped into five or six sections, which were then macerated in an Omni-Mixer homogenizer at 10,000 rpm for 1 min in 50 ml of a 2% polyvinyl pyrrolidone (PVP) 0.2% egg albumin in phosphate-buffered saline (PBS) solution, pH 7.0 (25).

Virus quantification. Tissue homogenates were tested for PVX by using the enzyme-linked immunosorbent assay (ELISA) (25). Anti-PVX immunogammaglobulin (IgG) was purified via stepwise ammonium sulfate precipitation. Partially purified IgG was then passed through a Whatman cellulose DEAE-22 column, which was prepared according to manufacturer's directions and pre-equilibrated with PBS. Fractions were collected and pooled to give an absorbance at 280 nm (A_{280}) of 0.76. Concentration of PVX IgG was obtained by dialysis against PVP (40,000 MW) to an A_{280} of 1.4 and globulin was subsequently conjugated with alkaline phosphatase type VII (Sigma P-4502). Dynatech microtitre plates (Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria, VA 22314) were used for ELISA. PVX IgG coating was used at 1.25 μg/ml. Conjugated PVX IgG was diluted 1:800 before use. Results were evaluated visually and photometrically. A_{405} was determined with a Beckman 25 spectrophotometer. Samples were tested in duplicate with appropriate controls on each plate. Five samples from each plate were retested on an additional plate to obtain a measure of interplate variability.

Fungal quantification. The tissue homogenates used for viral quantification also were used to determine the populations of *V. dahliae* and *C. atramentarium* in the stem tissues. For this purpose, a modification of the technique of Saalting (10) was used. Five-milliliter aliquots of the tissue homogenates were pipetted into warm (44 C) ethanol-water agar containing 100 μg streptomycin sulfate and 50 μg each of chlorotetracycline and chloramphenicol per liter. The mixture was shaken and then poured into five petri dishes that were incubated in the dark for 2–3 wk. Colonies of *V. dahliae* and colonies of *C. atramentarium* were counted and numbers of fungal propagules per centimeter of stem tissue were calculated as an estimate of incidence and amount of stem inoculum of each pathogen.

Data analysis. Koenig (17) indicated that the viral strain being tested may influence the A_{405} of ELISA reactions. Preliminary work proved that our antiserum satisfactorily detected both mild and severe PVX strains. The strain specificity, however, of PVX for ELISA has not been completely determined. Consequently, plants were classified as PVX-free if the absorbance at 405 nm (A_{405}) obtained from ELISA was $\leq 2\times$ the background level. Plants with an $A_{405} > 2\times$ the background level were classified as PVX-infected. Plants with an $A_{405} > 5\times$ the background level were classified as PVX-high and assumed to have a high level of virus concentration. The high-contrast rating scale was used to deemphasize minor variations in the A_{405} values.

The stem populations of *V. dahliae* and *C. atramentarium* propagules were calculated by averaging the number of propagules per centimeter of stem at the stem base for each plant. Point-biserial correlation analysis (12) was used to compare the propagule levels of *V. dahliae* and *C. atramentarium*, respectively, in PVX-infected and PVX-free plants. Percentage of infected plants was examined by chi-square analysis.

TABLE 1. Incidence of potato virus X (PVX), *Verticillium dahliae*, and *Colletotrichum atramentarium* in three Russet Burbank seed potato lots grown at two locations in the Columbia Basin of eastern Oregon

Seed source	Plot location	Infection (%) by: PVX	*V. dahliae*	*C. atramentarium*
A	New field[x]	82.2 a[z]	18.1 a	10.4 a
	Old field[y]	70.6 a	85.9 b	27.8 b
B	New field	46.1 b	23.3 a	9.6 a
	Old field	39.5 b	81.9 b	26.1 b
C	New field	74.3 c	19.9 a	14.0 a
	Old field	62.4 c	90.8 b	24.1 b

[x]New field = first year of potato production.
[y]Old field = 4 of the past 5 yr in potatoes.
[z]Means with different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.

RESULTS AND DISCUSSION

Occurrence of potato virus X, *V. dahliae*, and *C. atramentarium*. The number of plants infected with PVX varied with the seed source. While seed sources A and C produced 76.4 and 68.3% PVX-infected plants, respectively, seed source B produced a significantly lower level of virus infection; eg, 42.8%. The random probability that seed source had no effect on the percentage of virus infected plants was $P = 0.01$. The percentage of PVX infection was significantly greater in the new field regardless of the seed source (Table 1). The effect of location on final total virus infection observed at the end of the season had a random $P = 0.05$. The difference observed between locations was presumably due to different cultural practices at the two locations and the ease with which PVX is mechanically transmitted (9,24).

There was a greater percentage of plants infected by *C. atramentarium* in the old field with an average incidence across all treatments of 26.0% versus an average of 11.3% for plants from the new field (Table 1). There was no significant variation in plant infection by *C. atramentarium* among different seed sources.

The percentage of plants infected with *V. dahliae* also was higher in the old field with an average incidence across all treatments of 86.2% versus an average of 20.4% for plants from the new field (Table 1). These results agree with the characterization of *V. dahliae* infection as a problem associated with continuous potato production. Ninety-five to 99% of the plants in the old field showed symptoms of early dying disease on 15 August. Less than 5% of the

plants in the new field showed symptoms at that same time. There was no increase in percentage of plant infection due to seed-piece inoculation in the old field where the level of inoculum in the soil was high (153 microsclerotia per gram of soil). However, on new ground where the amount of soilborne inoculum was nine microsclerotia per gram of soil, inoculum externally applied to the seed piece was associated with slightly (9.5%) increased *V. dahliae* infections. There was no significant difference in the level of *V. dahliae* infection in the three seed sources.

Coincidence of PVX and fungal infections. Coincidence of PVX and *C. atramentarium* infections in individual plants was examined using a chi-square analysis comparing observed versus expected frequencies of plants coinfected with PVX and *C. atramentarium*. Coincident infection by PVX and *C. atramentarium* occurred at a much lower frequency than would be expected by chance. The probability that the observed incidence was due to random occurrence was $P = 0.01$. No correlation was found between PVX and *V. dahliae* incidence in individual plants ($\chi^2 = 1.4$). This was confirmed by regression analysis on a per plot basis ($R = 0.01$). We conclude that PVX is not capable of influencing *V. dahliae* incidence.

Coincidence of infection by *V. dahliae* and *C. atramentarium*. A tendency was observed for *V. dahliae* or *C. atramentarium* to occur separately in individual plants. Coincident infection was lower than expected in the 1,200 Russet Burbank plants tested ($P = 0.01$). In the old field, only 56.2% of the expected cases of coincident infections by the two fungi were observed. When examined individually, all three seed sources showed more cases of sole fungal infection by *V. dahliae* or *C. atramentarium* and significantly fewer instances of concurrent infection than expected (Table 2). This result is in partial disagreement with observations by others (7,13,22) that the incidence of *C. atramentarium* is increased by the presence of *V. dahliae*.

Internal populations of *C. atramentarium* in individual plants. High levels of propagules of *C. atramentarium* in the stem cortical tissues of potato plants consistently occurred in PVX-free plants in all treatments from both fields. The populations averaged 12,168 and 8,454 propagules per centimeter for PVX-infected and PVX-free plants, respectively ($P = 0.01$). Plants with a high concentration of PVX averaged 7,347 propagules per centimeter of stem at the stem base. This correlation was most pronounced in the old field with a random probability of 0.01 versus a random probability of 0.10 for the new field (Fig. 1). These results are not in keeping with the characterization of *C. atramentarium* as a saprophyte or weak pathogen, which would lead to the expectation of a high level of *C. atramentarium* infection in the less vigorous virus-infected plants. Although unexpected, this correlation may be due in part to a deleterious effect of PVX on plant nutritional levels which have been reported to influence *C. atramentarium* colonization of potato plants (8).

Internal population of propagules of *V. dahliae* in individual plants. Although the presence of PVX did not significantly ($P < 0.10$) influence incidence of plant infection by *V. dahliae*, the presence of PVX may have stimulated growth of *V. dahliae* in the plant; ie, a higher average number of *V. dahliae* propagules was found in the plants infected with PVX (16,281 propagules per

TABLE 2. Observed and expected numbers of plants infected with *Verticillium dahliae* and *Colletotrichum atramentarium* in the three Russet Burbank seed potato lots planted in a field with a history of continuous potato production[x]

Seed source	Infected by: Neither fungus	*V. dahliae*	*C. atramentarium*	Both fungi	*P*
A	28 (40)	87 (75)	28 (16)	18 (30)	0.01
B	23 (42)	115 (96)	35 (16)	17 (36)	0.001
C	10 (24)	140 (126)	21 (7)	24 (38)	0.001
A,B, and C	61 (107)	342 (295)	84 (38)	59 (105)	0.001

[x] Expected number of plants is shown in parentheses.

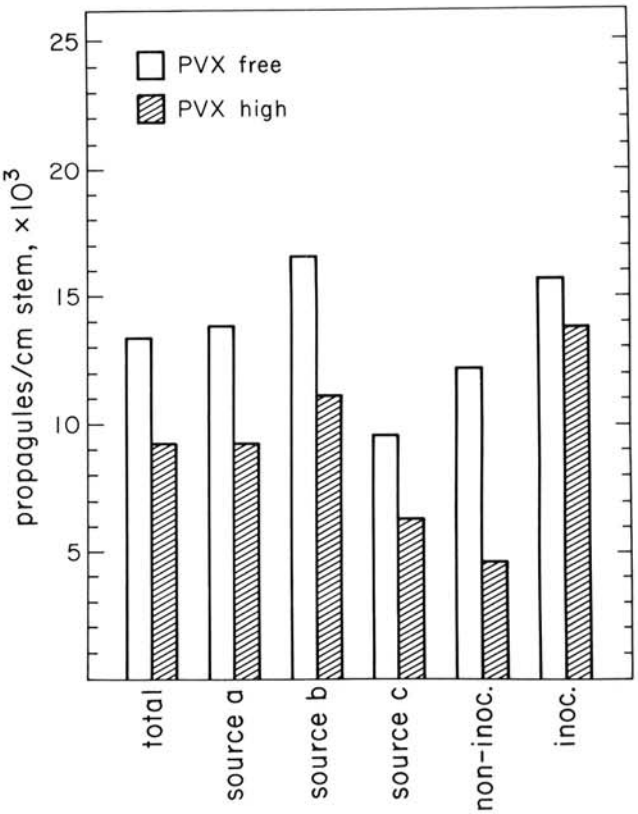

Fig. 1. The effect of potato virus X (PVX) infection on the mean population levels of *Colletotrichum atramentarium* in the stems of infected plants in a field with a history of continuous potato production. Plants designated PVX-high contain a high concentration of PVX as indicated by ELISA.

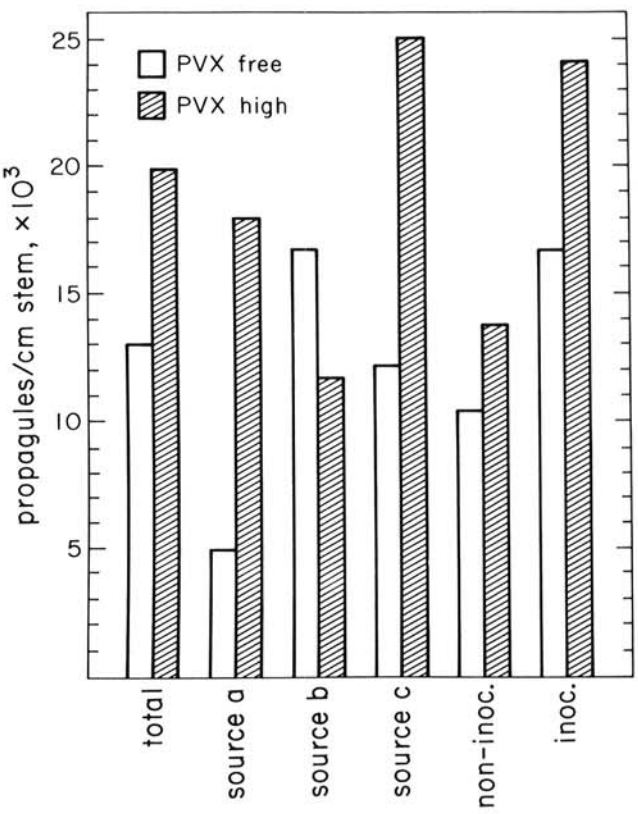

Fig. 2. The effect of potato virus X (PVX) infection on the mean internal population of *Verticillium dahliae* propagules in infected plants in a field with a history of continuous potato production. Plants designated PVX-high contain a high concentration of PVX indicated by ELISA.

centimeter of stem) than in PVX-free plants (12,993 propagules per centimeter of stem). The probability of this correlation occurring by chance was $< P = 0.01$. In seed source B, the mean *V. dahliae* level was higher in PVX-free plants. The difference between *V. dahliae* levels in PVX-present and PVX-free plants was most pronounced in the old field ($P < 0.01$) vs the new field ($P > 0.20$). Inoculation of the seed pieces with *V. dahliae* did not affect the percentage of plants infected with PVX, but did lead to higher internal propagule numbers of *V. dahliae* in PVX-infected plants.

The relationship between PVX infection and the level of internal stem populations of *V. dahliae* propagules was even more apparent when PVX-free plants were contrasted with just those plants that were infected with a high concentration of PVX (Fig. 2). Under these conditions, the overall random probability for the effect of PVX on internal populations of *V. dahliae* was <0.01. .The mean internal stem populations of *V. dahliae* propagules in PVX-free and PVX-high plants were 12,315 and 19,836 per cm at the stem base, respectively. The influence of PVX on growth of *V. dahliae* in potato stems could be due to increased disease susceptibility resulting from a general loss of vitality in the PVX-infected plants. This study does not suggest a causal role for PVX in the early dying disease, but the virus does significantly increase the level of host colonization by *V. dahliae*, a major factor in the yield reduction observed in fields continuously cropped to potatoes.

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