

Distribution and Development of Black Dot, Verticillium Wilt, and Powdery Scab on Russet Burbank Potatoes in Washington State

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ABSTRACT

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Plant samples were collected from 10 commercial fields of Russet Burbank potatoes in south-central Washington and assayed for *Colletotrichum coccodes*, *Verticillium dahliae*, and *Spongospora subterranea* 13 times between 21 May and 29 August 1990. Three fields were likewise surveyed seven times between 30 May and 20 August 1991. Colony-forming units of *C. coccodes* and *V. dahliae* were quantified in sap expressed from stem sections. Powdery scab galls were counted on roots. *C. coccodes* was detected 38 and 15 days after plant emergence in 1990 and 1991, respectively. *V. dahliae* was detected 10-15 days after plant emergence both years, and galls caused by *S. subterranea* were first seen 66 and 47 days after plant emergence in 1990 and 1991, respectively. *C. coccodes* was isolated from plants in all fields by 29 June 1990 and 18 June 1991. *C. coccodes* and *V. dahliae* were isolated from 70-100% of the randomly sampled plants by mid-July and mid-August, respectively, both years. Colony-forming units of *C. coccodes* and *V. dahliae* per centimeter of aboveground stem tissue increased as the season progressed. Infection of potato by *C. coccodes* did not appear to depend on concurrent or preceding infection by *V. dahliae*.

Black dot of potato (*Solanum tuberosum* L.), caused by *Colletotrichum coccodes* (Wallr.) S.J. Hughes, is characterized by the development of abundant, small, black sclerotia on senescent and dead potato roots, stolons, and stems (3,6). The fungus persists in soil, on plant debris, and on tubers (1,5,11). Infection of potato by *C. coccodes* has been considered of minor importance (6,12,15) and has consequently attracted little attention. *C. coccodes* in potato was reported to be associated with *Verticillium dahliae* Kleb. (13), other soil-borne pathogens (7), and environmental stresses (17). However, a recent report (1) and an earlier study (17) demonstrated loss of yield potential and infection of potato foliage by *C. coccodes* (1).

High incidences of black dot by the end of the season were observed by growers in Washington State during the mid-1980s. Incidence of galls caused by *Spongospora subterranea* (Wallr.) Lagerh. f. sp. *subterranea* Tomlinson also increased on roots of the cultivar Russet Burbank in the early 1980s. However, the extent and geographic distribution of these diseases and the time of the season when infection occurred were not recorded. Time of infection and

increase of Verticillium wilt, caused by *V. dahliae*, also are not well understood. The purpose of this work was to determine the geographic distribution and to quantify the development of black dot, powdery scab, and Verticillium wilt in fields of the potato cultivar Russet Burbank in Washington.

MATERIALS AND METHODS

Sampling. Plant samples were collected from 10 commercial fields of the potato cultivar Russet Burbank during 1990 and three fields during 1991. Each plant was assayed for *C. coccodes*, *V. dahliae*, and galls on roots caused by *S. subterranea*. All fields were center-pivot irrigated and located in the Columbia Basin of south-central Washington near George, Moses Lake, Warden, Othello, Connell, Pasco, and Alderdale. This is the major potato-growing region of the state, and fields were selected to represent different parts of the region. Samples were collected from 21 May to 29 August 1990 (13 sample dates) and from 30 May to 20 August 1991 (seven sample dates) at weekly or biweekly intervals.

Planting dates ranged from 26 April to 15 May in 1990 and from 2 to 15 May in 1991. Soil types of sampled fields were sandy loam, loamy sand, fine sandy loam, and silt loam. Number of previous potato crops ranged from one to six; preceding crops were wheat, corn, alfalfa, pea, bean, buckwheat, and potato.

Sampling locations within each field were determined by randomly selecting numbers to choose a wheel track of the irrigation system to be used as a transect

for sampling. The starting point was where the road leading to the center of the pivot system intersected the selected wheel track. Direction of sampling along the wheel track was determined by randomly selecting between left and right. Samples were collected approximately 3 m perpendicular to the wheel track. Adjacent samples were at a distance of 10 paces (about 10 rows) and on alternate sides of the wheel track. Each selected plant was carefully excavated, excess soil was shaken from the roots, and the plant was placed in a plastic bag. Samples were transported to the laboratory and stored at 5 C until the next day when isolations were made. In 1990, 10 plants were sampled per field except on 14 and 29 August, when eight were sampled. In 1991, 20 plants per field were sampled from 30 May to 1 July, 14 plants per field on 16 and 29 July, and 10 plants per field on 20 August.

Assays. Belowground stems and root systems were washed in running water and the number of powdery scab galls were counted (pustules caused by *S. subterranea* do not usually occur on tubers of Russet Burbank in Washington State). Stems were divided at the soil line with a knife and washed in running water for at least 3 min. Additional cuts were made with a sterile knife 1-2 cm above the lower end of the aboveground stem and below the upper end of the belowground stem. Plant segments were divided into two groups for two different assays. The first assay determined the presence of *C. coccodes* and *V. dahliae* and the second assay quantified the number of colony-forming units associated with the sections. Both assays were used to estimate disease incidence.

Qualitative assay. In 1990, two 3-cm pieces were cut from the lower end of the aboveground stem and the entire belowground stem was cut into 1- to 2-cm pieces. The stem pieces were washed for 3 min in running tap water, surface-disinfected for 3 min in 0.5% NaOCl, rinsed in sterile water, and placed in a glass petri dish lined with moistened filter paper. Stem pieces were incubated at 23 C and observed for 2-3 wk for fungal growth. Colonies suspected to be *V. dahliae* were transferred to petri dishes containing potato-dextrose agar and observed for microsclerotia and verticillate conidiophores.

This procedure was modified in 1991. Five 2-cm pieces were cut from random

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sites along 30 cm of the lower end of the aboveground stem and along the entire belowground stem. These were surface-disinfected in 0.5% NaOCl solution for at least 3 min, rinsed in sterile water, and immersed in a 0.015% solution of Gramoxone (20.4% paraquat), a desiccant herbicide, for 45–60 sec (16). Stem pieces were then placed in glass petri dishes with moistened filter paper at 23 C.

Quantitative assay. Plants were washed and a 5-cm segment was cut from the lower end of the aboveground stem and from the upper end of the belowground stem. The segments were washed with running tap water, surface-disinfected in 0.5% NaOCl for at least 3 min, and rinsed in sterile distilled water. Plant sap was expressed by passing the stem sections through an electric roller press consisting of two aluminum shafts, tightly positioned one above the other, that rotated in opposite directions. The shafts were slanted at an angle so that sap adhered to the lower shaft, ran to the lower end, and dropped off into a petri dish. To aid movement of sap into the petri dish, 1 ml of sterile distilled water was pipetted onto the shafts as they rotated. The shafts were thoroughly cleaned between samples with distilled water, disinfected with 70% ethanol, and rinsed with sterile distilled water. Warm (45 C) potato extract agar was poured over the expressed sap, the mixture was agitated to blend sap and agar, and the dish was incubated at 23 C for 3 wk. The agar medium contained 6 g of polygalacturonic acid, 2 g of potassium thiocyanate (KSCN), 1.5 g of monobasic potassium phosphate (KH₂PO₄), 4 g of dibasic potassium phosphate (K₂HPO₄), 15 g of agar, 10 mg of streptomycin sulfate, and 5 mg of penicillin G per liter of water extract from potato tissue. This extract was prepared by boiling 2 g of dried potato leaves in 200 ml of distilled water, filtering the product through four layers of cheesecloth, and adding distilled water to the filtrate to increase the volume to 1.0 L. Colonies of sclerotia of *C. coccodes* and *V. dahliae* growing in plates were counted with a stereomicroscope.

The incidence of plants with powdery scab galls was used to calculate the area under the disease progress curve (AUDPC) (10). Regression analysis for powdery scab incidence in 1990 was done with planting dates as independent variables and AUDPCs as dependent variables. Soil temperature at a depth of 20 cm was measured continuously in 1990 near Othello with a model 105T thermocouple (Campbell Scientific, Inc., Logan, UT).

RESULTS

Symptoms of early dying disease were seen both years in all fields by the end of July. Differences in disease severity were not evident among fields. Neither disease incidence nor severity of the three

diseases studied could be related to soil type, number of previous potato crops, or preceding crop.

C. coccodes was found during both years in all potato fields sampled. Percentage of plants with *C. coccodes* was similar in each field and during both seasons (Fig. 1). In 1990, *C. coccodes* was first isolated on 8 June from belowground stems in two fields, one near

George (Fig. 1) and the other near Moses Lake, 43 and 38 days after crop emergence, respectively. By 29 June, *C. coccodes* was isolated from belowground stems from all fields and from 20–100% of sampled plants. Thereafter, the percentage of plants from which *C. coccodes* was isolated rarely fell below 50 (Fig. 1). Aboveground stems were first sampled in 1990 on 13 July, and *C. coccodes*

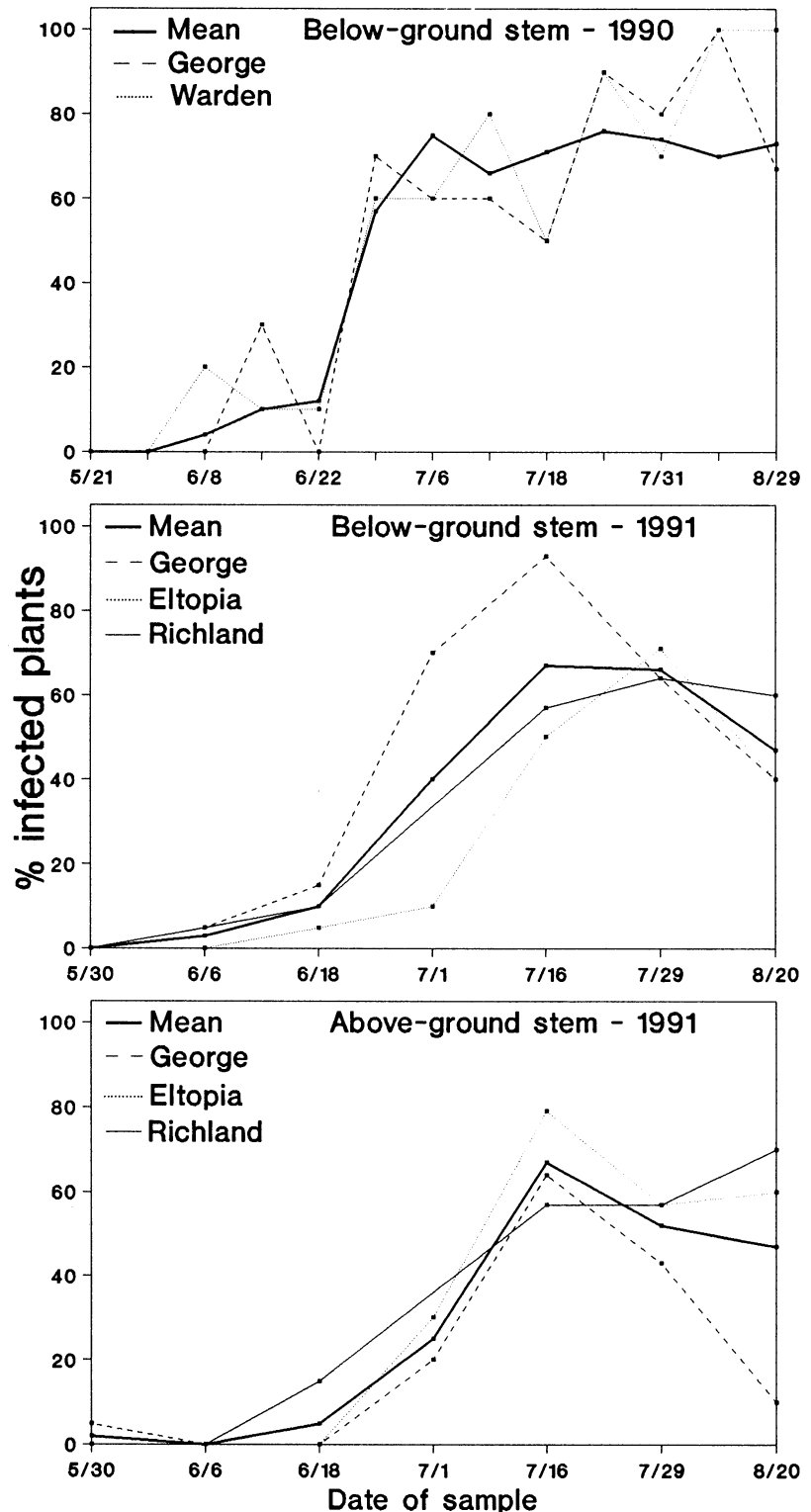


Fig. 1. Percentage of potato plants from which *Colletotrichum coccodes* was isolated from belowground stems from two representative fields and a mean for 10 fields in 1990 and from belowground and aboveground stems from three fields and their mean in 1991.

was isolated from plants from nine of the 10 fields at a mean percentage of 52.

In 1991, *C. coccodes* was first isolated from upper stems on 30 May (15 days postemergence; Fig. 1) and from belowground stems on 6 June (22 days post-emergence). During early July, *C. coccodes* was isolated from plants in all fields, and on 16 July the mean percent-

age of plants in which *C. coccodes* was isolated was above 60 for both aboveground and belowground stems (Fig. 1).

During both years, colony-forming units of *C. coccodes* per centimeter of belowground stem varied from one sampling date to the next, with no consistent trend as the season progressed (Fig. 2). For aboveground stems, mean

colony-forming units of *C. coccodes* increased as the season progressed (Fig. 2). More colony-forming units of *C. coccodes* per centimeter of stem were usually isolated from belowground than from aboveground stems (Fig. 2).

Disease progress curves for the percentage of plants infected with *V. dahliae* were similar for all fields both years. Because isolations of *V. dahliae* from aboveground and belowground stems gave similar disease progress curves, data for only aboveground stems are shown (Fig. 3). The fungus was isolated in the first samples collected from four of 10 fields in 1990 (21 May, 10–26 days after crop emergence) and from all three fields in 1991 (30 May, 15–28 days post-emergence). Subsequent samples early in the growing season showed sporadic occurrences and low percentages of infected plants (Fig. 3). The percentage of *V. dahliae* infected stems increased rapidly in all fields from mid-July to late August and reached a mean incidence of 92% in 1990 (Fig. 3) and of 87% in 1991.

Mean colony-forming units of *V. dahliae* per centimeter of stem showed a gradual increase in all fields through the end of July, then a very rapid rise during August in both years (Fig. 3). More colony-forming units of *V. dahliae* per centimeter of stem were isolated from aboveground than from belowground stems in August.

In 1990 and 1991, 37 and 48%, respectively, of the sampled plants from which either *C. coccodes* or *V. dahliae* was isolated also yielded the other fungus. The most rapid increase in the percentage of infected plants occurred 2 wk earlier for plants infected with *C. coccodes* than for plants infected with *V. dahliae* in 1991 (Fig. 4). The same relationship was observed in 1990 (Figs. 1 and 3).

Galls caused by *S. subterranea* were first noted in 1990 on 6 July; they were found on two of 10 plants in each of two fields near Warden (71 days and 60 days postemergence). During the rest of July, the number of infected plants, the number of fields with diseased plants, and the number of galls per infected plant increased rapidly (Fig. 5). In 1991, galls were first noted on 1 July (47 days postemergence), and all of the sampled plants in the three fields were infected on 20 August 1991, with a mean of 30 galls per plant.

In 1990, three of the 10 fields had few or no plants with powdery scab. These fields were planted 16–24 April and had AUDPC values of 0, 181, and 195. Fields with high levels of powdery scab were planted on 3–16 April and had AUDPC values ranging from 3,435 to 5,605. Regression analysis with date of planting as the independent variable and AUDPC as the dependent variable was significant ($P < 0.05$) and explained 48% ($R^2 = 0.48$) of the variation for AUDPC. By 2 May

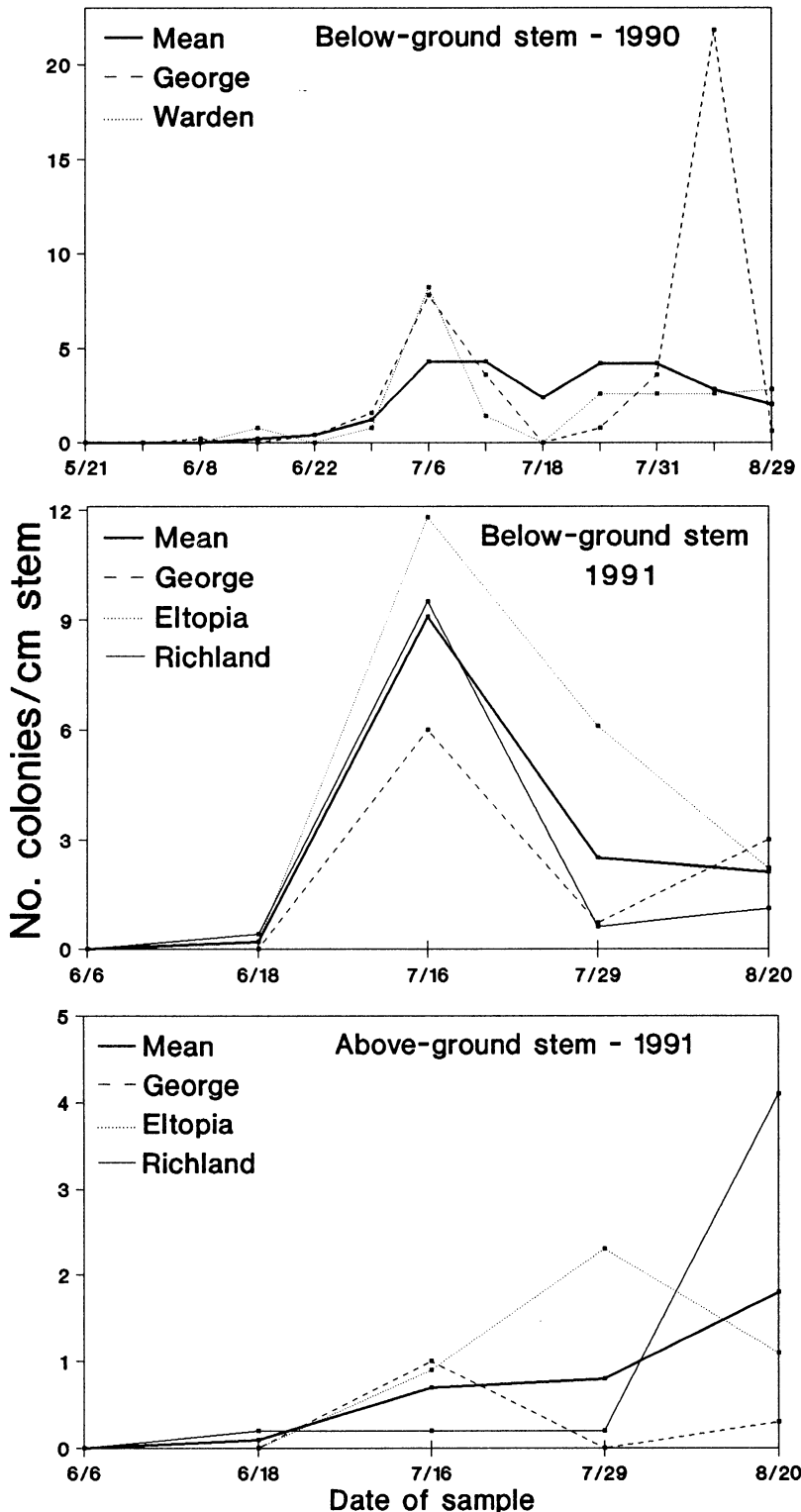


Fig. 2. Number of colonies of *Colletotrichum coccodes* per centimeter of belowground potato stems from two fields and the mean of 10 fields sampled in 1990 and of belowground and aboveground stems from three fields and their mean in 1991.

1990, mean daily soil temperatures increased from a 10-day mean of 12.3 C to daily means of over 14 C.

DISCUSSION

C. coccodes was widespread in potato fields in south-central Washington. The fungus has also been reported to be widely spread in potato fields in Idaho (1), Indiana (17), and North Dakota (13), in some fields of north-central Oregon (7), and in Australia (8).

C. coccodes was isolated early in the growing season and from a high proportion of plants by mid-July in all fields both in 1990 and in 1991. This study showed that *C. coccodes* was associated with potato for most of the growing season. Pathogenicity was not demonstrated, and the fungus was not necessarily isolated from interior tissues of potato stems and could have been epiphytic on stems. However, pathogenicity of *C. coccodes* on potato foliage was recently demonstrated (1). Large values for colony-forming units of *C. coccodes* (*C. atramentarium*) were reported for stem cortical tissues of potato plants grown in north-central Oregon (7), and the fungus was isolated from internal tissues of stems in North Dakota (13). The association of *C. coccodes* with potato plants during most of the growing season and the foliar infections and yield reductions caused by the fungus (1) suggest that *C. coccodes* may play an important role in potato production. This role requires further investigation.

The number of colony-forming units of *C. coccodes* from belowground and aboveground stems showed different patterns over time (Fig. 2). This may reflect distinct phases for the disease belowground and aboveground, and assays should continue to be taken from both as the role of *C. coccodes* in potato is investigated.

Verticillium wilt is a major disease of potato in Washington State. Infection of some plants occurred early in the growing season, and most plants were infected and had large numbers of colony-forming units of *V. dahliae* per centimeter of stem toward the end of the season. A rapid increase in colony-forming units was observed in early August, about 14–17 wk after planting. Hoyos et al (9) also observed a rapid increase in colonization of vascular tissue by *V. dahliae* in Russet Burbank 100–130 days (14.3–18.6 wk) after planting. This increase in colony-forming units over time represents the proliferation of the pathogen within a susceptible host. Hoyos et al (9) also demonstrated that the basal portion of stems was the most consistent site for isolating and quantifying levels of *V. dahliae* in potato (9). We recovered higher levels of *V. dahliae* in August from the base of aboveground stems than from belowground stems and

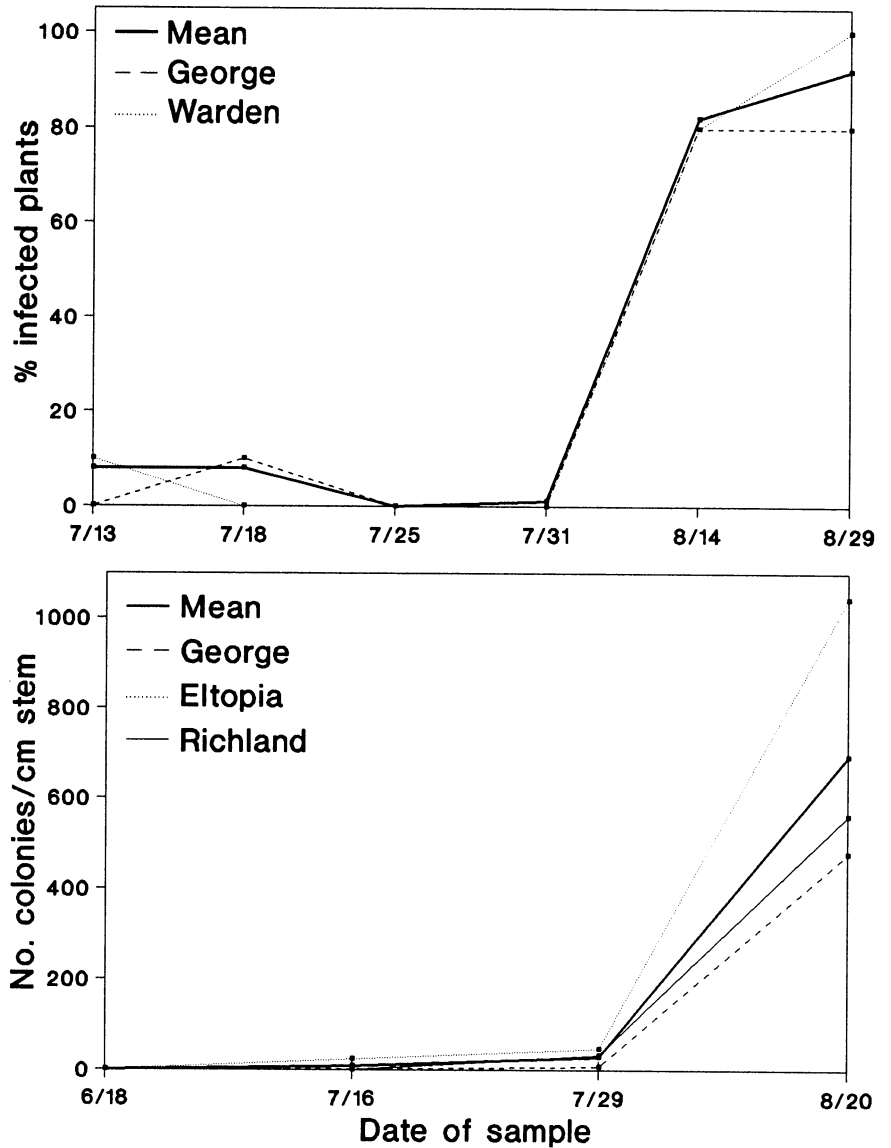


Fig. 3. Percentage of potato plants from which *Verticillium dahliae* was isolated from aboveground stems from two representative fields and a mean for 10 fields in 1990 and number of colonies of *V. dahliae* per centimeter of aboveground stems from three fields and their mean in 1991.

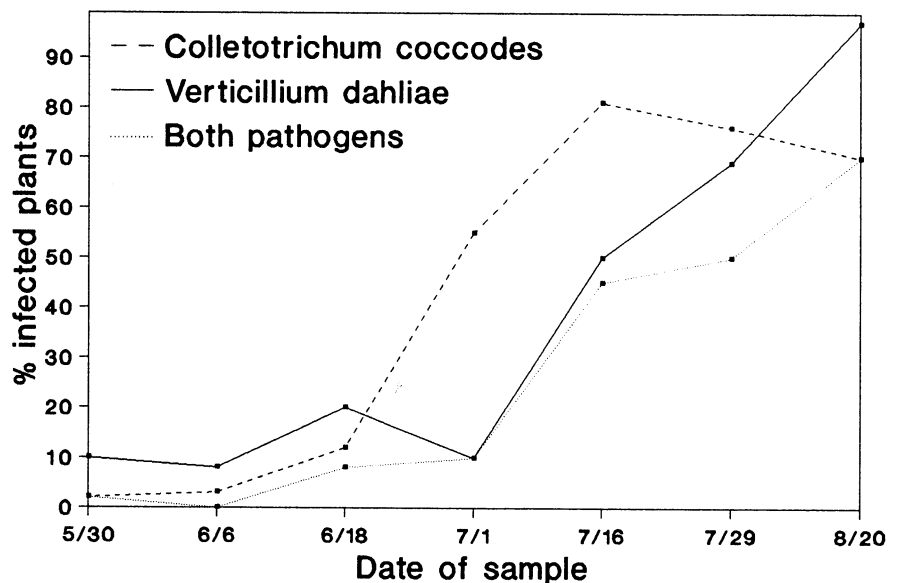


Fig. 4. Percentage of potato plants infected with *Colletotrichum coccodes* only, *Verticillium dahliae* only, and both fungi from three fields in 1991.

consider the former to be a better sampling site. The likelihood of isolating soilborne fungi that are morphologically similar to *V. dahliae* is probably less from aboveground than from belowground stems.

The association of *C. coccodes* with potato did not appear to depend on an infection by *V. dahliae* in that the proportion of plants infected with *C. coccodes* increased and peaked earlier than the proportion of plants infected with *V. dahliae*. In another study (7), coincident infection by *C. coccodes* and *V. dahliae* was less than expected, if infections were associated. However, some researchers (13) have reported that infection by *C. coccodes* was increased by the presence of *V. dahliae*.

The assay procedure used in this study, i.e., extracting sap from fresh tissue, was similar to that suggested by Hoyos et al (9). With this technique, *V. dahliae* in the vascular tissue and *C. coccodes* in or on stems can be quantified during the growing season because fresh tissue is

assayed. Also, relatively large numbers of stems can be sampled.

The wide variation in percentage of plants with *C. coccodes* at different times during the growing season in 1990 was probably due to the small number of plants sampled per field and the random selection of plants in relatively large fields. The mean for all fields in 1990 showed a more consistent rise in incidence of plants with *C. coccodes* after initial detection and peaked in early July. The reduction in incidence of plants with *C. coccodes* in some fields during the end of August in both years may have been due to sampling senescent plants with little sap; our assay procedure depended on plant sap.

Galls caused by *S. subterranea* were not seen until midseason, but infection would have occurred much earlier (2). In another study (2), symptoms on tubers were first noted, depending on cultivar, 23–50 days after artificial inoculation. Fields planted late had fewer infected plants than fields planted early. Cool,

moist seasons are most favorable for development of powdery scab (14). Center-pivot irrigated fields in Washington are usually fairly moist. Soil temperatures increase in early May, so late-planted crops are grown in cool soil for a shorter time, giving *S. subterranea* less time to infect and develop. In another study (2), percentage of tuber infection was less at 15 C than at 10 C. In our study, soil temperatures at Othello were above 14 C by 2 May 1990, and the higher temperatures appeared to reduce scab of potatoes planted later. In Pennsylvania (4), early-planted potatoes also had more powdery scab on tubers than potatoes planted later.

The importance of our study is that *C. coccodes* was shown to be associated with potato relatively early in the growing season and to be associated with a large proportion of plants over a large geographic area. Colonization of potato by *C. coccodes* did not depend on prior infection of plants by *V. dahliae*. These characteristics and the recent work from Idaho (1) indicate that the role of *C. coccodes* in potato production may be much more important than previously thought by some researchers (5,12,15).

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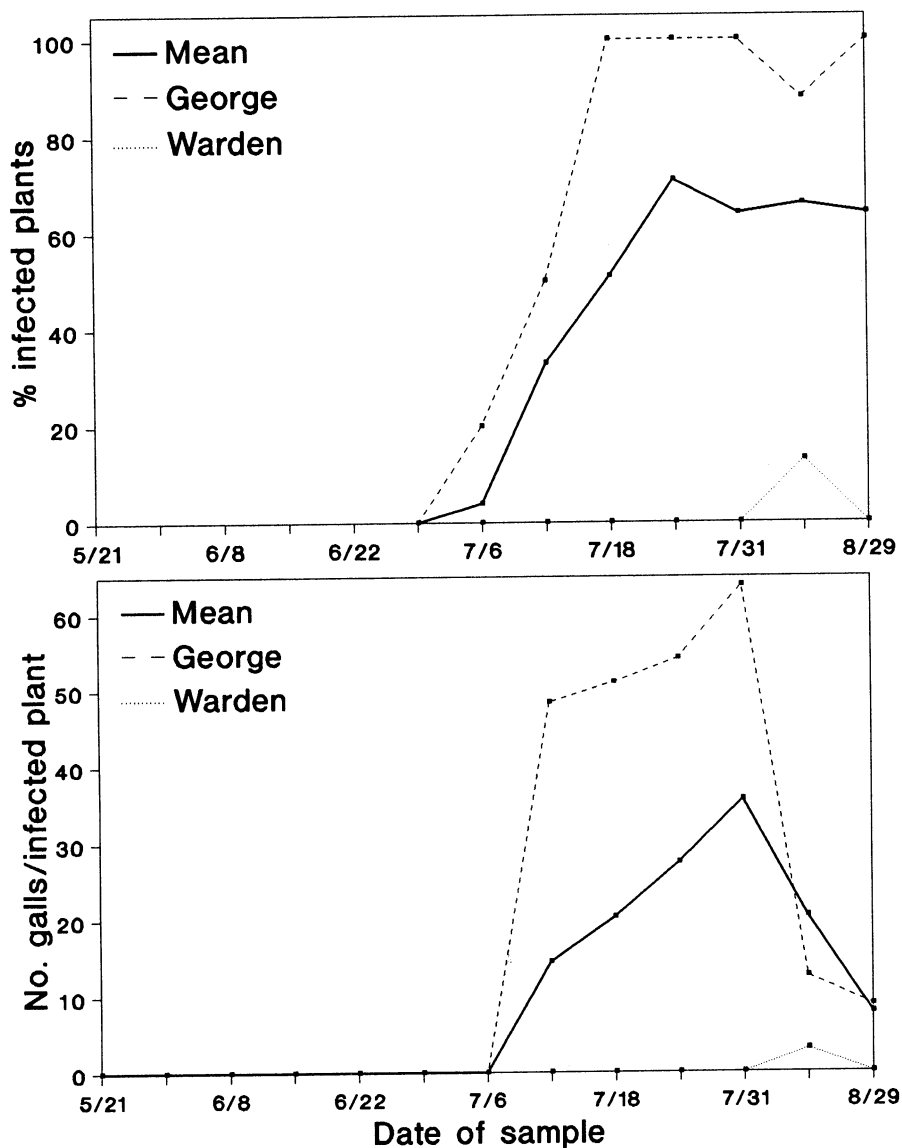


Fig. 5. Percentage of potato plants with galls and number of galls of *Spongospora subterranea* per infected plant in two fields and the mean of 10 fields in 1990.

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