# Effect of Foliar Infection Caused by Colletotrichum coccodes on Yield of Russet Burbank Potato

D. A. JOHNSON, Plant Pathologist, Department of Plant Pathology, Washington State University, Pullman 99164

### **ABSTRACT**

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The effect of foliar infection by Colletotrichum coccodes on yield of Russet Burbank potato was investigated in the greenhouse and field. Foliage of plants in both the greenhouse and field was wounded with sterile sand blown from a sandblaster before inoculation with a spore suspension of C. coccodes. In two experiments in the greenhouse, total yield was significantly reduced by 32 and 19% and mean tuber weight was significantly reduced by 29 and 43%, respectively. In the field, total tuber weight was significantly reduced by 7% in 1991, 12% in 1992, and 11% in 1993. Weights of potatoes graded U.S. No. 1 were significantly reduced by 12% in 1991, 18% in 1992, and 16% in 1993. The number of No. 1 tubers per plant was significantly reduced in the field. Specific gravity was not reduced. Sandblasting by itself did not affect yield.

Colletotrichum coccodes (Wallr.) S.J. Hughes, the cause of black dot of potato (Solanum tuberosum L.), is widespread throughout the potato-growing areas of the world (6,8,12,19,20). Roots, belowground main stems, stolons, tubers, and foliage are infected (1,5,10,14). Minute black sclerotia of the pathogen are commonly found at the end of the season on senescent and dead plant tissue, including decaying roots, stolons, and stems (5).

Infection of potato by C. coccodes has been considered of minor importance (6, 13,18) and has consequently attracted little attention. However, several studies question this assumption. C. coccodes was isolated early in the growing season from potato cv. Russet Burbank and associated with a high proportion of plants in fields throughout central Washington by midseason (11). Tuberborne inoculum has been shown to be a means of disseminating the pathogen (1,12) and of causing early-season plant infection (12). Inoculation with conidia or sclerotia after wounding plant foliage with sterile sand in the greenhouse and field resulted in foliar lesions, chlorosis, and wilting

PPNS No. 0183, Department of Plant Pathology, Washington State University, Project 0674, Agricultural Research Center, Pullman.

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(1,10,14). Work in Idaho (1,14) demonstrated loss of yield and lower quality of tubers after infection of foliage by *C. coccodes*, and an earlier study (19) demonstrated loss of yield when potato was grown in soil infested with *C. coccodes* in the greenhouse.

Additional information is needed on the effect of *C. coccodes* on potato yield and quality (12). The purpose of this study was to quantify the effect of foliar infection by *C. coccodes* on yield and quality of Russet Burbank potato.

## **MATERIALS AND METHODS**

Greenhouse studies. Certified seed of potato cv. Russet Burbank was used to propagate plants for tests in the greenhouse. Seed was washed thoroughly in running water, surface-disinfected in 20% sodium hypochlorite for 15 min, rinsed with running water, and then airdried. Tissue pieces from the stem end of tubers were plated on potato extract agar (11), incubated for 14 days at 21–23 C, and then observed for colonies of *C. coccodes*. Tubers that did not give rise to colonies of *C. coccodes* were used for plant propagation.

Plants were grown in pots 32 cm in diameter and 23 cm in height. Growing buds cut from tubers with a sterile dish-shaped cutter to produce a sphere of tuber tissue weighing about 13 g were used to propagate plants. Soil was a sandy loam from virgin sagebrush land supplemented with 2.5, 4.3, and 3.8 g per pot of actual nitrogen, phosphate, and

potassium, respectively, prior to planting (7). About 30, 45, and 60 days after planting, 0.18 g of actual nitrogen was added to the pots.

The experiments in the greenhouse were done twice. Mean height of plants at inoculation was 31 cm in the first experiment and 50 cm in the second. Plants were wounded before inoculation by making two vertical passes 50 cm from each shoot with a hand-held sandblaster (Speedaire Model 22632A, W. W. Grainger, Inc., Chicago, IL), using 16-grit autoclaved silica sand. Each pass took about 0.5 sec. Air pressure of the sandblaster was 166 kPa, creating a wind velocity of 14.5 km/hr at the plant surface

Isolate C-14 of C. coccodes, obtained from an aboveground stem of a Russet Burbank potato plant from a field near Quincy, Washington, was used. Conidia for inoculation were produced on acid PDA in petri dishes placed under continuous fluorescent light for 7 days at 21-23 C. The conidia were scraped and washed with water from the agar, filtered through four layers of cheesecloth, and then sprayed onto plants in the greenhouse with a mini-spray gun (Model 364-15502, Sears Roebuck and Co., Chicago) at 90 kPa. One drop of Tween 20 per 500 ml of water was added to the inoculum. Concentration of inoculum, as determined with a hemacytometer, was  $11 \times 10^6$  conidia per milliliter of water. After inoculation, plants were placed in a plastic mist chamber for 24 hr and then in the greenhouse. Plants that were wounded but not inoculated were used as controls. Inoculated and noninoculated plants were randomized into nine pairs.

Temperature in the mist chamber was maintained close to 20 C, and greenhouse temperatures ranged from 13 C at night to 27 C during the day. Natural light supplemented with fluorescent lamps (approximately 40  $\mu$ E·m<sup>-2</sup>·s<sup>-1</sup>) provided a photoperiod of 15 hr per day.

The length of inoculated shoots was measured at the time of inoculation, and the number of lesions per linear centimeter of stem was counted 7 days later. Plants were grown for 34 days before and 78 days after inoculation in the first test and for 47 days before and 85 days after inoculation in the second test. Tubers from each pot were hand-harvested, weighed, and counted. Student's t test was used to analyze data.

At harvest, 5-cm stem segments cut 20 cm above the soil line of the aboveground stems and 2-cm stem segments cut 1 cm below the soil line of the belowground stems were assayed for quantity of C. coccodes. The sections 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 and into a petri dish (11). Warm (45 C) potato extract agar (11) 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. Colonies of C. coccodes sclerotia growing in dishes were counted with a stereomicroscope.

Inoculation in the field. Certified seed pieces of potato cv. Russet Burbank were planted in April in a fine silt loam soil at the Irrigated Agriculture Research and Extension Center near Prosser, Washington, in 1991 and 1993 and in a sandy soil near Paterson, Washington, in 1992. Two weeks before planting in 1991, the soil was treated with methyl bromide (448.5 kg/ha) under polyethylene tarps. Potatoes had not been previously grown at the Paterson and Prosser locations in 1992 and 1993. Sweet corn had been rotated with potato for several years at the Prosser location used in 1991. Rows each year were 0.86 m apart, and seed pieces were spaced 0.23 m apart. Plots were single rows 6.1 m long (27 seed pieces per plot) with two buffer rows between plots in 1991 and 1993; buffer rows were not used in 1992. Seed pieces were uniformly planted by hand in 1991 and with an assist feed planter in 1992 and 1993 (9). During the 3 yr of this study, recommended practices of fertilization, irrigation, and pest control were followed (2,7,21). Plots were sprinklerirrigated.

Inoculum was prepared as described for greenhouse inoculations. *C. coccodes* isolate C-14 was used in 1991 and 1992 and isolate C-36, obtained from the vascular tissue of the aboveground stem of a cv. Norkotah Russet plant collected north of Pasco, Washington, in 1992, was used in 1993.

Plants in plots were wounded with 16-grit autoclaved silica sand, using a Speedaire sandblaster at 193 kPa. Individual plants were blasted with sand in a vertical pass for approximately 0.5 sec. The wounded plants in plots were inoculated with a conidial suspension of *C. coccodes* on the evening they were wounded and then sprinkler-irrigated. In 1991 and 1993, plants were sprinkler-irrigated for 13 hr using 2.78-mm nozzles following inoculation. In 1992, plots were sprinkler-irrigated 10 min/hr for 13 hr. Concentration of inoculum was 7 × 106 in 1991 and 1993 and 11 × 106 in 1992.

In 1991, plants were wounded with one vertical pass (light wounding) or two vertical passes (severe wounding) or not wounded before inoculation. Plants in plots were wounded and inoculated on 19 June (early inoculation) and 10 July (late inoculation). Control plants were not wounded and not inoculated, lightly wounded and not inoculated, and severely wounded and not inoculated. All controls were used both dates of inoculation. Treatments were arranged as randomized complete blocks with four replicates.

In 1992 and 1993, plants were wounded with one vertical pass with the sand-blaster or were not wounded. In 1992, plants were not wounded and not inoculated, wounded and not inoculated, and wounded and inoculated. Treatments were the same in 1993 as in 1992 but included plants that were not wounded but were inoculated. Treatments were arranged as randomized complete blocks with six replicates in 1992 and five replicates in 1993.

A 5-cm section about 30 cm from the soil line of an aboveground stem and a 5-cm section beginning about 3 cm below the soil line of the belowground stem of

two plants per plot were assayed to quantify *C. coccodes* in plant sap as described for assays of plants inoculated in the greenhouse.

Plots were harvested on 3 September 1991, 10 September 1992, and 15 September 1993 with a single-row potato harvester. Tubers were graded and weighed and specific gravity was determined each year. Tubers weighing more than 113 g in weight and without major deformity were graded U.S. No. 1. Tubers were counted in 1991 and 1992 but not in 1993.

Data from the field were analyzed as a randomized complete block design using analysis of variance. Data for colonies of C. coccodes per centimeter of stem were transformed to  $\log(1+x)$  to standardize variances before analysis. Predetermined single degree of freedom contrasts were used to compare the non-wounded/noninoculated treatment with the wounded/noninoculated treatment with the wounded/noninoculated treatment with the wounded/noninoculated and nonwounded/noninoculated treatments.

#### RESULTS

Lesions and chlorosis developed on wounded and inoculated plants in the greenhouse and field as previously described (6). Wounds on noninoculated plants appeared as white flecks less than 0.3 mm in diameter. C. coccodes was isolated from lesions on stems, petioles, and leaflets of the wounded and inoculated plants in the greenhouse and field but not from wounded and noninoculated plants.

Mean numbers of lesions per centimeter of stem on the wounded and inoculated plants in the two tests in the greenhouse were 4.87 and 4.91, respectively. Lesions did not appear on wounded but noninoculated plants. The number of colonies of C. coccodes from plant sap was significantly higher (P = 0.05) for the wounded and inoculated plants than for the wounded and noninoculated plants in the first test but not in the second (Table 1). Mean total weight of tubers (P = 0.01 and 0.05) and mean weight of tubers (P = 0.05) were significantly less for the wounded and inoculated plants than for the wounded and noninoculated plants (Table 1). The number of tubers per plant did not vary significantly between the two treatments (P = 0.05). Percentage of reduction for the inoculated and noninoculated treatments was 32.2 and 18.7%, respectively, for mean total weight and 29.2 and 43%, respectively, for mean weight of tubers.

In the field, 5-15% of the surface area of plants wounded and inoculated had lesions and chlorosis, whereas these symptoms were not observed on plants that were not wounded and not inoculated in all 3 yr or that were not wounded and inoculated in 1993. The number of

Table 1. Number of colonies of *Colletotrichum coccodes* per centimeter of stem and yield of Russet Burbank potato when foliage was wounded with blowing sand and inoculated or not inoculated in the greenhouse<sup>a</sup>

Experiment	Colonies/cm of stem	Total weight (g)	Mean weight of tubers (g)	
First				
Wounded/not inoculated	2.9	256.1	48.0	
Wounded/inoculated	13.1*b	173.7**	34.0*	
Second				
Wounded/not inoculated	1.7	263.2	62.1	
Wounded/inoculated	3.5	214.1*	35.4*	

<sup>&</sup>lt;sup>a</sup>Values are the means of nine replications.

<sup>&</sup>lt;sup>b</sup>Significantly different from noninoculated treatment at \* = P = 0.05 and \*\* = P = 0.01 according to Student's *t* test.

colonies of *C. coccodes* per centimeter of stem from sap of plants wounded and inoculated in the field was significantly higher (P = 0.01 in 1991 and 1992 and P = 0.05 in 1993) than from sap of plants not wounded and not inoculated (Table 2).

In the field in 1991, extent of wounding (light vs. severe) and time of inoculation (early vs. late) had no effect on yield. There was no significant difference in yield between the nonwounded/noninoculated treatment and the wounded/noninoculated treatment in any of the 3 yr (P = 0.05).

Total weight was significantly less (P = 0.05) in 1991 and 1992 and weight of tubers greater than 113 g (No. 1 grade) was significantly less (P = 0.05) in all 3 yr for plants that were wounded and inoculated than for plants that were not inoculated (Table 2). In 1993, total weight was significantly less (P = 0.06)for wounded and inoculated plants than for noninoculated plants. The number of tubers greater than 113 g per plant was significantly lower (P = 0.05) for wounded and inoculated plants than for noninoculated plants (Table 2). The total number of tubers per plant and the specific gravity of tubers did not differ significantly between the inoculated and noninoculated treatments (P = 0.05). For the 3 yr, total weight was reduced 7, 12, and 11%, respectively, and mean weight of No. 1 tubers was reduced 12, 18, and 16%, respectively.

## **DISCUSSION**

Recent research conducted in the greenhouse and field demonstrated that infection of potato foliage by C. coccodes resulted in foliar lesions, blight, chlorosis, and wilt (1,10,14). A high proportion of isolates of C. coccodes inoculated onto potato foliage was pathogenic (1,10) and was reisolated from internal stem tissue (10). Barkdoll and Davis (1) demonstrated yield reductions after foliar infection of potato by C. coccodes, and this work confirmed a loss in total yield and yield of No. 1 tubers due to C. coccodes. In an earlier study (19), tuber yield and plant growth were markedly reduced in the greenhouse when field soil was infested with sclerotia of C. coccodes.

A larger reduction in yield may have been observed in this study if C. coccodes had been entirely eliminated from plants in plots that were not inoculated. C. coccodes was isolated from plant sap of noninoculated plants, but lesions were not observed on these plants. The background level of C. coccodes could have come from seed tubers, soil, and windblown conidia and sclerotia (1,12,16). Results on yield are valid because larger quantities of the fungus were always recovered from inoculated plants than from noninoculated plants and because disease symptoms were always associated with plants that were wounded and inoculated. Large pots that did not restrict

Table 2. Number of colonies of *Colletotrichum coccodes* per centimeter of stem and yield of Russet Burbank potato when foliage was wounded or not wounded with blowing sand and inoculated or not inoculated in the field<sup>a</sup>

Year, location Treatment	Colonies/cm of stem	Yield (kg/plot)		No. of tubers/plant	
		Total	No. 1 grade	Total	No. 1 grade
1991, Prosser					
Not wounded/not inoculated	2.2	40.5	31.2	6.7	4.6
Wounded/not inoculated	1.6	41.2	31.2	6.7	4.5
Wounded/inoculated	9.9** <sup>b</sup>	37.9*	27.6*	6.5	4.1*
1992, Paterson					
Not wounded/not inoculated	0.2	30.6	24.4	9.6	5.9
Wounded/not inoculated	0	28.6	22.5	9.3	5.5
Wounded/inoculated	2.4**	26.2*	19.3**	8.9	4.9*
1993, Prosser					
Not wounded/not inoculated	0.1	40.5	27.0		
Not wounded/inoculated	6.9**	39.2	26.8		
Wounded/not inoculated	0.8	40.6	28.6		
Wounded/inoculated	2.6*	36.2	23.4*		

<sup>&</sup>lt;sup>a</sup> Values are the means of four, six, and five replications in 1991, 1992, and 1993, respectively. <sup>b</sup> Significantly different from noninoculated treatment in wounded or nonwounded category at \* = P = 0.05 and \*\* = P = 0.01 according to single degree of freedom contrasts.

root and tuber growth were used to grow plants in the greenhouse, and the relative differences in yield between inoculated and noninoculated plants presumably reflect potential differences in the field.

C. coccodes has been referred to as an elusive pathogen of potato because damage is not readily associated with symptoms unless there are careful and timely observations (20). Barkdoll and Davis (1) observed yield loss of potato with very little symptom expression. Symptoms may also be overlooked because they are frequently expressed late in the growing season and confused with natural senescence in potato (1) as well as weed hosts of C. coccodes (17).

Latent infection in tomato by C. coccodes is documented (4,16). The isolation of C. coccodes from internal stem tissue of potato (10,15) and potato stem segments throughout the season (11) by a technique specifically for detection of latent infections by Colletotrichum spp. (4) suggests that latent infections by C. coccodes are possible in potato. The rapid development of C. coccodes sclerotia on a large proportion of senescent and dying potato stems suggests latent infection or prior colonization (3). Establishment in host tissue through parasitism gives the parasite a competitive advantage over saprophytes for the substrate when the host dies (3). The extent of latent infection in potato crops and its effect on yield and quality need to be established.

C. coccodes has the potential for infecting potato foliage and reducing yields in the semiarid regions of south-central Washington and southern Idaho because the fungus is widely distributed in this area, frequent windstorms early in the growing season may increase infections, and sprinkler irrigation is used (1). The association of C. coccodes with a high proportion of potato plants in south-central Washington by midseason (11)

and the reduction in yield reported earlier (1,19) and in this study support the above hypothesis.

The need for more research on the effect of *C. coccodes* on potato production has been acknowledged for many years (6,8,12). The results reported in this and earlier papers (1,10,11,14,19,20) demonstrate a broader potential of *C. coccodes* as a potato pathogen than generally accepted and establish a need for further research to fully elucidate the role of *C. coccodes* in potato production.

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