Effects of Wounding and Wetting Duration on Infection of Potato Foliage by *Colletotrichum coccodes*

DENNIS A. JOHNSON, Plant Pathologist, and E. R. MILICZKY, Research Technician II, Washington State University Irrigated Agriculture Research and Extension Center, Prosser 99350

ABSTRACT

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Dark brown to black lesions developed on leaves, petioles, and stems of Russet Burbank potato after wounding by sandblasting and inoculating with either conidia or sclerotia of *Colletotrichum coccodes* in the greenhouse and with conidia in the field. Conidia from 10 isolates and four collections of sclerotia of *C. coccodes* from various areas of Washington and Idaho were pathogenic on potato foliage. Significantly ($P < 0.01$) more lesions developed on foliage that was wounded than on foliage that was not wounded just before inoculation. Inoculated plants wilted, and lower and middle leaves became chlorotic and sometimes blighted. *C. coccodes* was reisolated from lesions and blighted areas and from internal stem tissues of inoculated plants. Disease symptoms did not develop on plants that were wounded but not inoculated. Successful infections decreased significantly ($P < 0.001$) as the interval between wounding and inoculation increased from 0 to 7 days, with most of the reduction occurring at an interval of 2 days. Lesion numbers on foliage of wounded and inoculated plants increased significantly ($P < 0.001$) as the duration of the postinoculation wet period increased from 2 to 48 hr.

Colletotrichum coccodes (Wallr.) S. J. Hughes, the cause of black dot of potato (*Solanum tuberosum* L.), is a soilborne pathogen of potato in many potato-growing regions of the world (6,13,14). Roots, belowground main stems, stolons, and tubers are infected. Minute black sclerotia of the pathogen are commonly found at the end of the season on senescent and dead plant tissue and on decaying roots and stems (3). Infection of potato foliage has been recently reported in Idaho (1,2,10). Foliar inoculations with conidia in the presence of mist or sprinkler irrigation and with or without wounding resulted in disease (2). A potential for foliar infection exists in central Washington, because blowing sand is frequent, and most potato crops are sprinkler-irrigated (2). We isolated *C. coccodes* from leaves, petioles, and upper stems of potato plants collected from fields in Washington State (8). Little is known about environmental conditions favoring infection of foliage. The purpose of this study was to confirm observations in Idaho concerning infection of foliage and to investigate the role of wounds from blowing sand and the length of postinoculation wet period on infection of potato foliage by *C. coccodes*.

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MATERIALS AND METHODS

Whole tubers (73–99 g) of certified potato seed (cv. Russet Burbank) were planted in pots, 16 cm in diameter, in the greenhouse. Soil was a silt loam from virgin sagebrush land supplemented with 5 g of fertilizer (6.6% ammoniacal N, 7.4% nitrate N, 14% P_2O_5, and 14% K_2O per pot) prior to planting. Two shoots per pot were grown (except where noted), and additional shoots were pruned about 2 cm above the soil level. Mean height of plants was 50–62 cm when inoculated for the various experiments.

Plants were wounded before inoculation by making a single vertical pass 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. Air pressure of the sandblaster was set at 166 kPa, creating a wind velocity of 14.5 km/hr at the plant surface. Shoots were tied to bamboo stakes for stability during wounding. Each pass took about 0.7 sec to complete. Control plants were left unwounded and inoculated or were wounded and not inoculated.

An isolate of *C. coccodes* (C-14) was obtained from an aboveground stem of a Russet Burbank potato plant from a field near Quincy, Washington, in 1990. Conidia for inoculation were produced on V8 juice agar medium in petri dishes placed under continuous fluorescent light for 7–8 days at 20–23 C. Conidia were scraped and washed with water from the agar, filtered through four layers of cheesecloth, and sprayed on test plants with a mini-spray gun (model 364-15502, Sears, Roebuck and Co., Chicago, IL) at 90 kPa. One drop of Tween 20 per 500 ml of water was added to the inoculum. Concentration of inoculum was determined with a hemacytometer.

After inoculation, plants were placed in a plastic mist chamber for a predetermined time, removed, dried as quickly as possible with forced air from a fan, and then placed in the greenhouse. Temperature in the mist chamber was maintained close to 20 C, and greenhouse temperatures ranged from 10 C at night to 27 C during the day. Inoculations were done from April to August 1991 and 1992; supplemental lighting was not used.

The length of each inoculated shoot was measured at time of inoculation, and lesions were counted on main stems and 12 petioles per shoot 7–10 days after inoculation. When lesions coalesced, individual lesions could still be identified and individually counted. The number of lesions per linear centimeter of stem was tabulated for each shoot, and a mean was calculated for each pot. The mean number of lesions per petiole was determined for each pot. Experiments were designed as a randomized complete block.

Tissue from the margin of 12–18 lesions on stems, petioles, and leaflets of inoculated plants from all treatments in each replicate and experiment were plated on potato-dextrose agar (PDA) to reisolate *C. coccodes*. Plant parts were first thoroughly washed in running water, disinfected in 10% NaOCl for 3 min, and rinsed in sterile distilled water.

Age of wound. To determine the effect of wound age on infection, plants were wounded 0, 1, 2, 3, 6, 8, and 10 days before inoculation. Unwounded plants were inoculated as a control. Concentration of inoculum was 11×10^6 conidia per milliliter. Three replicates were used, with two shoots per replicate, and the experiment was repeated with plants being wounded 0, 1, 2, 3, 4 and 7 days before inoculation. Data were analyzed by regression, with age of wound before inoculation as the independent variable

and lesion number per centimeter of stem as the dependent variable. The unwounded control was compared to plants wounded on various days before inoculation with Fisher's protected LSD.

Length of wet period. To determine the effect of length of wet period on infection by *C. coccodes*, plants were wounded, inoculated with a conidial suspension of 11.6×10^6 conidia per milliliter, and removed from the mist chamber 3, 4, 6, 9, 12, and 24 hr after inoculation in one experiment. In a second experiment they were wounded, inoculated with 11×10^6 conidia per milliliter of inoculum, and removed from the mist chamber 2, 3, 6, 9, 12, 24, and 48 hr after inoculation. The elapsed time from washing conidia from agar to inoculation and placing plants in the mist chamber was about 1 hr. Control plants were wounded but not inoculated and were left in the mist chamber for 24 and 48 hr, respectively, for the two experiments. An additional control for the second experiment consisted of plants not wounded before inoculation and left in the mist chamber for 48 hr. Three replicates of each treatment with two shoots per replicate were used. Data were analyzed by regression. Length of the postinoculation wet period was the independent variable, and lesions per centimeter of stem or lesions per petiole was the dependent variable.

A third experiment was conducted to repeat the infection of potato foliage at short wet periods after inoculation. This was replicated three times and consisted of wounding foliage, inoculating with 13×10^6 conidia per milliliter, and placing plants in the mist chamber for 2, 3, and 9 hr. Elapsed time from washing conidia from agar to inoculation was 1 hr. Control plants were wounded but not inoculated.

Pathogenicity of isolates. A total of 10 isolates of *C. coccodes* were tested for pathogenicity on potato foliage. Six isolates (C11, C12, C13, C14, C18, and C19) were obtained from below- and aboveground stem lesions or stem sap of potato plants collected throughout the Columbia Basin in Washington in 1990 and 1991. A seventh isolate (C21) was obtained in the spring of 1991 from dried, aboveground stem debris that had overwintered in a field near Richland, Washington. Two isolates (C16 and C20) were obtained from seed tubers grown in northwestern Washington, and a 10th isolate (C22) was obtained from a dried, aboveground stem collected near Ririe, Idaho, soon after potatoes were harvested in 1991.

Fig. 1. Blighted leaflet and lesions on leaflet and petiole of potato wounded with sand and inoculated with *Colletotrichum coccodes* in the field.

Plants were grown in a sandy loam soil from virgin sagebrush land supplemented with 4.5 g of a 16-16-16 (NPK) fertilizer at planting and with 0.35 g of 34-0-0 fertilizer 18 and 34 days later. Plants were grown from growing buds cut from certified seed tubers with a disk-shaped cutter that produced a sphere of tuber tissue about 13 g in weight. Plants were wounded and inoculated when about 35 cm high with the 10 isolates of *C. coccodes* in a randomized complete block design with five replicates in one test and three replicates in a second test. Concentration of inoculum for each isolate was approximately 20×10^6 in the first test and 9×10^6 in the second. Length of wet periods for both tests was 24 hr.

Internal tissues of potato stems were plated on PDA 17 and 20 days after both inoculation tests with the 10 isolates of *C. coccodes* to test for internal colonization of stems by the fungus. Three methods were used to disinfect external tissues. First, the epidermis and necrotic tissue on stems were cut off with a scalpel, and the stem was cross-sectioned into 2-cm sections with a sterile scalpel. Stem pieces were then placed in a 2.63% solution of NaOCl (50% bleach) for 15 min, rinsed in sterile distilled water, cut longitudinally, and placed on PDA in a petri dish. Second, the epidermis and necrotic tissue on stems were cut off, and stem pieces were cut into 2-cm sections as above. Stem pieces were then placed in 90% ethyl alcohol for 10 sec, passed over a gas flame, and allowed to burn for about 7 sec. They were then cut longitudinally with a sterile scalpel and placed on PDA. Third, stems about 10 mm in diameter were cross-sectioned, and the cut surface was placed in 90% ethyl alcohol and then passed through a gas flame and allowed to burn. The internal tissues of the stem were then removed, beginning at the flamed cross-cut, with a 6-mm-diameter cork borer and plated on PDA.

Sclerotial inoculation. Dead, aboveground potato stems with sclerotia of *C. coccodes* were collected after harvest from three fields located in different areas of the Columbia Basin and Yakima valley in Washington. Stems were stored in the laboratory for 7 mo at room temperature and then washed with running water for about 10 min. Sclerotia were scraped from stems with a scalpel. Thin epidermal strips with adhering sclerotia came off stems and were cut perpendicular to their length into pieces about 2 mm long. Sclerotia from each location were applied to recently wounded foliage of potato plants (one plant per collection of sclerotia) by laying stems horizontally on a plastic sheet and pouring 20 ml of distilled water, with approximately 1,200 sclerotia per milliliter, over the stems before gently moving stems around in the water that landed on the plastic. Concentration of sclerotia was determined in a nematode counting dish. After inoculation, plants were placed in a mist chamber for 24 hr. A sample of sclerotia for each collection was observed through a microscope for acervuli and conidia after plants had been in the mist chamber for 22 hr. Plants were then placed in the greenhouse for 14 days, and afterward disease symptoms were recorded and diseased tissue was plated on PDA to reisolate the pathogen. This experiment was preceded with a similar test with a collection of sclerotia from a location in the Columbia Basin. However, the foliage of two plants was and was not wounded before inoculation, the length of time of the wet period was 55 hr, and sclerotia were not observed for acervuli.

Inoculation in the field. Certified seed pieces of potato (cv. Russet Burbank) were planted by hand in a fine silt loam soil at the Irrigated Agriculture Research and Extension Center near Prosser, Washington, on 19 April 1991. At 2 wk before planting, the soil was treated with methyl bromide (448.5 kg/ha) under polyethylene tarps. Rows 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. Plants emerged on 17 May. Plants in plots were wounded with 16-grit autoclaved silica sand, using a Speedaire sandblaster at 193 kPa, on either 19 June or 10 July 1991. The field trial was repeated in 1992, with a planting date of 10 April and inoculation dates of 21 and 28 May. Individual plants were blasted with sand in a vertical pass both years 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 for 13 hr on 19–20 June and 16 hr on 10–11 July 1991, using 2.776-mm nozzles. In 1992, plots were sprinkler-irrigated 10 min/hr for 13 hr. For comparison, both wounded and unwounded plots were not inoculated. Plants in an individual plot were wounded and inoculated once. Treatments were arranged in a randomized complete block design with four replicates. Concentrations of inocula were 7×10^6 on 19 June and 4×10^6 on 10 July 1991, and 11×10^6 in 1992; one drop of Tween 20 per liter of water was added to the inocula.

RESULTS

Disease symptoms developed on the foliage of plants wounded and then inoculated with conidia in the greenhouse and field (Fig. 1). Lesions initially had a water-soaked appearance, then turned dark brown to black, and were usually associated with wounds. Uninoculated wounds appeared as white flecks less than 0.3 mm. Lesions on leaflets were generally similar to those caused by *Alternaria solani* Sorauer but did not contain concentric rings. Lesion diameters ranged from 0.3 to more than 7 mm on leaflets and 0.5–5 mm on petioles and stems. Inoculated plants sometimes wilted, and lower and middle leaves frequently became chlorotic and sometimes blighted. Symptoms on wounded and inoculated plants in the greenhouse and the field were similar. Symptom development did not differ on plants wounded and inoculated in the field on the different dates or years. Lesions became evident 2 days after plants were inoculated in the greenhouse and field. Plants that were wounded but not inoculated did not develop lesions or severe chlorosis. *C. coccodes* was reisolated from lesions and blighted leaves of all inoculated treatments and replicates.

Age of wounding. Successful infections decreased significantly ($P < 0.001$) as the interval between wounding and inoculation increased from 0 to 10 and from 0 to 7 days (Fig 2). Most of the reduction in number of lesions per centimeter of stem in both experiments occurred at an interval of 2 days. The quadratic equations $\hat{y} = 2.915 - 0.952x + 0.069x^2 (R^2 = 0.90)$, and $= \hat{y} = 3.990 - 1.697x + 0.165x^2 (R^2 = 0.91)$, described the decrease in number of lesions per centimeter of stem with increase of wound age for both experiments, respectively. The mean number of lesions per centimeter of stem for plants not wounded before inoculation was 0.01 and 0.30, respectively, for the two experiments, which was significantly less ($P < 0.01$) than plants wounded 2 and 1 days before inoculation and on the day of inoculation. *C. coccodes* was reisolated from lesions on inoculated plants of all treatments.

Length of wet period. Lesions developed on all plants placed in the mist chamber for 2 and 3 hr after wounding and inoculation. Lesion numbers increased significantly ($P < 0.001$) on stems and petioles as the wet period increased from 3 to 24 hr and from 2 to 48 hr, respectively. Quadratic equations described the increase in number of lesions on stems and petioles with time of wet periods from 3 to 24 hr (Fig. 3) and from 2 to 48 hr (Fig. 4). Coefficients of determination for number of lesions per centimeter of stem and petiole, respectively, were both 0.96 when wet periods ranged from 3 to 24 hr (Fig. 3),

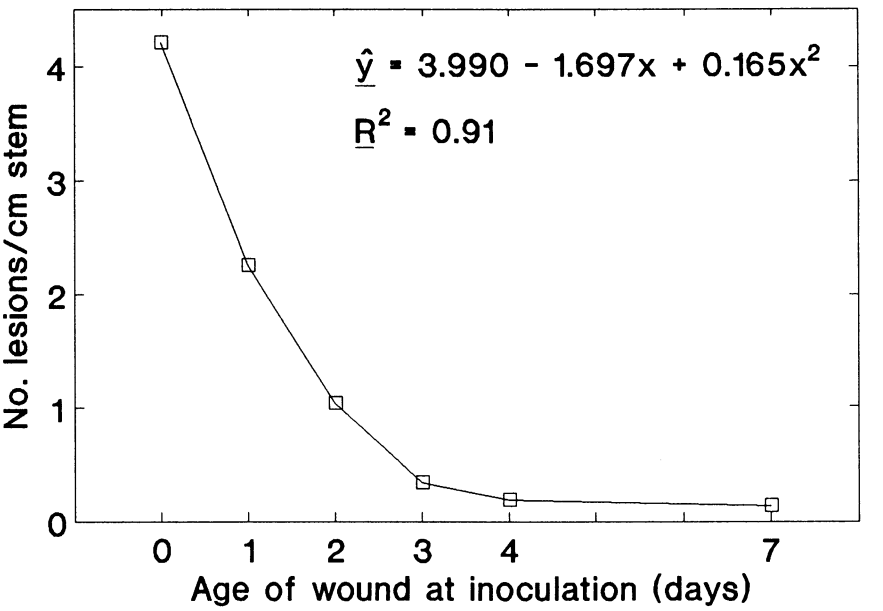

Fig. 2. Lesions per linear centimeter of potato stems wounded with air-driven sand and inoculated with *Colletotrichum coccodes* 0, 1, 2, 3, 4, and 7 days after wounding in the greenhouse. The quadratic equation was significant ($P < 0.001$).

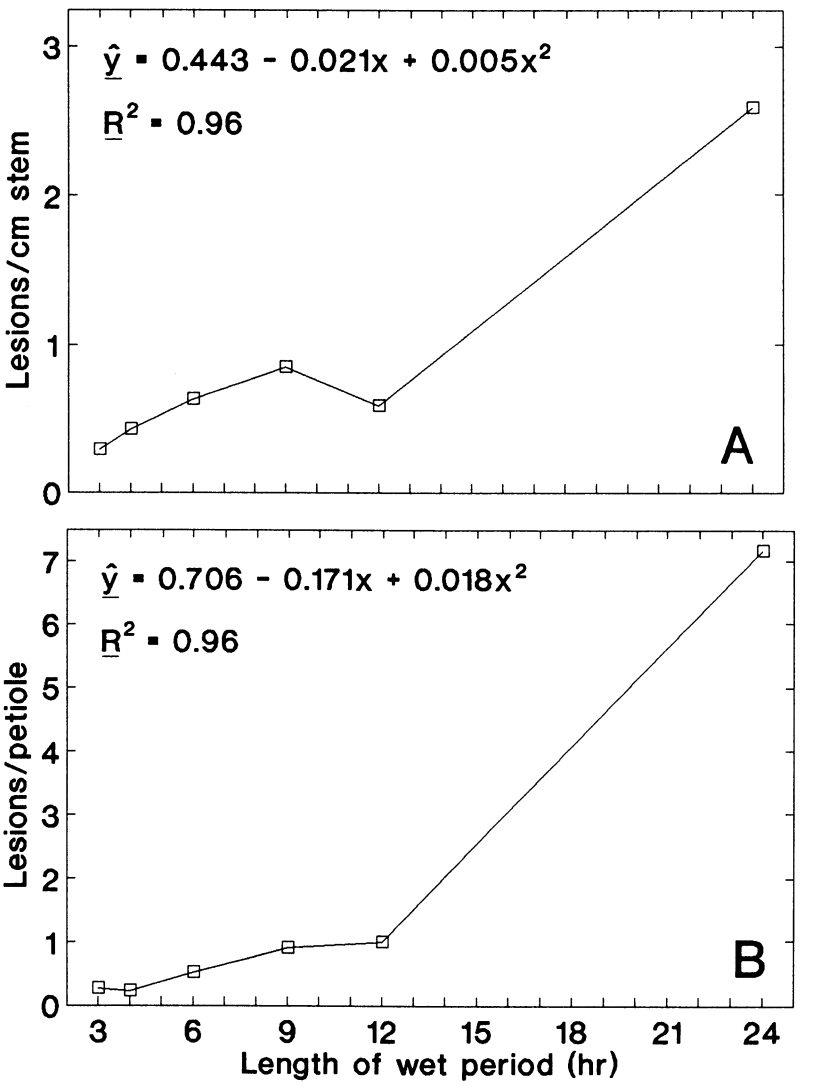

Fig. 3. Lesions per linear centimeter of stem **(A)** and per petiole **(B)** of potato plants wounded with air-driven sand, inoculated with *Colletotrichum coccodes* in the greenhouse, and placed in a mist chamber for 3–24 hr.

and were 0.96 and 0.93 (Fig. 4) when wet periods ranged from 2 to 48 hr. Lesions developed on all plants during the third experiment with wet periods of 2, 3, and 9 hr. *C. coccodes* was reisolated from lesions on plants inoculated and placed in all wet periods of the three tests.

Significantly more ($P < 0.01$) lesions developed on plant foliage that was wounded before inoculation than on unwounded foliage. Mean number of lesions per centimeter of stem and petiole of plants with a 48-hr postinoculation wet period was 0.13 and 0.17, respectively, for unwounded foliage and 13.7 and 50.1, respectively, for wounded foliage.

Pathogenicity of isolates. Lesions developed on stems, petioles, and leaves, and some chlorotic and blighted leaves resulted on the foliage of all plants wounded and then inoculated with conidia of the 10 isolates of *C. coccodes* in both experiments. Number of lesions per centimeter of stem did not differ significantly ($P = 0.05$) among the 10 isolates in both experiments. A difference among isolates in resulting chlorosis or blighting on plant foliage was not observed. The mean number of lesions per centimeter of stem ranged from 6.3 to 7.8 in the first test and from 2.5 to 5.0 in the second test.

All 10 isolates of *C. coccodes* were isolated from internal stem tissues of plants inoculated during the two tests. The fungus was isolated from nearly 60% of the stem pieces plated on PDA, using each of the three methods to disinfect stem pieces.

Sclerotial inoculation. Lesions on stems, petioles, and leaflets, and blighting and chlorosis of leaves as described for the inoculations with conidia developed on all plants wounded and inoculated with sclerotia. A mean of 0.16 lesions per centimeter of stem developed on plants placed in the mist chamber for 24 hr. A mean of 1.45 and 0.25 lesions per centimeter of stem developed on plants that were and were not sandblasted, respectively, before inoculation and had a 55-hr wet period. Acervuli, numerous conidia, and mycelial growth from sclerotia were observed when sclerotia were sampled and examined 22 hr after initiation of the wet period. *C. coccodes* was reisolated from diseased tissues of all plants inoculated with sclerotia.

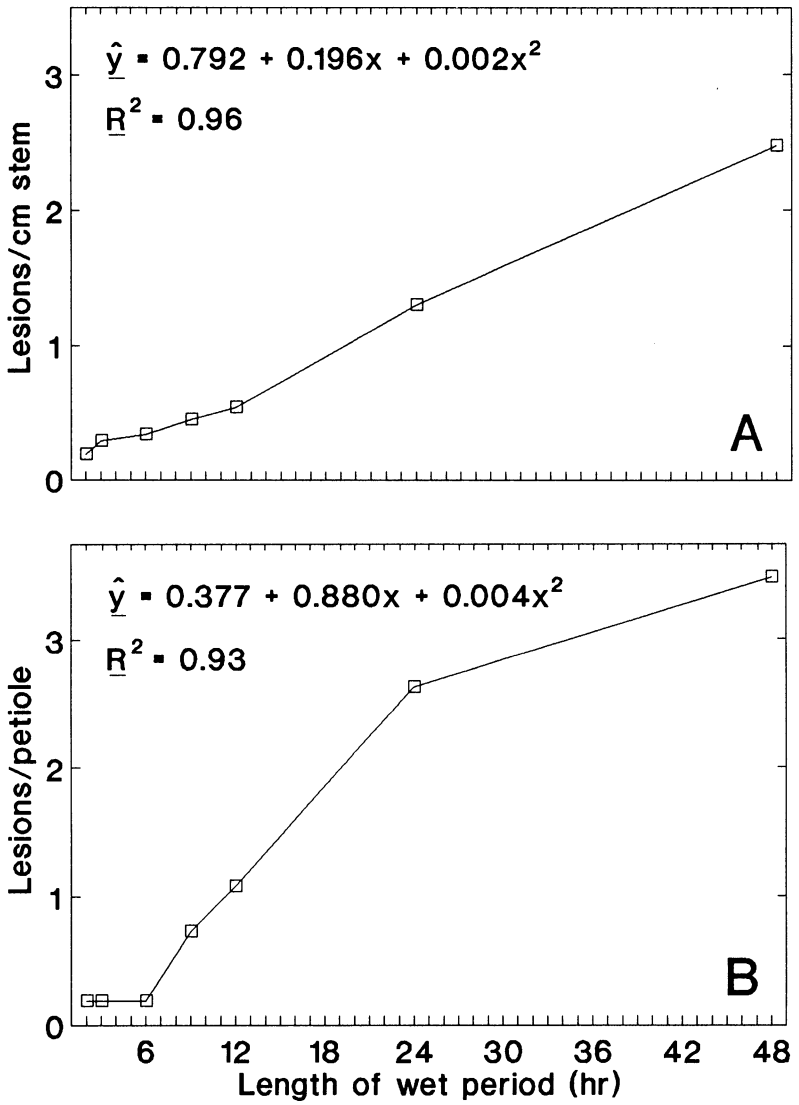

Fig. 4. Lesions per linear centimeter of stem **(A)** and per petiole **(B)** of potato plants wounded with air-driven sand, inoculated with *Colletotrichum coccodes* in the greenhouse, and placed in a mist chamber for 2–48 hr.

DISCUSSION

Black dot has historically been known as a root, stolon, and belowground stem disease of potato (3,6,14). Foliar symptoms of yellowing and wilting have been attributed to infection and rot of belowground stems and roots (4). Infection of belowground tissues by *C. coccodes* has been discounted by some researchers as playing a significant role in potato early dying disease (5,9,12). However, a significant reduction in yield of the cultivar Superior was measured in the greenhouse when sclerotia of *C. coccodes* were added to soil (13). Recently, yield reductions from foliar infections have been reported from field and greenhouse studies in Idaho (1,2,10).

Infections resulted on potato foliage inoculated with either conidia or sclerotia of *C. coccodes*. Sclerotia on plants in a moist environment produced both conidia and hyphae. Observations in Idaho (1,2,9) concerning foliar infection of potato by *C. coccodes* conidia have been confirmed in this study. Disease symptoms reported in the Idaho study (2) were the same as we observed.

Wounds from blowing sand that were 2 days or less in age increased infections on inoculated foliage. Blowing sand and soil are common during the growing season in the Columbia Basin of Washington, and sclerotia from soil are most likely carried with blowing soil. Such conditions may lead to infection of potato foliage during rain or sprinkler irrigation (2). We isolated *C. coccodes* from below- and aboveground stem tissues of the cultivar Russet Burbank in Washington, beginning 5 weeks after crop emergence (8), and from lesions on stems, petioles, and leaflets of potato foliage collected during mid-growing season in growers' fields that are similar to those lesions found on artificially wounded potato foliage inoculated with the fungus. This suggests that infection of potato foliage in the field occurs under natural conditions in Washington State.

Long wet periods of 24 and 48 hr were most conducive for infection. Lesions were initiated on plants by spraying with conidia that had been in a water solution for 1 hr, followed by a 2-hr wet period. This was a shorter time than required for germination of conidia in the laboratory. We observed germ tubes emerging from conidia on PDA after 4.5 hr of incubation at 21 C. Plant sap leaching from wounds or a moderately high

relative humidity (42–80%, with a mean of 60% during the first 24 hr after the wet period) may have supplied sufficient moisture for continued germination and infection, especially after initiation of germination in free water for 3 hr.

All isolates and collections of sclerotia of *C. coccodes* tested in this study were pathogenic on potato foliage. These were from central and western Washington and southeastern Idaho and were originally recovered from below- and aboveground stems, tubers, and potato stem debris. The 10 isolates tested for pathogenicity were recovered from internal tissues of potato stems. Large quantities of colony-forming units of *C. coccodes* (*C. atramentarium* (Berk. & Broome) Taubenhaus) were isolated from stem cortical tissues of potato plants grown in north central Oregon (7), and the fungus has been isolated from internal tissues of stems in North Dakota (11).

Yield reductions of potato resulted in Idaho after foliar inoculation with *C. coccodes* (2). The pathogen may play an important role in potato production in the northwestern United States by infecting foliage and invading internal stem tissues, and this role needs to be more extensively investigated.

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