Colonization of Potato Stems by the Black Dot Fungus, *Colletotrichum coccodes*

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Introduction

Potato black dot, caused by the fungus *Colletotrichum coccodes*, is characterized by small black sclerotia on the roots, stems, stolons, and progeny tubers of infected plants. Black dot is common in most potato growing areas in the world, and may cause up to 30% yield reduction. In the field, infection of potato plants by *C. coccodes* may be associated with soil-borne, tuber-borne and air-borne inoculum either solely or jointly in different combinations. In the Columbia Basin of central Washington, *C. coccodes* has been isolated relatively early in the growing season from below- and above-ground potato stems and from a high proportion of plants by mid-season. Disease symptoms often do not become evident until late in the growing season and numerous sclerotia develop on plant parts as plants senesce and die. Latent infections by *C. coccodes* in potato stems were suggested in an earlier study to explain the early infections and later expression of sclerotia.

The consensus from the literature is that soil-borne and tuber-borne inocula are the main inoculum sources for black dot development in potato plants. However, air-borne infection may be an important contributor to the initiation and development of the disease. The frequency of black dot development from aerial infection in potato plants under field conditions is not documented. In the Pacific Northwest and other regions with similar semi arid conditions, sand storms and sprinkler irrigation systems are common. Therefore, a high potential for foliar infection of potato plants is present. Sand storms are capable of wounding potato foliage, while sprinkler irrigation may disseminate conidia and sclerotia via splashing, and contribute the needed free water and relative humidity for inoculum germination and infection. Aerial infections of potato foliage by *C. coccodes* have been demonstrated in research trials in Idaho and the Columbia Basin. The foliage of Russet Burbank potato plants was sandblasted and later spray-inoculated with a conidial suspension of *C. coccodes*. The inoculated plants demonstrated foliar chlorosis, necrosis and dark brown to black lesions on the leaves, petioles, and stems. The results of field trials in Idaho indicated a significant yield reduction of 6.5 t/ha (about 10% in relation to contemporary average yield). The results of greenhouse trials in the Columbia Basin recorded a significant reduction of total yield (19 to 32%), and a reduction of mean tuber weight (29 to 43%). A significant reduction of total tuber weight by 7, 12 and 11% and a significant reduction of U.S. No. 1 potatoes weight by 12, 18, and 16% was documented over three years in field trials in the Columbia Basin.

The purpose of this paper is to report on studies conducted in 2003 and 2004 in which the colonization of potato stems by *C. coccodes* was quantified after aerial infections of stems. Such knowledge would be helpful in understanding the development of *C. coccodes* on foliage in the field and in developing disease management strategies for black dot.

Inoculation of Stems

Plants of Russet Burbank were grown in the greenhouse from nuclear tubers that were free from *C. coccodes*. Plants were inoculated with one of two isolates of the black dot fungus in the middle of the stem, half way between the soil surface and the plant apex, 45 days after planting. The amount of chlorosis and necrosis, due to either disease development or natural plant senescence was assessed using a visual scale from 0 to 6.

Plant stems were divided equally into three stem sections (lower, middle and apex) to facilitate the assessment. The scale was as follows: 0 = absence of chlorosis and necrosis – green, healthy appearing plant; 1 = the lower leaves with moderate chlorosis (flaccid yellow leaves), the middle leaves with mild chlorosis (turgid, pale green leaves), and the apex with green leaves; 2 = the lower leaves with severe chlorosis (flaccid leaves with orange/brownish discoloration) and with necrosis on the tips of leaflets, the middle leaves with moderate chlorosis, and the plant apex with mild chlorosis; 3 = the lower leaves with necrosis, all middle leaves with moderate chlorosis and 10-30% of them with necrosis, the plant apex with moderate chlorosis; 4 = the lower and middle leaves with necrosis or fell off the plant, and the plant apex with severe chlorosis; 5 = the lower and the middle leaves with necrosis or fell of the plant, the plant apex is flaccid with severe chlorosis, and the stem is soft with dark orange discoloration; 6 = dry plants - plants were left to dry in the pots, on the greenhouse bench until sclerotia were visible (10-14 days). The beginning of plant senescence was considered as symptoms reached category 3 (generally100 days after planting).

Stems were collected and sampled for *C. coccodes* over the course of the experiment. The stems were divided into 3 sections. The first stem section was the inoculation court that was used as a positive control for infection. The second and third stem sections were the upper section - above the inoculation court, and the lower section - below the inoculation court, respectively. The upper and lower stem sections were dissected entirely to 1 cm segments, progressing by 1 cm from the inoculation court. The stem segments were placed onto modified PDA and were incubated for 10 days at 25°C in the dark.

Infection and Colonization of Stems

The black dot fungus was isolated from 47 of 60 (78%), and 52 of 60 (86.6%) potato plants that were stem-inoculated in 2003 and 2004, respectively. The fungus was not isolated from the non-inoculated control plants. *C. coccodes* colonized the stems of infected plants to a maximum height of 9.4 inches above the inoculation court (mean 4.4 and 5.6 inches in 2003 and 2004, respectively). The lower stem sections were completely colonized both years (mean 5.2 and 4.7 inches in 2003 and 2004, respectively). On the dry plants (chlorosis and necrosis severity category 6) sclerotia were observed on both stems and roots of inoculated plants. Both fungal isolates had similar patterns of colonization above and below the inoculation court. In both years, fungal colonization significantly decreased as the distance away from the inoculation court increased. Fungal colonization was also significantly greater below than above the inoculation court both years (Figs. 2, 5).

The severity of chlorosis and necrosis and the time of their expression did not vary between inoculated and non-inoculated control plants (Fig. 1). However, fungal colonization had a curvilinear relationship with the severity of chlorosis and necrosis both years. In 2003 the fungal colonization of the upper stem sections declined from 0.8 to 0.4 as the severity of chlorosis and necrosis progressed from category 1 to 3. The colonization of the upper stem sections in 2003 increased to 0.9 as the severity of chlorosis and necrosis exceeded category 3 (plant senescence). The increased colonization is indicated by the upward curvature of the regression surface (Fig 2). In 2004, the fungal colonization of the upper stem sections was restricted between 0.0 and 0.2 as chlorosis and necrosis severity developed from category 1 to 3. Similar to the results of 2003, the fungal colonization in 2004 increased from 0.2 to 0.9 as the severity of chlorosis and necrosis exceeded category 3 (Fig 3).

On the lower stem sections, fungal colonization was similar to that of the upper stem sections.

Fungal colonization was restricted until the severity of chlorosis and necrosis exceeded category 3 (plant senescence), after which it greatly increased. In contrast to the upper stem sections, the colonization of the lower stem sections did not decrease as quickly as the distance from the inoculation court increased (Figs 2, 3).

Relationship of Chlorosis to Stem Colonization

The similar expression of chlorosis and necrosis on infected and non-infected control plants indicates that natural senescence was the cause of chlorosis and necrosis development and not the infection and colonization of the plants by *C. coccodes.* A curvilinear relationship between fungal colonization and the development of chlorosis and necrosis is shown in the Figures 2 – 5, and indicates that natural senescence significantly influences *C. coccodes* colonization of potato plants. The restricted colonization of plant stems by *C. coccodes* demonstrated a latency period that was overcome as the potato plants started to senesce. *C. coccodes* colonization was then activated between 95 to 105 days after planting. Comparisons between growth stages of potato plants grown in the green house and in the field should only be made for relative purposes. With this in mind, at 100 days after planting long season potatoes, such as Russet Burbank, in the Columbia Basin of Washington State are generally starting the last half of the linear bulking in growth stage 4. During this period, which generally ends at 120 to 130 days after planting, the vegetative growth stops, sugars are translocated to the maturing tubers, and plants start to senesce.

Infection, followed by a latency and then rapid colonization has been observed in potato and other hosts for *Colletotrichum* spp. A lack of foliar symptoms was noted on inoculated potato plants in the field until senescence in Idaho. Infection of *C. coccodes* in tomato fruit and of *C. piperatum* and *C. gloeosporioides* in pepper, mango and avocado demonstrated latency prior to fruit ripening and a break of latency when fruit ripened.

Colonization Model

Based on the present study, a model of stem colonization after aerial infection by *Colletotrichum coccodes* in potato is proposed. Conidia or sclerotia are deposited on plant leaves and stems, germinate and infect the inoculated tissues. Sand storms and water splashing in the presence of irrigation promote the process. Infection of leaves then results in leaf lesions that expand over time under humid conditions within the potato canopy. Infected leaves low in the canopy turn chlorotic and drop. Stem infections are latent until tubers bulk and plants start to senesce. The fungus then colonizes internal stem tissues. The internal colonization of stems greatly increases with a greater affinity towards the roots, stolons and tubers than towards the foliage. Increased levels of endogenous ethylene may trigger the fungus to overcome latency. A similar colonization process up stems may also occur from tuber-borne and soil-borne inoculums.

Conclusions

The black dot fungus colonized and expanded in potato stems; rapid expansion within stems was associated with plant senescence. Foliar symptoms such as chlorosis and necrosis were an inaccurate tool to diagnose infection in potato by *C. coccodes,* and did not necessarily indicate the presence or amount of *C. coccodes* in potato plants. Disease symptoms of black dot in previous field studies have been noted not to appear or to be confused with those caused by *Verticillium dahliae* or natural senescence. The lack of specific disease symptoms on potato foliage due to *C. coccodes* has made detection of the disease and the development of control practices for black dot difficult.

Because black dot symptoms are not readily evident on foliage, other methods of disease and pathogen detection are needed such as examination of roots for cortical rot, isolation and quantification of the fungus from stems and roots on semi-selective media or Polymerize Chain Reaction (PCR) techniques. Multiple assessments for *C. coccodes* in potato tissues should be made and should begin before plant senescence. Contact fungicides applied after initial infections are not likely to effectively reduce disease incidence because of latent infection and internal expansion of *C. coccodes* in potato stems points. Therefore, systemic fungicides, cultural practices, or host resistance will be needed to achieve economic control of potato black dot.

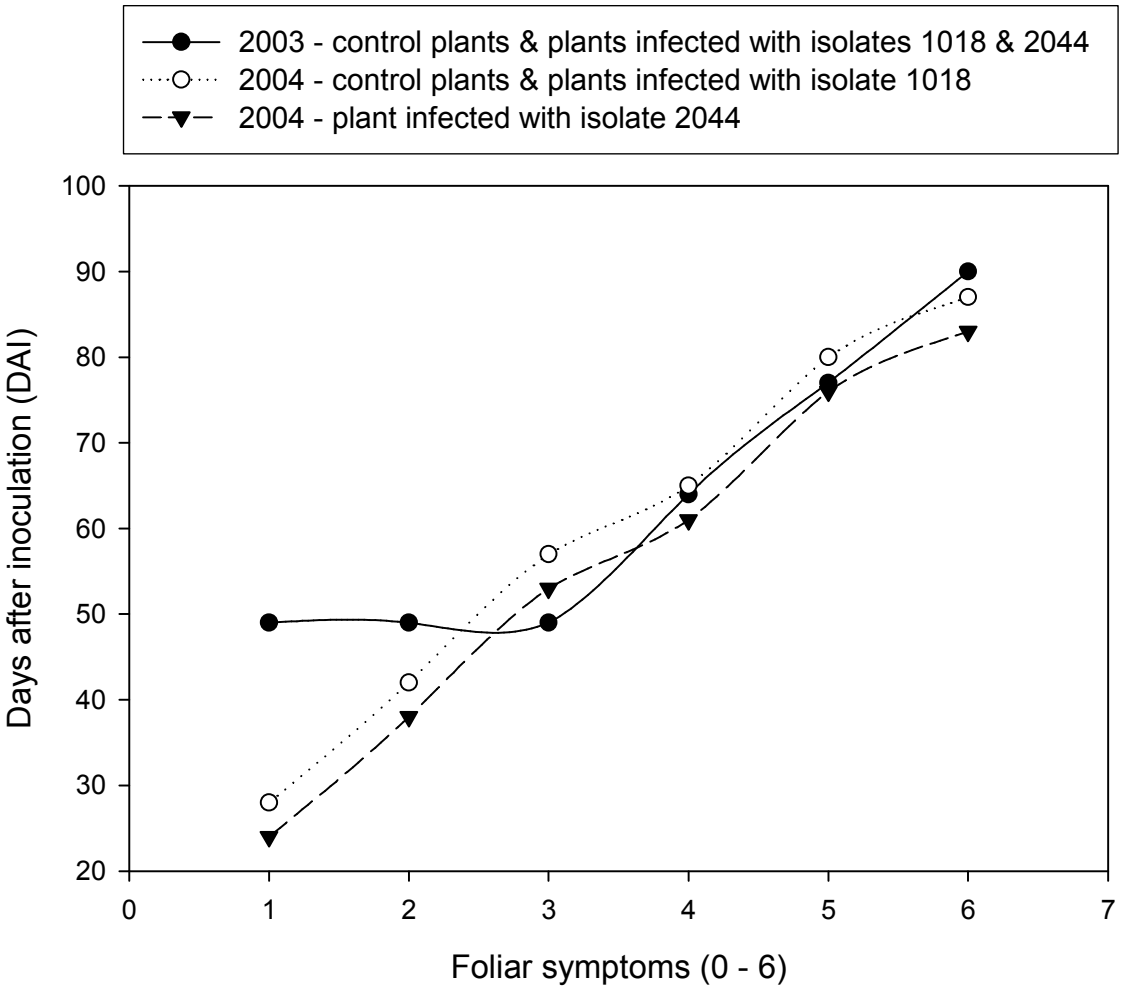

Fig 1. Progress curve for the development of foliar symptoms on potato plants that were either infected or non-infected with *C. coccodes* isolates 1018 and 2044 at the stem in 2003 and 2004 in the greenhouse.

Figure 2 A

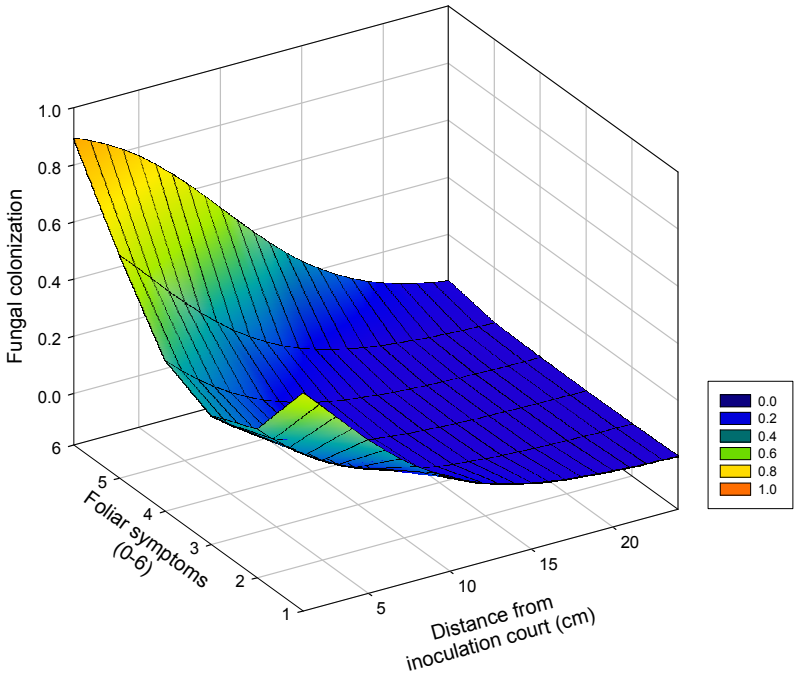

Figure 2 B

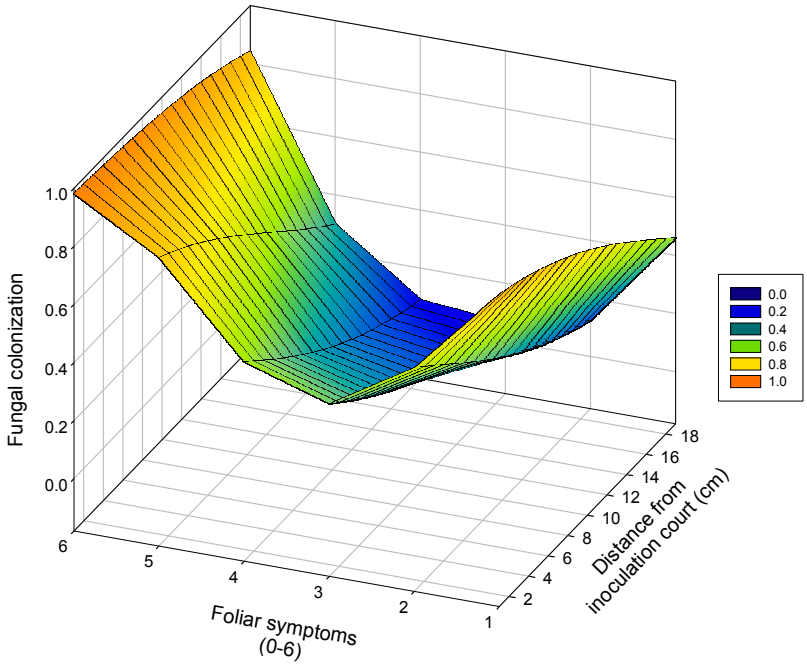

Fig 2. The relationship among the fungal colonization of the upper (A), and lower (B) stem sections, the foliar symptoms severity and the distance from the inoculation court in plants infected with *C. coccodes* isolates 1018 and 2044 in 2003.

Figure 3 A

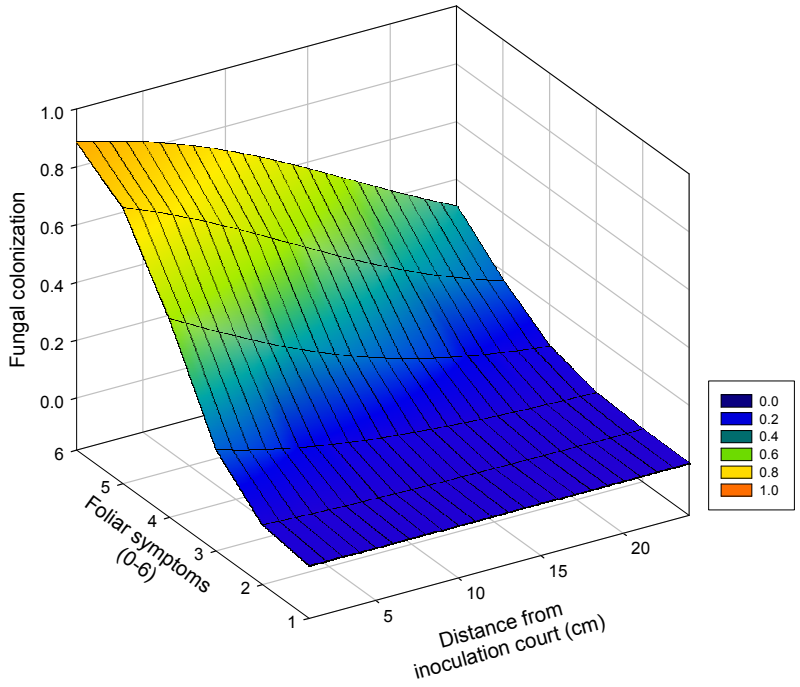

Figure 3 B

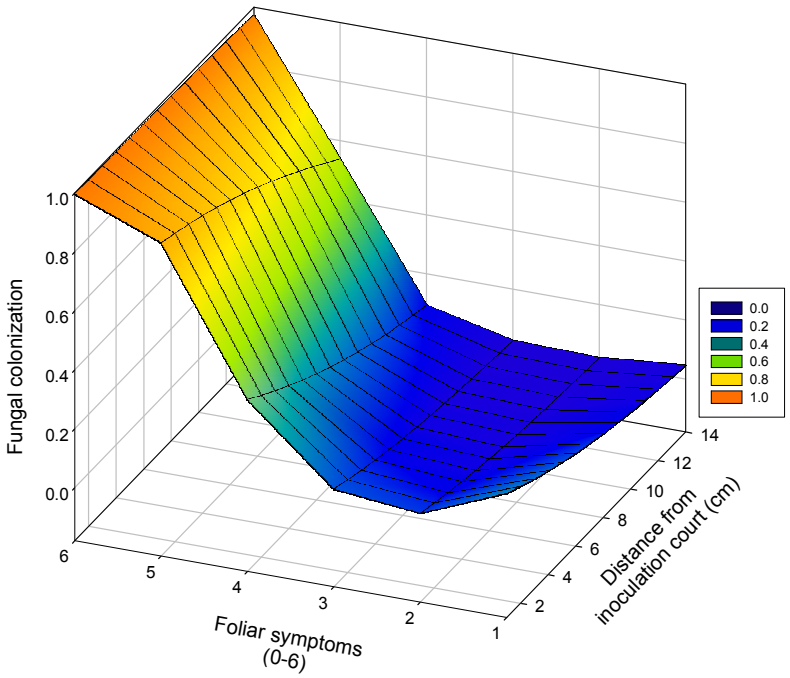

Fig 3. The relationship among the fungal colonization of the upper (A), and lower (B) stem sections, the foliar symptoms severity and the distance from the inoculation court in plants infected with *C. coccodes* isolates 1018 and 2044 in 2004.