SPREAD OF *COLLETOTRICHUM COCCODES* FROM INFECTED POTATO SEED TUBERS AND EFFECT OF FUNGICIDES ON STEM INFECTION

By

JASON TIMOTHY INGRAM

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Department of Plant Pathology

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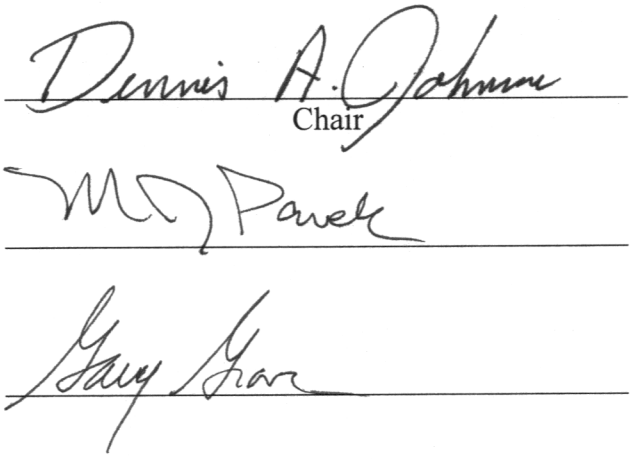
To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of JASON TIMOTHY INGRAM find it satisfactory and recommend that it be accepted.



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SPREAD OF *COLLETOTRICHUM COCCODES* FROM INFECTED POTATO SEED TUBERS AND EFFECT OF FUNGICIDES ON STEM INFECTION

Abstract

Jason Timothy Ingram M.S.
Washington State University
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Chair: Dennis A. Johnson

Colletotrichum coccodes, the cause of potato black dot is one of several seedborne pathogens. The quality of seed-tubers would likely improve if the interactions between seedborne inoculum and the crop were better understood. The rate of spread of *C. coccodes* from the seed-tuber was quantified on roots and stolons of 'Russet Burbank' plants grown in the greenhouse. The effect of seedborne inoculum of *C. coccodes* on final disease severity and potato yields was assessed in the field, and specific fungicides were evaluated for disease management. Expansion of *C. coccodes* foci along roots in two trials resulted in a linear growth model of 1 mm/day ($P < 0.01$, $R^2 = 0.83$). Colonization always radiated from the seed-tuber to form compact foci of infected tissue. In the field, severity of host plant colonization in belowground stems was related to percentage of infected tubers in seed lots in two of four fields ($P < 0.05$). Yields were not related to percentage of seed-tubers infected with *C. coccodes* ($P > 0.1$). Black dot was not a yield limiting factor under the conditions of this study, but differences in yield among seed lots ($P < 0.05$) from other factors illustrated the importance of selecting the

highest quality seed available to maximize yields. A live plant stem assay was used to evaluate the effect of fungicide application timing and active ingredient on *C. coccodes*. Fungicide application by center pivot irrigation (chemigation) in commercial potato fields was evaluated as a potential black dot management strategy. Fungicide application post-inoculation did not significantly reduce infection. Several fungicides were successful in preventing infection by *C. coccodes w*hen applied prior to inoculation ($P < 0.05$). Belowground stems were sampled from the areas where azoxystrobin was chemigated onto the crop at 50- and 67-days after planting (DAP). Less stem surface area was covered with microsclerotia in treated areas at 79 DAP in both years ($P < 0.04$), but consistent differences between treated and non-treated areas were not detected at 102- and 140-DAP.

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DEDICATION

To Dennis Johnson for giving me the freedom to make mistakes,

Tom Cummings for helping me fix them,

Jeremiah Dung for laughing at them with me,

and my wife Rebecca for bringing us all together.

PREFACE

The chapters included in this thesis have been prepared for submission to professional journals. The manuscripts from chapters one and two will be submitted to Plant Disease. Chapter three will be submitted to American Journal of Potato Research. Citations within each chapter refer to the "Literature Cited" section of that chapter and follow the format used in Plant Disease and American Journal of Potato Research. Citations made in the "Introduction" section are listed under the heading "References" at the end of this thesis.

INTRODUCTION

Colletotrichum coccodes causes black dot of potato (*Solanum tuberosum* L.). The name black dot is highly descriptive and is based on the appearance of the microsclerotia of *C. coccodes* that develop on infected host tissues. The pathogen is not restricted to potato but is pathogenic to many *Solanum* species and other select species within 13 families of Angiospermae (15, 20, 47). *Colletotrichum coccodes* has a wide geographic distribution and had been reported in association with potato by 1926 in North America, South America, Europe, Australia and Africa (19). *Colletotrichum coccodes* can frequently be found in potato fields of the Columbia Basin of Washington State (34, 35).

Black dot has been described both as a disease of minor importance (19) and one capable of reducing yield (7, 32, 63). Annual losses in market quality due to black dot and silver scurf were estimated at £5 million in the United Kingdom (39). Reduced value of tubers that can be attributed directly to black dot is difficult for processors to determine since symptoms on infected tubers are similar to those of silver scurf (19). Complete potato crop failure or product rejection has not been reported with *Colletotrichum coccodes.*

The Pathogen

Colletotrichum coccodes (Wallr.) Hughes, is an imperfect fungus originally described and a voucher specimen deposited in a permanent collection in Strasbourg, France as *Chaetomium coccodes* (29, 64). The fungus causing potato black dot has had many synonyms. The most common synonyms by which the pathogen has been known in 20th century plant pathology literature include *Colletotrichum atramentarium, C. phomoides*, *C. solanicolum and*

Vermicularia atramentarium. These species were resolved as *Colletotrichum atramentarium* by the efforts of von Arx in 1957 (5). In 1958 Hughes (29) reassigned the name and primary authority of the species to Wallroth. Cross inoculation and pathogenicity on both potato and tomato was successfully demonstrated by Illman in 1959 (30).

Colletotrichum coccodes produces sclerotia and setae in abundance on host tissue and in culture. Sclerotia are a survival structures for *C. coccodes* and have been considered incipient acervuli (9, 26). Sclerotia are a dense arrangement of melanized hyphae with abundant setae, and size can range from 150- to 230-µm (9, 26, 61). Sclerotia morphology can vary among isolates and be affected by substrate.

Conidia bearing acervuli can develop from stroma that develops within the infected host periderm. Acervuli can also develop from superficial sclerotia in the absence of periderm penetration given the proper environmental stimulus (40). Acervulus generation from *C. coccodes* microsclerotia has been demonstrated (24, 56) and described using the previously defined termed sporogenic germination (16).

Conidia are produced in a gelatinous matrix (6). The complete function of the matrix is not yet determined but one hypothesis includes sequestration of the conidia in the acervuli until the moisture requirements needed for germination and successful host penetration are present (6). The genus is generally acknowledged to have their conidia dispersed and distributed by the impaction of water drops on the hydrophilic matrix and conidia in the acervulus. Conidia are strait, hyaline, aseptate and 16- to 24-µm by 3- to 4-µm in size. Shape is generally fusiform with an abrupt taper (9, 61). Conidia can germinate in periods as short as 2 hours, and production of melanized appressoria can occur in 22 hours on tomato (12).

Microsclerotia and conidia are both capable of infecting potato and this feature may add a level of complexity to the disease cycle. Direct germination of the microsclerotia (myceliogenic) to penetrate the host via mycelia is possible, or the microsclerotia may form an acervulus and conidia production becomes possible (sporogenic) (56). Viability of conidia in soil is a matter of weeks (9, 24). It is therefore believed that the microsclerotia are largely responsible for disease in potato. *Colletotrichum coccodes* can frequently be detected in soils with previous potato rotations. In Idaho, *C. coccodes* was not detected in soil samples from virgin sage brush within potato growing regions, but populations as high as 211 colony forming units (cfu)/g soil were detected in nearby potato fields (7). In France, *C. coccodes* was detected in soil from 37 of 37 fields sampled (3). The frequency of isolation and cfu levels detected are likely significant since soil counts as low as 3 conidia/g soil were sufficient to cause 100% disease incidence in research plots (13). When recovered at eight years after burial in fallow ground, *C. coccodes* microsclerotia maintained 0, 90 and 88% viability at burial depths of 0, 10 and 20 cm (22). This longevity was obtained in fallow ground and burial depth did affect survival, but likely represents some level of the pathogens ability to survive in soil. If applicable to cultivated ground, this soil longevity represents a time period longer than most rotational schemes used in potato production.

The Disease

Colletotrichum coccodes can be introduced to a field via certified potato seed-tubers. Incidence of tubers infected with *C. coccodes* in a seed lot has been reported as high as 90% (35). The seed-tuber may serve an important role in the distribution of the pathogen to new locations, or the dissemination of more virulent isolates (37). It has been demonstrated that

isolates of *C. coccodes* segregated based on the North American Vegetative Compatibility Group (NA VCG), have different levels of pathogenicity (4, 28, 48). Differences in pathogenicity by isolate or VCG is not surprising since this has previously been established for *Verticillium dahliae* in potato (31).

The process by which *C. coccodes* infects tomato (*Solanum lycopersicum* L.) has been well documented. Histological studies of conidia germination and tomato host penetration have corroborated field observations of disease progress and symptomatology. When *C. coccodes* affects tomato fruit the disease is known as fruit anthracnose. Symptoms found on fruit due to *C. coccodes* do not become apparent until fruit ripening (25). Despite this, protectant fungicide applications are needed as soon as fruit formation begins in order to minimize losses from the disease (11, 23, 25). This field observation was explained by histological studies of conidia germination and appressoria formation on tomato fruit and leaves. Through monitoring the infection process using microscopy, conidia present on immature fruit and leaves were demonstrated to form appressoria and penetrate the cuticle via physical force (12, 25, 30, 36). After successful penetration, only a limited colonization of the neighboring cells takes place (12, 30, 36). Then at fruit maturity, an unknown signal causes the latent infection to spread dramatically, become symptomatic, and cause the dark sunken anthracnose lesions that can lead to a completely unmarketable crop within days (12, 25, 30, 36). When protectant fungicides are applied throughout the entire period of fruit development (11, 25), latent infections are prevented. The disease is not controlled by a single fungicide application just prior to crop maturity when anthracnose symptoms normally appear.

Knowledge of the host-pathogen interaction has not been as well studied in potato, but evidence exists for a similar latent type of infection as in tomato, where the host tissues are

infected and colonized, but without symptoms or signs (46, 50, 58, 62). Roots and stolons have been reported the most susceptible potato tissues to infection (2, 57). Whether this is due to the host-pathogen interaction or the influence of the soil environment compared to that of the foliar is unknown.

The assumption that *C. coccodes* is a weak pathogen and requires a predisposed host for infection and colonization may have come about from the frequency with which it is isolated from plants along with other pathogens (19). Alternatively, this conclusion could be based on biased sampling, because symptoms from a potato plant infected exclusively with *C. coccodes* can be remarkably non-distinct (7, 50, 62). Perhaps instead, the association of *C. coccodes* with other pathogens that elicit more apparent host symptoms was required to initiate investigation (62). *Colletotrichum coccodes* has been isolated from plants of healthy appearance (50). A valid assessment as to the requirement of a predisposed host for infection and colonization by *C. coccodes* is needed.

The effects of *C. coccodes* on potato production is not as clear as for other pathogens since it does not lead to the rejection of an entire crop as for processing tomatoes (11, 21), or major stand loss in potato fields as from *Phytophthora infestans* (33). Total crop losses have not been reported due to *C. coccodes* and incremental losses have been difficult to obtain consistently. Of 10 peer reviewed publications where yields were taken after potato plants were challenged with *C. coccodes,* mixed results among years, cultivars and locations are evident within the same publication (4, 7, 32, 38, 41, 45, 53, 58, 60, 63). In an attempt to clarify the frequency with which yields were affected by *C. coccodes* in those 10 publications, each event in which a potato was challenged with *C. coccodes* was tallied. A challenge event was judged to occur whenever year, inoculation method, cultivar, isolate, location, etc, differed within the

experiment. Then the number of challenge events where significant yield reductions were observed was recorded. This resulted in a total yield loss occurring 54% of the time when potato was challenged by *C. coccodes*. Inoculation methods described were foliar, seedborne, and soilborne and only total yield figures were used. Authors who recorded no yield loss include Scholte, Read and Hide, and Kotcon (38, 53, 58). Yield losses have been reported due to *C. coccodes* by seven authors (4, 7, 32, 41, 45, 60, 63). The inconsistency in losses could be due to the pathogen being relatively unimportant except under certain conditions such as high inoculum levels or plant stress, or that the effects from the pathogen are masked when another more serious yield limiting factor is present. Alternatively, *C. coccodes* has been reported in the negative controls (non-inoculated) of 10 refereed publications about black dot in potato and tomato (14, 25, 32, 35, 37, 38, 47, 50, 53, 63). This could partially explain the inconsistencies in losses attributed to black dot throughout the literature. When present in negative controls, a component of variance between treatments was unknowingly removed and would make detection of yield differences less likely. Maintaining vigilance on the matter of negative control contamination may be as critical as any to determining the true impact of *C. coccodes* on yields. This observation also makes a point about the ubiquitous nature of the pathogen.

Current Management Tactics

Recommended best practices for management of black dot is based on the rotation interval between potato or other susceptible crops (19, 24). The recommended intervals are a minimum of three years under the most favorable of conditions (51). No potato cultivar has been identified as resistant to *C. coccodes*. Trials designed to determine the efficacy of fungicides for black dot management have been conducted since 1976 (60) and have included treatment for

seed, soil fumigation and foliar fungicide application. Most fungicide studies for foliar or seed-tuber treatment have not generated favorable results for control of black dot. With the exception of fenpiclonil as a seed treatment (54), no significant chemical control was reported in field studies until the release of the quinone outside inhibiting (QoI; strobilurin) fungicides (44). The QoI fungicides are a class of synthetic fungicides first marketed in 1996 (8). Since that time, several additional fungicides with modes of action different to the more traditional potato fungicides have become available to potato growers and need to be evaluated for control of *C. coccodes*.

Reasons for Additional Study of Potato Black Dot.

Based on the biology of the pathogen, continued research should be viewed as managing risk. The extended viability of microsclerotia of *C. coccodes* in soil, could lead to continually increasing soil inoculum levels under current rotational systems in the Columbia Basin. *Verticillium dahliae*, cause of Verticillium wilt, also overwinters in potato fields as microsclerotia and is a major limiting factor in potato production. However, *Verticillium dahliae* populations can be reduced by soil fumigation (55). When soil fumigation is combined with resistant cultivars, losses due to Verticillium wilt can be managed. Neither option is available to growers for managing black dot. With the exception of methyl-bromide (18) no soil fumigant has delivered significant reduction in *C. coccodes* populations (43, 54, 60) and some evidence exists that the pathogen may also be tolerant to soil solarization treatment (17), and organic amendments (49). Furthermore, cultivars acceptable to consumers or processors have not been identified as resistant to *C. coccodes* (1, 3, 53).

In addition to a soil viability period that may exceed current potato rotation periods, alternative hosts such as eastern black nightshade (*Solanum ptycanthum*), yellow mustard (*Guillenia flavescens*) and alfalfa (*Medicaigo sativa*) (47) have been identified. These crops and weeds are common in regions where potatoes are produced and could serve as a bridge for the pathogen between crops. With continually increasing populations of the pathogen in the soil, *C. coccodes* may become a major pathogen simply from the aspect of extremely high inoculum levels. This is a concern because evidence exists for the greater importance of soilborne inoculum relative to that which is available from an infected seed-tuber (18, 47, 52). Continued work to understand pathogen biology, identify host resistance, and clarify black dot etiology may be crucial to future disease management strategies.

Objectives

The objective of this thesis research was to provide information to help develop a management strategy for potato black dot. Of particular interest was the colonization process of the potato stem and root system from infected seed-pieces. A yield assessment based on the effects from different seed sources with different proportions of tubers infected with *C. coccodes* would also provide needed information to potato growers.

A second component of the study was to determine whether newly available fungicides could provide a measure of control and when they need to be applied in relation to the infection process. Effectiveness of a QoI fungicide that had previously shown promise in reducing black dot in research plots was also evaluated in replicated plots in a chemigated commercial potato field. The specific objectives that were addressed in the three chapters of this study include:

1. Quantify the rate and identify the pattern of colonization of the potato root system when the primary source of inoculum is the seed-tuber.

2. Identify effective fungicides and application timings that prevent infection and restrict colonization of *C. coccodes* in potato stems.

3. Determine effectiveness of fungicides in reducing severity of black dot in commercial potato fields.

4. Quantify the association of incidence of infected seed-tubers with yield and disease severity in the field.

Management Recommendations Based on this Study.

When potato is challenged by *C. coccodes,* a yield reduction is noted approximately one out of two times in the available literature. Total yield losses in studies that used field grown potatoes were as high as 30% in Israel and 12% in the Columbia Basin (32, 63). Given that frequency and magnitude of loss, growers can consider the costs of increasing rotation interval or the use of more expensive fungicides that have efficacy on *C. coccodes*. In this study, potato yield was not related to the percentage of the seed lot infected with *C. coccodes*. However, yields were different among the seed sources. Growers that choose to plant high quality seed (59) are starting with the best available yield potential. High quality seed is also likely to have low levels of tuberborne pathogens such as *Fusarium* sp. and *Verticillium* sp. as well as *C. coccodes* (10, 59).

Field selection is a second step in managing black dot and other soilborne diseases. Rotation intervals are often driven by economic considerations (42). The most effective use of rotation is as a preventative step to restrict the development of high populations of pathogens. As a general recommendation, a three year period without potato is often sufficient if pathogen levels are low (51). In two fields in the Columbia Basin used for the chemigation treatments in this study, less than 5% of the seed was infected with *C. coccodes*, but the pathogen was detected in nearly 100% of the plants sampled from the fields. In those fields, neither 2 nor 3 years without a potato crop were sufficient to reduce the percentage of infected plants below 100% in the no-fungicide treatment. Rotation crops that were not colonized by *C. coccodes* in a greenhouse study include barley, maize, rye and wheat. However, failure to manage velvetleaf (*Abutilon theophrasti*) and nightshade would leave two highly susceptible hosts in the field and could reduce the benefits of rotation (47).

Dramatic increases in crop vigor, and reduced host plant colonization were observed in this study when potatoes were planted in virgin or long rotation fields, as compared to common rotation fields. This conclusion is only the result of a subjective comparison since type of field was not a replicated treatment. However, maintaining low pathogen levels in a field through selection of clean seed and long intervals between potato are more likely to emulate the conditions of the virgin fields.

Good cultural practices will ensure the continued quality of the crop once decisions regarding seed source and field selection have been made. Tactics that exclude pathogens from fields should not be overlooked. Cleaning equipment in the field before it is moved will reduce the rate of pathogen spread between fields. The looming threat of potato cyst nematode should be enough to ensure the adoption of this practice. From a practical standpoint, *C. coccodes* is found

in such a high proportion of seed potatoes that sanitation of equipment is unlikely to reduce the spread of the pathogen.

To minimize host predisposition to infection, water management should be carefully exercised. Available soil moisture should be maintained at levels specified for the growth stage of the crop. Overwatering predisposes both the plant and tubers to infection by many pathogens (27). Resistant cultivars that are suitable to the industry are not available at this time. Based on the widespread distribution of *C. coccodes* in potato fields, perhaps more recently released cultivars will have unknowingly been selected for *C. coccodes* resistance, or at least highly susceptible lines culled based on poor performance in evaluation trials. If resistant cultivars acceptable to the industry are not identified, and economic constraints restrict potato rotation intervals, then development of a management plan for black dot will likely rely on fungicides.

Scouting for black dot is impractical because infection remains asymptomatic (46), or ambiguous as to the causal agent (19). However, the consistency with which the pathogen can be isolated from plants early in the growing season may eliminate the need for scouting. If the pathogen if known to be present in the field, it can be expected to have infected the crop by tuber initiation (34, 54, 63). If a field has a history of black dot, preventative application of a fungicide effective against *C. coccodes* can be used. However, the fungicide must also be highly effective on the pathogens of primary importance as determined from field scouting, forecasting, or crop development stage targeted applications such as for white mold. Fungicides effective against *C. coccodes* should be applied prior to infection. Severity of black dot was reduced in a commercial potato field in this study when azoxystrobin was applied through the irrigation system at tuber initiation; however, the effects did not persist throughout the growing season. The proximity of the plant to sclerotia in soil, lack of fungitoxic fungicides, and the long growing season place the

host at continual risk for infection. An additional, later season fungicide application targeted against *C. coccodes* could be needed. The precise interval between these sprays was not determined, but a 30 day interval between applications is probably too long based on disease progress observed in this research. The key concept with fungicide control of black dot is to prevent infection, and only as part of the regular spray program used for the major foliar pathogens. In this fashion, extra expenses are not incurred for management of black dot. The fungicide selections should also be made so as to alternate modes of action in order to prevent resistance and preserve the tools for growers. The information contained in this thesis should be useful in developing a plan to manage potato black dot.

Specific findings and conclusions for this study include the following:

1. *Colletotrichum coccodes* spread throughout the plants root system from the seed-tuber. In the greenhouse this occurred at a rate of 1 mm/day and was always along continuous plant tissue. This implies that black dot is a monocyclic disease in the soil environment.

2. Progeny tubers were infected via the stolon as *C. coccodes* colonized the host along continuous tissues from tuberborne inoculum.

3. QoI fungicides, azoxystrobin and pyraclostrobin, when applied to potato prevented infection by *C. coccodes*, but did not have curative activity.

4. Azoxystrobin applied at tuber initiation, and again 17 days later via chemigation reduced black dot severity on the belowground stems of plants grown in a commercial potato field.

5. Famoxadone, fluoxastrobin, mandipropamid + difenoconazole, penthiopyrad and polyoxin prevented infection by *C. coccodes* in one of two trials.

6. Severity of black dot in the field was significantly related to the percentage of seed-tubers infected in two of two fields in 2008, but not in two of two fields in 2007.

7. Potato yield was not significantly related to percentage of *C. coccodes* infected tubers in the seed lot.

Colonization of Potato Roots and Stolons by *Colletotrichum coccodes* from Tuberborne Inoculum

Jason Ingram

Department of Plant Pathology, Washington State University, Pullman, WA 99164

Colonization of Potato Roots and Stolons by *Colletotrichum coccodes* from Tuberborne Inoculum.
J.T. INGRAM. Dept. Plant Pathology, Washington State Univ., Pullman, WA 99164

ABSTRACT

The rate of spread of *Colletotrichum coccodes*, cause of potato black dot, was quantified on roots and stolons of cv. 'Russet Burbank' potato plants. Greenhouse grown plants arising from seed-tubers with natural and artificially inoculated infections of *C. coccodes* were observed. The distance that *C. coccodes* colonized potato roots was measured at 21 days after plant emergence and continued at 14 day intervals until crop death. Distance was measured from the seed-tuber to the distal edge of sclerotial development along the roots. Visual assessment of disease progress was confirmed by isolation from diseased and non-diseased tissues onto nutrient agar. Expansion of *C. coccodes* foci along the roots in two trials resulted in a linear growth model of 1 mm/day. ($P < 0.01$, $R^2 = 0.83$). Colonization always radiated from the seed-tuber to form compact foci of infected tissue; no secondary foci were observed. Results suggest that *C. coccodes* expands linearly from an inoculum source and that for multiple disease foci to develop on roots, multiple primary infections must occur from over wintering sclerotia in the soil. The lack of secondary infections from a single inoculum source also suggests that overwintering inoculum in the soil or seed is exclusively responsible for disease of roots in the field and that

management tactics that reduce the effects of overwintering inoculum or inhibit the rate of disease development on plants should be emphasized.

INTRODUCTION

Black dot of potato, caused by the fungus *Colletotrichum coccodes* (Wallr.) Hughes is aptly named from the melanized microsclerotia that develop on infected tissues. Detection of the pathogen can be accomplished through direct observation of the characteristic microsclerotia that become prevalent on host tissues late in the growing season. However, early season detection can be difficult due to latent infection that is without signs of the pathogen (12, 18, 19, 23, 28, 34, 40) and because infected host symptoms are often similar to those caused by other soilborne pathogens (36, 41). Symptoms of *C. coccodes* infection can include sloughing of the root cortex and brown lesions on roots typical of cortical rot pathogens (7, 41). The symptom ambiguity can make field detection of the pathogen difficult as demonstrated when *C. coccodes* was isolated from asymptomatic plants in commercial potato fields (32). In addition to latent infection and non-distinct symptoms, detection is made difficult because disease development occurs in the soil. These factors likely contribute to some of the knowledge gaps in black dot etiology.

One knowledge gap is the lack of a quantitative measure of the relative aggressiveness of *C. coccodes* as a pathogen and whether predisposition is required prior to infection and disease development. It is also unclear whether secondary infections occur. While not absolute, many soilborne pathogens are monocyclic (13, 36) and this generalization has not been demonstrated otherwise for *C. coccodes* in potato. Therefore, when considering the root-colonizing phase of the disease, the likelihood that an infected potato plant would serve as a source of inoculum for an adjacent plant is low. However, one point of interest is the process of fungal ramification

within the individual host (13). The potential presence of a secondary infection cycle that occurs within the same plant is likely important in understanding black dot etiology. One final component of the disease that is not clearly understood is the process of progeny tuber infection that leads to the tuber blemish form of the disease that affects fresh pack and storage quality of the crop.

Primary infection of plants can occur from soilborne microsclerotia routinely found in fields with previous potato crops, or from the seed-pieces cut from an infected seed-tuber. (3, 6, 7, 22, 43). Previous work has shown that seedborne inoculum is less important than soilborne inoculum for causing disease (6, 30, 37). However, the study of disease increase from seed-pieces infected with *C. coccodes* provides an opportunity to assess disease progress from a point source of inoculum. The resulting spatial pattern of disease, and the rate of colonization of a potato plants root system are two unknown components of the disease cycle. Observation of the spatial pattern of diseased tissues and the rate of disease increase throughout crop development could provide quantitative evidence as to whether *C. coccodes* is able to colonize healthy plants. Knowledge of the spatial pattern of disease within root systems would also provide some evidence for whether secondary inoculum is produced that leads to a non-linear increase in the proportion of infected host tissue. Observation of the spatial pattern of disease could also clarify the potential mechanism of progeny tuber infection. All three of these characteristics of disease etiology are important for selection of disease management tactics.

The purpose of this study was to characterize and quantify the spread of *C. coccodes* within intact potato root systems when infection originated from the single point source of inoculum at the seed-piece. This paper outlines the experimental methods used to validate a visual assessment for both presence and absence of *C. coccodes* and also provides evidence that

C. coccodes can successfully colonize a non-compromised potato host throughout all phases of plant development. The observed spatial pattern of disease increase is described and interpreted for impact on management decisions for black dot and the possible mechanism of progeny tuber infection. The temporal-spatial results of disease progress are also contrasted to the known colonization patterns of the aerial parts of the potato plant, the growth rate of *C. coccodes* in culture and the colonization of potato roots described by Andrivon in 1998 (1).

MATERIALS AND METHODS

Inoculum, plant material and inoculation. Colonies of *C. coccodes* were grown on V-8 agar (35) under constant florescent light (10 W/m^2) at 22°C. The cultured isolate was CR-102 belonging to NA-VCG 2 (29), and had originally been collected from a certified seed potato. Two Petri dishes containing nine day old colonies were used to prepare a conidial suspension, which was used to inoculate seed-tubers. Sterile distilled water (1.0 ml) was added to each dish, the colony surface was scraped using a sterile scalpel and the resulting suspension strained through four layers of cheesecloth to remove microsclerotia. The conidial suspension was adjusted to a concentration of $1.3x10^5$ conidia/ml with aid of a hemocytometer.

Certified seed-tubers of *Solanum tuberosum* L. cv. 'Russet Burbank', were assayed to detect natural infection by *C. coccodes*. The seed-tubers, graded as Generation 3 according to the Washington limited generation scale (26) were obtained from a commercial seed source. Prior to assay, seed-tubers were removed from 4°C storage and warmed to 22°C for 12 hours. They were then washed with warm soapy water, gently scrubbed with a sponge, rinsed with distilled water and air-dried. A disk of tissue from the basal end of each tuber was aseptically removed using a scalpel and plated on modified potato dextrose agar (MPDA) (21). Each disk of tissue was

approximately 1.5 cm in diameter, 3 mm thick, centered on the stolon scar and consisted of periderm, cortex and vascular tissue. MPDA was prepared using 750 ml distilled water, 0.75 g Difco PDA, 15 g Bacto agar, 4.5 ml ethanol 95%, 0.2 g streptomycin sulfate (31). Tuber disks were incubated in the dark at 22°C for 14 days and then evaluated for presence of *C. coccodes*. Seed-tubers were discarded when the assay disk primarily yielded other fungi that confounded the accurate detection of *C. coccodes*. Seed-tubers were considered naturally infected (N+) when sclerotia of *C. coccodes* developed on the excised disks. Seed-tubers were considered as *C. coccodes* non-detected (N-) when sclerotia did not develop on the excised disks.

N+ and N- tubers were randomly selected for inoculation with either a conidial suspension of *C. coccodes* or distilled water. Tubers were inoculated by placing a 1 cm^2 piece of filter paper soaked with a conidia suspension of *C. coccodes* onto the most apical bud, a mid-tuber bud and upon the suberized wound from the basal end assay. Tubers receiving the conidial suspension were coded (I+) and those receiving sterile distilled water were coded (I-). The four groups of tubers (N+I+, N+I-, N-I+, N-I-) were placed atop a plastic screen with a 1 mm^2 mesh that covered seven, moistened, unbleached paper towels inside glass baking pans (23- x 33- x 5.5-cm, 2.8 liter). Pans were placed in loose fitting plastic bags and the bag ends were tied loosely to facilitate air exchange. Tubers were then stored in the dark at 20°C for 5 days. After the 5-day incubation period, the tubers were cut into seed-pieces that averaged 57 g, placed into nylon mesh bags (27- x 45-cm with 5 mm mesh) and stored at 10°C with 95% RH. Seed-pieces were planted 14 days after cutting.

The experiment was completed twice. A total of 102 seed-pieces were planted in the first trial (March 13, 2007), and 105 seed-pieces planted in the second trial (May 6, 2007). Quantity of seed-pieces for each method of seed-piece inoculation was dependant on seed-tuber assay

results. The final seed-piece inoculation ratio in both trials was approximately 5,N+I+ : 3,N+I- : 3,N-I+ : 3,N-I-. Pots containing the seed-pieces were considered experimental units and were arranged in the greenhouse in a completely randomized design with unbalanced replication.

Seed-pieces were planted at an approximate depth of 5.5 cm below the soil surface in 3.7 liter pots (20 cm dia. x 15 cm deep) that contained 830 g of LC1 Soil Mix (Sun Gro Horticulture, Canada Ltd.) amended with 18 g of 16-16-16 NPK fertilizer (Agriliance Agronomy Company, St. Paul, Minnesota). The plants were grown in a greenhouse under natural light and 22/15°C day/night average temperatures.

Root collection. Intact root systems were collected throughout crop development in order to evaluate root colonization by *C. coccodes*. The root collection schedule was based upon days after crop emergence (DAE). Crop emergence was defined as the date upon which 90% of the pots contained an emerged stem. Ninety percent emergence (DAE = 0) took place 41 days after planting in the first trial and 26 days after the planting date in the second trial.

Roots were collected at 21-, 35-, 49-, 63-, 77-, 91-, and 115-DAE. The first six collections encompassed potato growth stages II-V (27) including vegetative plant establishment prior to tuber initiation through full maturity. The final collection was made 15 days after natural crop death due to senescence, however, those root systems were not able to be recovered as intact as for the previous collections. Prior to root collection, water was withheld on 14 randomly selected pots in order to push plants to the wilting point and remove available water from the soil. One day later, foliage was excised at the soil line and the root system and soil were allowed to dry for three wks in the pot. Any foliage regrowth was removed during the three wk period. After the three wk period, roots were washed, then arranged such that the whole of the root system sat upon the cut surface of the stem and the roots radiated from the stem in their natural

orientation. The root systems were then air-dried for 48 hours. Each root system was then placed in a separate paper bag and stored at 21°C for later disease assessment.

Disease assessment. The dried root systems were visually assessed for *C. coccodes* based on the presence of microsclerotia observed with a dissecting microscope at 10- to 50-x magnification. The spatial pattern of disease was assessed qualitatively for whether or not there was a single disease focus per root system. For the purposes of this study, the term focus has been used to describe the concentrated area of infected root tissue surrounding the point source of initial inoculum at the seed-piece. Disease was also measured quantitatively by determining the radius of the black dot focus. This distance was taken from the point of seed-piece attachment on the primary stem to the outer edge of the disease focus along the roots. An average radius of the black dot focus was calculated for each root system using distances taken at four 90° intervals radiating from the seed-piece. (Figures 1 & 2.)

To confirm the visual assessment for diseased tissue, an assay was conducted to confirm the presence or absence of *C. coccodes* on a random selection from diseased plants; 13% from the first trial and 17% from the second trial. Root tissue samples were taken from diseased tissue located halfway between the seed-piece attachment point and the outer edge of the black dot focus. A second sample from the same root system was taken from roots halfway between the outer edge of the black dot focus and the outer edge of the root system (Figure 2). The latter sample was taken from a portion of an infected plants root system that would have been visually assessed as non-diseased. For both the diseased and non-diseased areas, tissue assays consisted of approximately 2 cm long root subsamples gathered from two areas located on opposite sides of the seed-piece. The subsamples were collected by pinching the designated area of roots with

flamed forcipes and placing the tissue pieces onto MPDA. The root segments were incubated at 22°C for 14 days and assessed for both microsclerotia and conidia of *C. coccodes*.

Growth of *C. coccodes* in culture. Growth rate experiments for two isolates of *C. coccodes* on nutrient agar were completed twice. One isolate was CR-102, the same isolate used for inoculation of seed-tubers. The second, designated CC08-1 had been recovered from a plant grown in the study that was naturally infected from the seed-piece (N+, I-). For each isolate, mycelial plugs 6 mm in diameter were excised from the margin of 14 day old colonies growing on potato dextrose agar (PDA, Becton, Dickinson and Company, Sparks, MD). A single plug was placed mycelia face down onto the center of an 8.5 cm diameter Petri dish containing PDA (ph 5.6). Petri dishes were incubated at 16°C in the dark and distance of fungal growth from the edge of the inoculum plug to the advancing mycelial edge was recorded at 4-, 8- and 15-days after plate inoculation. The Petri dishes were arranged in a completely randomized design in the incubator with four replications per isolate.

Data analysis. A linear regression model that described the rate of *C. coccodes* focus expansion was created using SAS 9.1 procreg (SAS Institute Inc., Cary, NC). Only data from the 21- thru 91-DAE collections were used because of the inconsistency in root recovery on the 115 DAE collections. Simple linear regression was conducted using the mean radius of black dot foci among infected plants for each assay date as the dependant variable, and time (DAE) as the independent variable. Only infected plants were used in the calculation of mean radius and the assumptions of normality and equal variance for the regression model were satisfied (25).

Previously, values for time and distance of *C. coccodes* expansion from a seed-piece have been reported, but no growth rate calculated (1). In order to calculate a rate from this previously published data, values were collected by interpreting data points directly from the uppermost plot

in Figure 2 of Andrivon 98'(1). Values for time and symptom extension were determined using a ruler and the plot axis from that figure. Using the data from that publication, growth rates for *C. coccodes* on the roots of potato cvs. 'Bintje' and 'Roseval' were then calculated. Values were converted from weeks to days and cm to mm, and symptom extension (mm) was regressed upon time (days) with SAS 9.1 procreg. Data for mean radius growth rate of *C. coccodes* on PDA were likewise analyzed with SAS 9.1 procreg.

Pearson's chi-square (χ^2) goodness-of-fit test was used to validate the visual assessment of diseased tissue. The χ^2 test statistic was calculated to determine whether the observed results from root isolations, when compared to the expected results from the visual assessments, lead to the same conclusion for presence or absence of *C. coccodes* (33).

RESULTS

On each infected root system, the disease was restricted to a compact focus that consisted of a dense cluster of roots covered with microsclerotia (Figures 1 & 2). Foci were rotationally symmetric, consisted of a continuous area of diseased roots that was sharply delimited from non-diseased root tissues outside that area and were centered on the point of seed-piece attachment to the stem. A single disease focus that radiated from the seed-piece was observed in 100% of the 63 *C. coccodes* infected potato root systems in the first trial. The same spatial pattern was observed in 100% of the 89 root systems infected with *C. coccodes* in the second trial. Additional or secondary foci originating separately from the seed-piece were not observed on any root system.

Twenty-five of 26 isolations from sampled roots yielded results identical to that of the visual assessment for diseased tissue in the first trial, and 35 of 36 were identical in the second

trial (Table 1). The two observations where visual assessment and root isolation did not agree were from root samples visually identified as diseased that did not develop *C. coccodes* in culture. Accuracy of visual assessments based on presence of microsclerotia on roots was not statistically different than detection of the pathogen by plating root samples on MPDA. The calculated χ^2 probabilities lead to failure to reject the assumption that the two methods of disease diagnosis yield the same results, $P > 0.78$ in the first trial and $P > 0.81$ in the second trial (Table 1).

The 115 DAE root collections appeared to have fewer roots successfully recovered when compared to previous assay dates, but this was not quantified. Root systems collected at 115 DAE had senesced and died, regardless of infection by *C. coccodes.* The proportion of plants that were infected with *C. coccodes* at 115 DAE was not significantly different from samples collected after 35 DAE in either trial. ($P \geq 0.13$, ANOVA, data not shown). Since percentage of plants infected was consistent, additional, late season spawning of seedborne inoculum with fewer days to colonize root systems were not likely the cause of several plants having small disease foci radius at 115 DAE (Figure 3). More likely, it was due to less overall root recovery during the root washing to remove soil. Whether root loss was due to disease or host tissue age was unclear, therefore, the 115 DAE observations were removed from the regression model.

Mean radius of black dot foci significantly increased linearly with time (DAE) until 91 DAE (Figure 3, Table 2). A linear regression model best described the relationship between mean black dot focus radius on a potato root system and time (Figure 3), as identified by R^2, P and statistical assumptions interpreted from residuals analysis (25). This regression model resulted in a parameter for time that represented *C. coccodes* advance along roots of actively growing plants at a rate 0.95 mm/day in the first trial and 0.99 mm/day in the second (Table 2).

Effects due to inoculation method of the seed-piece were inconclusive. The first attempt at regression analysis used observations of black dot focus radius for individual plants and time, but failed the assumption of equal variance (25). In the failed model for individual plant observations, inoculation method and interaction with DAE were not significant in either trial at the 95% confidence level but values for radius of disease foci for individual diseased plants had an increasing range that was related to increase in DAE. This relationship prevented the use of linear regression to draw any conclusions about effects due to inoculation method and the model was discarded. Alternatively, the regression model that calculated the 0.95 to 0.99 mm/day growth rate used the mean radius of black dot foci for all diseased plants on each assay date, regardless of inoculation method (Figure 3). This step eliminated the violation of the assumption of equal variance but prevented drawing any conclusions about differences in inoculation method.

The rate of root colonization by *C. coccodes,* as derived from plotted data taken from Andrivon (1) was determined to be 1 mm/day on the cv. 'Bintje' and 1 mm/day for cv. 'Roseval' ($R^2 > 0.95$, $P < 0.001$). Visual interpretation of the plot in that publication initially resulted in the notion that there was a strong linear association between the cortical rot symptom extension from inoculated seed-pieces and time. This initial visual interpretation was confirmed with the resulting linear regressions that generated $R^2 > 0.95$.

The daily growth rate of *C. coccodes* on PDA at 16°C was 2.5 to 2.9 mm/day. The linear model's parameter for time (growth, mm/day) represents the daily increase in radius of the fungal colony in culture (Table 2). There was no significant effect due to isolate and the growth rate represented is that of both isolates combined. The rates observed for these two isolates were

lower, but comparable to previously described rates of growth for isolates of *C. coccodes* at 16°C on V8 agar (8) and 1% PDA (14) of 3.3 mm/day.

DISCUSSION

Rate of root colonization by *C. coccodes* was constant throughout plant development and diseased tissue was always continuous from the seed-piece. The margin of diseased tissue advanced at a rate of approximately 1 mm/day on roots of cv. 'Russet Burbank' in this study. This value is in agreement with black dot symptom extension originating from the seed-piece that was described by Andrivon in a study that compared cultivar-isolate interactions (1). In that study, *C. coccodes* colonization on roots of cvs. 'Bintje' and 'Roseval' was determined visually using cortical rot symptoms or sclerotia, and the data was plotted to compare disease extension between inoculated and control plants. In that plot, a linear increase of symptom extension on roots culminated with 8 cm at 12 wks after inoculation. Regression analysis of that authors data, which was completed during this study, resulted in an average daily growth rate of 1 mm/day. Between the two studies, no major difference in rate of root colonization was observed across three potato cultivars, two known *C. coccodes* isolates, and the uncharacterized isolates in the naturally infected commercial potato seed-tubers.

The pattern of linear disease increase on roots throughout all plant development stages departs from the disease increase reported in potato plant foliage and aerial stems (31). The pattern of colonization in aerial tissues is typically rapid, non-constant throughout the entire growing season and is associated with the later growth stages or senescence (19, 28, 31). Whether this difference is due to the host, pathogen, or the influence of the soil environment relative to the foliar environment is unclear.

Based on the consistency of the pathogens growth rate on roots, a method to formally test the assertion that *C. coccodes* is a weak pathogen of potato could be derived. This could be accomplished by comparing the *in vitro* temperature response growth curve of *C. coccodes* to an *in vivo* temperature response growth curve created from observations made on healthy potato roots. As described by Bruehl (5) for *Rhizoctonia solani*, causal agent of sore shin of cotton, the well established temperature response growth curves of *C. coccodes* in culture (1, 8) could be compared to a temperature response growth curve of *C. coccodes* on potato roots. Either similarity or divergence from the growth curve of the fungus in culture would end the debate as to whether *C. coccodes* is a weak pathogen and whether or not it is dominant in the host-pathogen relationship. That relationship could not be determined from this study since potato plants were not grown over a range of different temperatures.

The average night and mean lowest soil temperature that *C. coccodes* was exposed to while colonizing plant roots in this study was 16°C. Therefore, this temperature was selected for the growth rate analysis of *C. coccodes* isolates cultured on PDA. The 16°C culture temperature was less than the optimum temperature of 28°C (1, 8) for mycelial development of *C. coccodes,* yet the mycelial growth rate in culture was 2.5 times greater than on potato roots. Fungal growth rate on roots and PDA were linear over time and the assessment of isolate growth rate *in vitro* also confirmed that the isolates used in this study of root colonization were similar to the growth rate in culture previously reported for *C. coccodes*.

Precedents for measuring the spatial pattern of disease within a single plant's root system were sparse (10, 15) and examples describing the spatial pattern of disease within intact root systems has not been previously reported. Therefore, the term focus, used conventionally in populations of plants, was used to describe disease increase within the population of roots for

individual infected plants. For the purposes of this study, the term focus has been used to describe the concentrated area of infected root tissue surrounding the point source of initial inoculum at the seed-piece. Vanderplank (44) references Zadoks (45), who used the term focus to define a group of 3 or 4 closely spaced leaves infected with stripe rust, and Huisman (17) suggested that for mycorrhizae and cortical rot disease data, a quantitative measure of both the area of diseased tissue and infection foci be recorded. Use of the term focus in this study might then be appropriate when viewed under this context. If multiple primary or secondary infections within the same root system can be had, then it becomes more intuitive that an individual root system can be treated as a population.

Colletotrichum coccodes actively colonized healthy potato root systems in single distinct foci that originated from a point source at the seed-piece. A distinct margin that separated diseased and non-diseased tissue was always observed and no examples of secondary sites of infection or daughter foci developed in any of the plants root systems. That spatial pattern of diseased tissue on potato roots during vegetative plant establishment through maturation (growth stages II-V) (27) indicates that *C. coccodes* was actively parasitic and colonized healthy roots. Diseased tissue areas were densely concentrated and the pathogen did not appear to show preference for specific roots that might have been weakened. Additionally, this colonization was not associated with senescence or other factors that had weakened the plant as is often reported with this pathogen (7, 28, 32, 40).

Within plant root systems, the spatial pattern of diseased tissues covered with *C. coccodes* microsclerotia was consistent, and measurements of foci radius within 1 cm were typically observed in the four cardinal directions extending laterally from the seed-piece. This consistent radial pattern, and the agreement between visual and root isolation methods for the

detection of *C. coccodes* from diseased and non-diseased areas within root systems leads to several hypotheses. First, it may be explained that *C. coccodes* grows vegetatively and ramifies and colonizes potato plant roots as a latent infection that leads to the linear and continuous single focus of disease observed in this study. The second hypothesis is based on the observation of tuber bearing stolons at various stages of fungal colonization, where *C. coccodes* appears to grow along roots and stolons to eventually reach the progeny tubers. This might explain the frequency with which *C. coccodes* has been associated with remnant stolons on tubers (3, 7, 14, 16, 42) and the greater frequency that it can be isolated from the stolon end than from other areas of the tuber (21). Based on the frequency of pathogen detection on the stolon end of the tuber and the spatial pattern of disease observed in this study, it seems likely that progeny tubers are infected via the stolon. Infection of a stolon that serves as the bridge to progeny tuber infection could potentially be initiated from either the seed-piece, or from contact with soilborne inoculum.

Disease progress on the underground plant parts appears to fit the monocyclic disease model within a single growing season. The lack of evidence for secondary infections developing within root systems leads to this conclusion from a biological standpoint and is not different from the generally accepted principles for most of the soilborne diseases of potato (36). However, the case for black dot being a monocyclic disease was not directly demonstrated. The rate of linear disease increase calculated in this study was that of the advance of the margin of diseased tissue rather than the rate of apparent infection as used by Vanderplank (44). Monocyclic and polycyclic diseases are categorized based on the rate of apparent infection, which was not quantified in this study, but the lack of daughter foci within an individual root system indicates that there were no successful secondary infections or resulting rate of apparent infection.

Microclimate can affect production of *C. coccodes* conidia *in vitro* (39). Based on soil atmospheric characteristics, Sanogo hypothesized that *C. coccodes* may not produce many conidia belowground (39). Furthermore, for the genus *Colletotrichum*, splashing water is the generally accepted concept for conidial spread from non-dried acervuli (2). If conidia are produced within the soil environment and are capable of percolation in the soil, spore dispersal without splashing would be contrary to the generally accepted method of dispersal for the genus (2). Determining whether conidia are produced on belowground plant tissue, and if they can be released and transported in the soil would be another logical step in clarifying black dot etiology. If so, the single focal pattern of *C. coccodes* infected tissues observed in this research could be an artifact of greenhouse study.

Through the use of a hypothetical example, Vanderplank demonstrated that doubling time available for disease increase has the same effect as doubling rate (44). Lack of secondary foci development and a 1 mm/day rate of advance of diseased tissues would seem to make for an unsubstantial total disease increase. However, *C. coccodes* can be isolated early in crop development in potato fields (9, 20, 38, 43) and a long interval between infection and crop death could attribute to the significant root colonization observed in commercial fields. For example, cv. 'Russet Burbank' can occupy fields for 160 days between planting and harvest and the time from infection to harvest could exceed 100 days. In addition to the long period of time that *C. coccodes* has for infection and ramification, overwintering soilborne inoculum could cause multiple focal infections within a single root system. Foci that coalesce over the course of the growing season could partially explain severe black dot infections. To assess this hypothesis, simulation of disease progress might be accomplished comparing single and multiple primary infections within a root system using the growth rates identified in this study and colony forming

unit soil populations identified by Barkdoll (3). Furthermore, the effect on total disease from an infection court on the belowground stem where focus expansion is possible in all directions along continuous plant tissue, could be compared to the two dimensions available for spread from an infection court on a single root.

The spatial pattern of *C. coccodes* expansion within potato root systems did not support a model for systemic growth or of having a secondary disease cycle because *C. coccodes* remained focal and never developed a pattern of scattered infections throughout the root system. Lack of internal or external spread of secondary inoculum indicates that only overwintering infective propagules that make plant contact cause disease.

The method of visual assessment for presence of *C. coccodes* that was validated in this study utilized plant tissues that were given adequate time to express latent infection. Visual assessment may not be accurate for discriminating non-diseased tissue when live plant samples are used. A latent period occurs between infection by *C. coccodes* and microsclerotia development (12, 18, 19, 23, 28, 34, 40) so the opportunity to develop microsclerotia may be critical for proper visual determination for presence, or more importantly the absence of the pathogen. The in-pot drying period after the foliage was excised assured the required time for development of microsclerotia from latent infection by *C. coccodes.* It also rendered any conidia non-viable prior to root washing. Blakeman identified a survival period for *C. coccodes* conidia in soil of less than three weeks (4, 11). The drying period reduced the possibility of conidia infecting healthy roots during root washing and this step may have been important in generating the similar results obtained from visual disease diagnosis and root isolations.

In conclusion, no secondary infection sites or additional areas of diseased tissue that resulted from secondary infection were observed in potato root systems produced in a

greenhouse. The diseased area within each root system was always continuous from the point source of initial inoculum and the margin of diseased tissue advanced continuously throughout all stages of plant development at approximately 1 mm/day. Furthermore, it can be inferred from these developmental characteristics that infected stolons frequently serve as a bridge for infection of progeny tubers. The results of this study lend support to the generally accepted principles for control of soilborne pathogens that concentrate on the reduction of effects from pathogen populations in the soil (24, 36, 44). For crops grown in fields with previous potato rotations, overwintered primary inoculum is likely the major concern for infection of the root system as multiple primary infections could occur. Therefore, based on the biology of a pathogen with no apparent secondary infection cycle, and a 1 mm/day growth rate that only occurs along continuous tissues, efforts should be concentrated on developing tactics that reduce the effects of initial inoculum. This would include efforts that reduce inoculum quantity and effectiveness, and the use of cultural practices that minimize host predisposition in order to minimize the number of initial infections that occur from perennation structures.

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Table 1. Number of *C. coccodes* infected plants exhibiting a single focal spatial pattern of diseased root tissue and comparative results of observed pathogen detection as determined on nutrient agar compared to the expected result from visual assessment.

Trial	Total Plants	Total Plants Infected	Total Plants with Single Focus*	Results of Isolation on Nutrient Media: Non-Diseased Roots Observed / Expected	Results of Isolation on Nutrient Media: Diseased Roots Observed / Expected	Chi-square P †
1	102	63	63	13 / 13	12 / 13	0.78
2	105	89	89	18 / 18	17 / 18	0.81

* Plants with compact area of diseased roots covered with microsclerotia of *C. coccodes* that radiated from seed-piece and was sharply delimited from non-diseased root portions located distally along the diseased roots.

† $P > 0.05$ indicate that presence of *C. coccodes* as observed using root isolation on nutrient agar was not different than the expected result based on visual assessment using presence of microsclerotia.

Table 2. Rate of growth (mm/day) with 95% confidence interval, coefficient of determination, and *P* for the slope when data for growth of *C. coccodes* on roots and potato dextrose agar was regressed over time in two trials.

Substrate	Growth* mm/day	95% Confidence Interval‡ Lower	Upper	R^2	*P*
Roots Trial 1	0.95	0.36	1.54	0.83	0.018
Roots Trial 2	0.99	0.45	1.53	0.86	0.007
PDA† Trial 1	2.88	2.75	3.02	0.98	0.001
PDA† Trial 2	2.51	2.22	2.80	0.93	0.001

* Growth represents the increase in mean radius (mm) of black dot focus on roots or *C. coccodes* colony in culture per 1 day increase in time.

‡ Confidence interval indicates 95% confidence range within which true value of growth (mm/day) resides.

† PDA incubated at 16°C to simulate the average nighttime soil temperature to which the greenhouse grown potato plants were exposed.

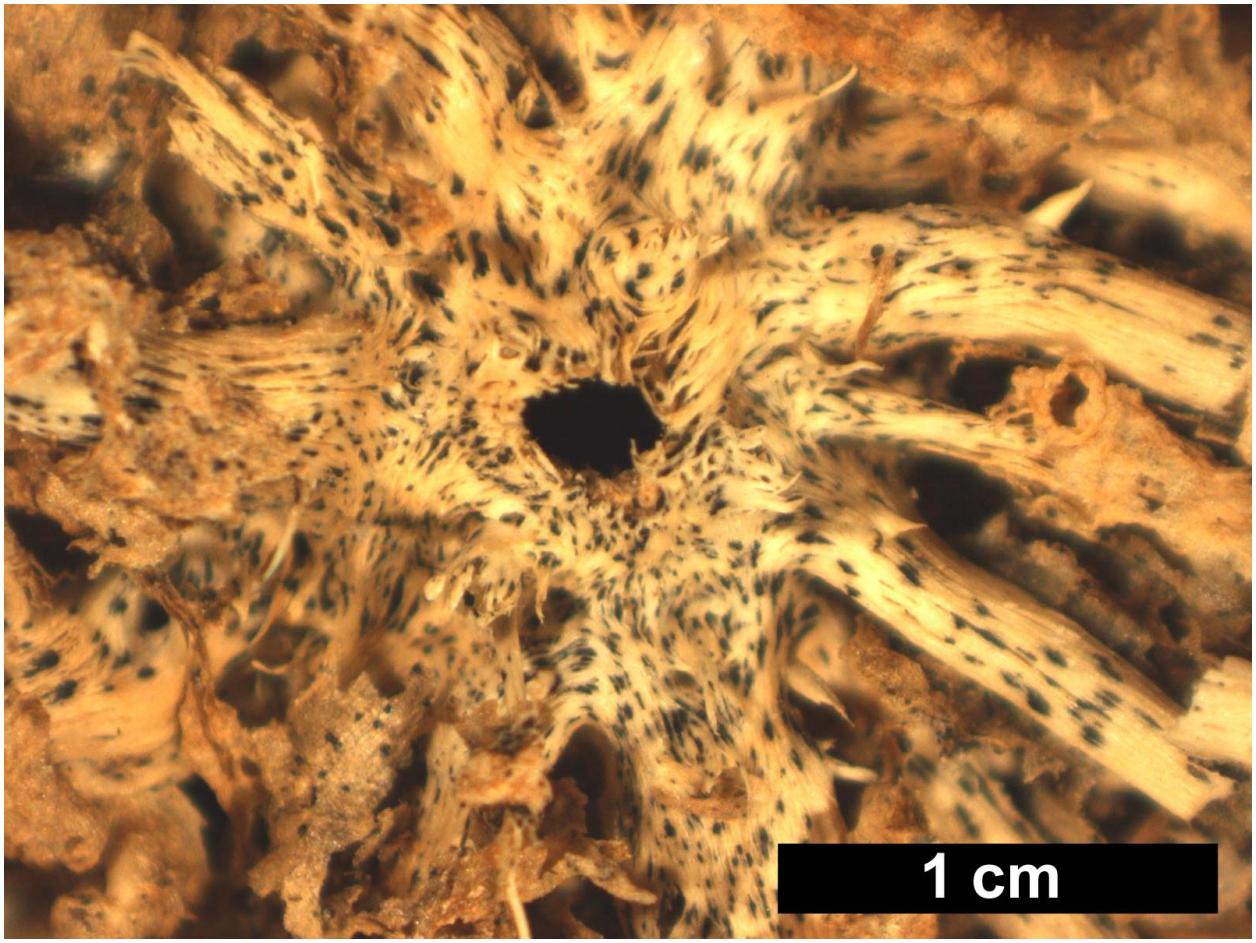

Figure 1. Base of potato stem at the point of seed-piece attachment. Microsclerotia of *C. coccodes* cover host tissues continuously and radiate distally along roots from the point source of infection at the seed-piece.

Figure 2. Mean radius of *C. coccodes* foci with infection originating at the seed-piece. The black disk overlaid the root system represents the shape and size to scale of the diseased tissue area relative to the root system averaged from two separate trials. The number on the white background represents the root collection date (DAE) as measured from 90% plant emergence in each trial.

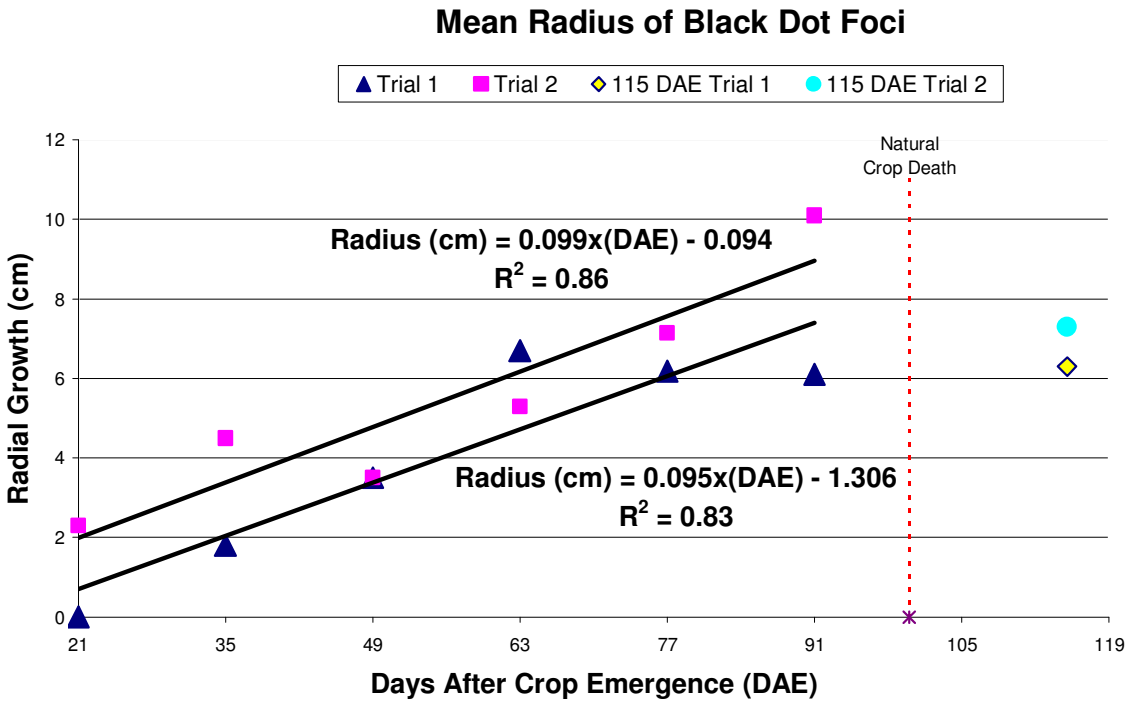

Figure 3. Mean radius of *C. coccodes* foci by assay date for Russet Burbank plants with infection arising from the seed-tuber. ($P < 0.01$)

Response of *Colletotrichum coccodes* to Selected Fungicides in Potato

Jason Ingram

Department of Plant Pathology, Washington State University, Pullman, WA 99164

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J.T. INGRAM. Dept. Plant Pathology, Washington State Univ., Pullman, WA 99164

ABSTRACT

A method to screen fungicides was used to evaluate the effect of application timing and active ingredient to prevent *Colletotrichum coccodes* from infecting potato plants. Additionally, fungicide application by center pivot chemigation in commercial potato fields was evaluated as a potential black dot management strategy. Incidence of infection in plants treated with azoxystrobin and pyraclostrobin was significantly less than inoculated control plants when the fungicides were applied prior to inoculation ($P < 0.05$). Fungicide application after inoculation did not significantly reduce infection. Belowground stems sampled from the areas where azoxystrobin was chemigated onto the crop at 50- and 67-days after planting (DAP) had a significantly smaller percent area covered with sclerotia at 79 DAP in both years ($P < 0.04$). However, the effect was not observed in both years at the subsequent 102- and 140-DAP collections. Latent infections were detected in non-symptomatic plants from the chemigated field despite a lack of visual sign of the pathogen or obvious host symptoms.

INTRODUCTION

Colletotrichum coccodes (Wallr.) Hughes causes black dot of potato (*Solanum tuberosum* L.). Small, black microsclerotia of the fungus develop on infected roots, stems and tubers and give the disease its name. In addition to black dot, the pathogen can also cause severe economic

losses in processing tomato (*Solanum lycopersicum* L.) in which infected fruit develops black sunken lesions, a condition known as anthracnose fruit rot (7, 12, 13, 16, 22).

Colletotrichum coccodes has been shown to inconsistently reduce yield and quality in potato (21, 23, 27, 30, 39-43), but the fungus does not cause the catastrophic losses associated with tomato fruit anthracnose (7, 11). Therefore, potential management tactics for potato black dot are tempered by the low risk associated with not controlling the disease. In light of this, identification of a fungicide effective against the major foliar potato diseases, early blight and late blight, as well as black dot, could be of benefit. Identification of a fungicide with efficacy against all three diseases, that could also be applied within existing spray schedules, would provide a cost and environmentally effective management tactic for a pathogen that causes inconsistent yield and quality losses in potato.

Several fungicides recently labeled or being considered for use on potato could fill this niche for black dot management. These potential fungicides represent five different Fungicide Resistance Action Committee (FRAC) groups (3). Most of the fungicides investigated in this study are either registered for early blight and late blight, or are experimental and believed to have activity on both. Grouped by mode of action these fungicides include: FRAC Group 11 quinone outside respiration inhibitors (QoI) azoxystrobin, famoxadone, fluoxastrobin and pyraclostrobin; a FRAC group 7, fungal respiration inhibitor, penthiopyrad; the sole member of FRAC group 19, a chitin synthase inhibitor, polyoxin; and FRAC groups 3 + 40, two fungal membrane synthesis inhibitors represented by a formulation of difenoconazole + mandipropamid. An additional component of interest is how these products affect *C. coccodes* compared to the multi-site mode of action fungicides that are industry standards, FRAC group M3 (dithiocarbamates or EBDC's) such as mancozeb, and FRAC group M5 (chloronitrile),

chlorothalonil, both labeled for early blight and late blight control in potato (Table 2). Of the fungicides evaluated, those that specifically list *C. coccodes* or control of black dot on the label include, azoxystrobin (Quadris, Syngenta, Wilmington, DE), chlorothalonil (claim varies by manufacturer), mandipropamid + difenoconazole (Revus Top, Syngenta, Wilmington, DE), and pyraclostrobin (Headline, BASF, Research Triangle Park, NC). Three potato fungicides still without an approved label and undetermined *C. coccodes* efficacy include penthiopyrad (DPX-LEM17, DuPont, Wilmington, DE), polyoxin, (Kaken Pharmaceutical, Tokyo, Japan) and famoxadone (Famoxate, DuPont, Wilmington, DE).

Chemigation is a common method of fungicide application for potatoes grown in the Columbia Basin of Washington (24). In this system, fungicides are injected into the overhead irrigation water and are distributed in a relatively high volume of water. Volumes over 18,708 liters/ha (2000 gallons/acre) are common, and are much greater than a typical ground sprayer at 327 liters/ha (35 gallons/acre) or aerial at 47 liters/ha (5 gallons/acre). The high water volume application used in chemigation has been demonstrated to provide a different effect on fungicide distribution in the foliage when compared to aerial application (17). Based on this, it is hypothesized that chemigated fungicides could be useful in managing potato black dot on belowground plant parts.

The purpose of this research was first to improve upon previously published fungicide screening techniques (2, 12, 44) by using a wounded, inoculated plant assay under controlled conditions rather than monitoring fungal growth on fungicide amended media. The second purpose was to quantify infection and colonization of *C. coccodes* in potato stems when fungicide applications using different active ingredients (a.i.) were made pre- and post-infection.

The third purpose was to evaluate fungicide application by center pivot chemigation for management of black dot in commercial potato fields.

MATERIALS AND METHODS

Tuber assay. Seed- and progeny-tubers of *Solanum tuberosum* L. cv. 'Russet Burbank' were evaluated for the presence of *C. coccodes* by sampling from the stolon end of tubers. Prior to assay, seed-tubers were removed from 4°C storage and warmed to 22°C for 12 hours. They were then washed with warm soapy water, gently scrubbed with a sponge, rinsed with distilled water and air-dried. A disk of tissue from the basal end of each tuber was aseptically removed using a scalpel and plated on modified potato dextrose agar (MPDA) (25). Each disk of tissue was approximately 1.5 cm in diameter, 3 mm thick, centered on the stolon scar and consisted of periderm, cortex and vascular tissue. MPDA was prepared using 750 ml distilled water, 0.75 g Difco PDA, 15 g Bacto agar, 4.5 ml ethanol 95%, 0.2 g streptomycin sulfate (33). Seed-tubers were returned to storage at 95% RH and 10°C until planting or seed cutting. Progeny tubers were discarded after basal end sampling. Tuber disks were incubated in the dark at 22°C for 14 days and then evaluated for presence of *C. coccodes*. Tubers were considered infected if the basal end tissue disk developed microsclerotia of *C. coccodes*.

Inoculum preparation. Colonies of *C. coccodes,* isolate CR-102 were grown on V-8 agar (37) under constant florescent light (10 W/m^2) at 22°C. Isolate CR-102 belongs to the NA-VCG 2 and was originally isolated from a seed potato obtained from a commercial seed lot. Pathogenicity of the isolate has been previously demonstrated (20, 31, 33, 34) and the isolate had been stored on rye berries at room temperature until culture on V8 media. Two Petri dishes containing nine-day-old colonies were used to prepare a conidial suspension for inoculation of

plants. Sterile distilled water (1.0 ml) was added to the dish, the colony surface was scraped using a sterile scalpel and the resulting suspension was then strained through four layers of cheesecloth to remove microsclerotia. The conidial suspension was adjusted to a specified concentration with aid of a hemocytometer.

Plant material. Seed-tubers were evaluated for the presence of *C. coccodes*. A seed-tuber was not used for propagating greenhouse plants if *C. coccodes* developed in culture from the assayed periderm disk. Seed-tubers or seed-pieces were planted 5.5 cm below the soil surface in 15 cm diameter x 18 cm tall, 3 liter pots. Pots contained 540 g LC1 Soil Mix (Sun Gro Horticulture, Canada Ltd.) and greenhouse conditions were maintained under natural light with mean day/night temperature of 22/14°C and relative humidity of 38/65%.

Plant inoculation. Inoculation of plants with *C. coccodes* was done by wounding the plant stem at a point halfway between the soil line and stem apex and then introducing a conidial suspension of the fungus. The most dominant stem of each plant was selected and a piece of 120 grit sandpaper (5 cm x 2 cm) was wrapped around an 8 mm diameter rod and dragged across the stem with light hand pressure. The sandpaper was dragged perpendicular to the stem longitudinal axis and only 3 cm of the total sandpaper length was dragged across the stem. Wounds approximately 4- x 3- x 1-mm deep were generated. Inoculation was done by placing 40 µl of the *C. coccodes* conidial suspension onto a 1 cm^2 piece of Whatman #1 filter paper and placing the paper over the wound (33). This area was considered the inoculation court. Plants in the non-inoculated control treatment received identical handling except that sterile distilled water was used for plant inoculation. Immediately after placement of each filter paper, the plants were placed in a mist chamber for 21 hours at 21°C. After the misting period, plants were removed

from the chamber and permitted to air dry before the inoculum soaked filter paper squares were removed.

Fungicide application timing. The effect of fungicide timing on preventing infection of potato stems by *C. coccodes* was studied in the greenhouse using three fungicides commercially available for potato. Certified, pre-nuclear generation seed-tubers (whole mini-tubers), with a size range of 9- to 35-g were planted in March, 2007. Potting soil (LC1 Soil Mix, Sun Gro Horticulture, Canada Ltd.) was amended with 18 g of 16-16-16 NPK fertilizer (Agriliance Agronomy Company, St. Paul, Minnesota). Additional fertilizer was applied at 57 days after planting at a rate of 2.65 g/plant (Miracle-Gro Tomato Plant Food, 18-18-21. Scotts Miracle-Gro Products, Inc. Marysville, Ohio). Plants were arranged in the greenhouse in a completely randomized design until 67 days after planting when most plants were at tuber initiation (growth stage III) (28). Plants were organized into four blocks based on stem height (18- to 35-cm height range) and inflorescence development at the time of inoculation. Plants were wounded with the sandpaper, then assigned to one of 14 treatments within each of the 4 blocks and resulted in a randomized complete block design with 4 replications per treatment. The no-fungicide control had an additional replication included in blocks two and three, and resulted in 6 replications for the no-fungicide control. After wounding, pre-inoculation fungicide treatments were sprayed and permitted to air dry on the foliage.

Plants were then inoculated at the wound site using a suspension adjusted to $6x10^4$ conidia/ml. The one day post-inoculation fungicide treatment was applied after plants were removed from the mist chamber and had dried. The remaining fungicide treatments were applied at the prescribed intervals over the subsequent ten days.

Fungicide application timing examined three active ingredients that were broadcast applied to the foliage using five different scheduled applications. The application timings included: an application prior to inoculation, post-inoculation applications made at 1 day, 5 days, or 10 days after inoculation, and a sequential application made at 1 & 10 days after inoculation. Azoxystrobin, 164 g a.i./ha (Quadris, 9 oz/acre) and pyraclostrobin, 165 g a.i./ha (Headline, 9 oz/acre) were both applied using all five application timings. Dicloran at 4.2 kg a.i./ha (Botran 5F, 3 quarts/acre, Gowan, Yuma, AZ) was applied at only 2 times, pre-inoculation and 1 day post-inoculation. All fungicides were applied within the manufacturers labeled rate per acre for potato (Table 1) in 355 liters/ha (38 gallons/acre) water carrier without surfactant and using a squeeze trigger sprayer (7800 Series, Impact Products, Toledo, OH). Individual plant dose was calculated by dividing combined volume of fungicide and water per hectare by a typical plant population for 'Russet Burbank' of 45,822 plants/ha (18,544 plants/acre). Two remaining treatments included a no-fungicide control on inoculated and non-inoculated plants.

Stems were collected for assay at 20 days after the last fungicide application. The entire experiment was repeated with a planting date in May, 2007. The period between final fungicide treatment and the assay for *C. coccodes* in the second trial was 35 days. All plants at the time of assay in both trials, were in the tuber bulking stage (growth stage IV) (28) and were not yet senescent.

Fungicide efficacy. To evaluate the effectiveness of selected fungicides as a protectant application against *C. coccodes*, a study was completed twice in 2008. Seed-tubers graded to Generation 1 of the Washington limited generation scale were used to propagate plants for inoculation (5). Tubers were cut into 42- to 85-g seed-pieces and stored at 10°C and 95% RH for 6 days before being planted. In order to promote early plant senescence, only 3.0 g of fertilizer

(16-16-16 NPK) was incorporated in the soil at planting, and no additional in season fertilizer was used. Plants were inoculated with *C. coccodes* when approximately 25% of plants were at primary flower or the end of growth stage III. This occurred at 55 days after planting in the first trial and 48 days after planting in the second trial. Plants were segregated into 4 blocks based on maturity. Each block contained a single replication of the 14 treatments, resulting in a randomized complete block design with four replications per treatment. Fungicide treatments included: chlorothalonil (1.05 kg a.i./ha, Bravo Wx Stick, Syngenta, Wilmington, DE); fluoxastrobin (101 g a.i./ha, Evito 480SC, Arysta Life Science, Cary, NC); penthiopyrad (140-, 245- and 351-g a.i./ha); famoxadone (76-, 114- and 152-g a.i./ha); mancozeb (1.05 kg a.i./ha, Penncozeb 75DF, Cerexagri, King of Prussia, PA); polyoxin D zinc salt (201 g a.i./ha); azoxystrobin (196 g a.i./ha); and mandipropamid + difenoconazole (114 g + 114 g a.i./ha) (Table 2). All fungicides were broadcast on foliage at a single time just after wounding but prior to inoculation. Fungicides were applied at the middle rate of the prescribed range on the manufacturers label for potato (Table 2). Products containing penthiopyrad, famoxadone and polyoxin did not have an approved label and were used at rates suggested by the manufacturer (Table 2). Inoculation with a $1.5x10^5$ conidia/ml suspension of *C. coccodes* was performed after the fungicides had dried on the foliage, approximately 1 hour after application.

The assay for *C. coccodes* stem colonization was done 35 days after inoculation. All plants at time of assay were chlorotic and the bottom two to four leaves were necrotic. To confirm that any *C. coccodes* detected in the stem was not due to colonization arising from infected seed-tubers, one stem cross-section was taken near the soil line of inoculated stems and placed onto MPDA. *Colletotrichum coccodes* was not detected in any of the soil line cross-sections.

Greenhouse stem assay. The inoculated stem of each plant was excised just above the soil line using flame-disinfested pruning shears, and petioles were removed using flamed scissors. The stems were soaked in a 10% bleach water solution for 3 minutes, rinsed with distilled water and permitted to air dry on a laboratory bench top. After drying, stem cross-sections were aseptically removed and placed onto MPDA to determine if *C. coccodes* was present. Cross-sections were taken from above and below the inoculation court beginning 5 mm from the edge of the inoculation court wound. Seven consecutive, 1 cm long stem cross-sections were taken from each side of the inoculation court in sequential order to assess a total distance of 7.5 cm in each direction from the inoculation court edge. Stem cross-sections were placed in tissue culture Petri dishes (100 x 20 mm, Sarstedt brand, Newton, NC) containing MPDA and incubated at 21°C for 14 days. The inoculation court for each stem was also cultured and served as the basis for evaluation of incidence of *C. coccodes*. Colonization of a segment was considered positive if the microsclerotia of *C. coccodes* developed. The diameter of the mycelial colony radiating from the inoculation court stem segment was recorded using two perpendicular measurements taken at 14 days after stem cross-sections were placed on MPDA. The difference in *C. coccodes* incidence within the cross-sections containing the inoculation court, the incidence in cross-sections distal to the inoculation court, and mycelial colony diameter due the combined treatment effect of the fungicide and application timing were then compared using ANOVA.

Application by chemigation. The experiment was designed to quantify efficacy of a fungicide applied by chemigation during early crop development, compared with a non-treated control. **'**Russet Burbank' certified seed graded as Generation 3 according to the Washington limited generation scale (5) was sampled prior to planting in a commercial potato field.

Approximately 60 tubers were collected and the incidence of seed-tubers infected with *C. coccodes* was determined.

Fields located east of Moses Lake, WA in the Columbia Basin were used in 2007 and 2008, and had each had a total of three previous potato crops (Figure 1). The field used in 2007 was in a four-year potato rotation, located (47°16'18.47"N, 118°52'03.64"W) and planted April 30. The field used in 2008 was in a three-year rotation, located (47°17'37.24"N, 118°51'44.90"W) and was planted on May 1. Soil types in both fields were Shano and Burke Silt Loams. Seed was treated with thiophanate-methyl, mancozeb and imidacloprid (Tops-MZ-Gaucho, 1:100 weight seed) and with the exception of the prescribed fungicide treatments, the fields were managed according standard regional practices.

The chemigated fungicide treatment and non-treated control were replicated three times and arranged in a center pivot irrigated field that had been divided into six adjacent 30° sections or wedges that totaled 180° (Figure 1). The fungicide treatment was azoxystrobin applied at a rate of 113 g a.i./ha delivered in 35,544 liters/ha water via center pivot chemigation (Quadris 6.2 oz/acre in 3800 gallons/acre or 0.14 acre-inches water carrier). Non-treated wedges received water but no fungicide on those dates. Fungicide treatments were applied to the three treated wedges at 50- and 67-days after planting (DAP) in 2007 (50 & 69 DAP in 2008). Plant and crop maturity at 50 DAP corresponded to tuber initiation or potato growth stage III and plant closure within row. At the time of the second treatment at 67 DAP, a maximum tuber size of 4.25 cm diameter and 90% between row closure was observed. Treated wedges were separated by a non-treated 30° wedge, and effects of fungicide application on black dot development in the three pairs of adjacent treated and non-treated wedges were designed for comparison using ANOVA (Figure 1 & Table 3).

The 50 DAP azoxystrobin treatment was the first fungicide application made to the field that growing season. Between the two experimental fungicide applications, the entire field was treated at 60 DAP with fluazinam (Omega 500F, 8 oz/acre, 292 g a.i./ha, FRAC 29) mixed with an aromatic PCNB (Blocker 4F at 64 oz/acre, 2.24 kg a.i./ha, FRAC 14). An EBDC fungicide, (Dithane DF) was applied to the entire field 14 days after the final azoxystrobin treatment and all conditions including the growers usual 14 day fungicide calendar spray were maintained alike across all wedges for the remainder of the season.

Extent of *Colletotrichum coccodes* colonization was determined by sampling at 79-, 102- and 140-days after planting. Plants were collected from each wedge (replication) between the two outer most pivot wheel tracks on a transect between the outer edge of the field toward the center pivot. The starting point of the transect was at the center of each treatment wedge as determined by half the number of paces required to walk the wedge perimeter. Aboveground stem samples were obtained by clipping six stems per wedge at the soil line using pruning shears. Petioles on the stems were then clipped so that the remaining stubs were approximately 3 cm in length. The stems were placed on ice in coolers, followed by overnight storage in 4°C refrigeration. The following day the remaining petiole stubs were removed using scissors that were flamed between each cut. The stems were then rinsed in tap water, placed in 10% bleach water solution for 5 minutes, rinsed in distilled water and air-dried. Stem cross-sections 5 mm thick were excised aseptically at 2- and 10-cm above the soil line at 79 DAP and at 10-, 18- and 26-cm above the soil line at 102- and 140-DAP. The cross-sections were placed onto MPDA and incubated for 14 days at 21°C, then evaluated for *C. coccodes* incidence based on the presence of microsclerotia. A quantitative disease severity was assigned to each subsample (stem) based on the number of cross-sections from which *C. coccodes* could be detected in culture. A stem cross-

section that developed *C. coccodes* was given a value of 1, and a cross-section in which *C. coccodes* was not detected was assigned a value of 0. The values assigned the cross-sections were then totaled within each subsample (stem) and the mean value of subsamples was calculated for each replication.

Extent of colonization of the belowground stem was determined by digging two plants per wedge. The top of each plant stem was removed at approximately 30 cm above the soil line. Petioles were trimmed and the belowground stems stored overnight as described for aboveground stems. The belowground stems were rinsed in tap water and placed in a 10% bleach water solution for 5 minutes. The stems and root systems were then rinsed in distilled water, the excess water was shaken from the stem and roots and then all subsamples (root systems) for each replication (wedge) were placed in a single plastic bag (20- x 10- x 45-cm) with the stems extending from the top of the bag. A twist tie was placed around the bag and stems just above the crown of the plant and the whole unit was stored upright for 12 days. The bag was secured loosely around the stems so that a pencil could be inserted into the neck. This permitted air exchange and inhibited bacterial decay. Microsclerotia of *C. coccodes* were never visible at the time of digging of the roots, however, after 12 days in the "improvised moist chamber" the roots could be evaluated for the extent of *C. coccodes* colonization by observing the microsclerotia. The extent of belowground stem colonization was recorded by measuring the stem from seed-piece to soil line, then the total length of the stem that was covered with diseased tissue was recorded. The stem was rotated 180 degrees about the longitudinal axis and again the length of the stem that was covered with diseased tissue was measured, then length of diseased tissue was totaled for both sides. The percent area of belowground stem that was diseased was then calculated for each subsample by dividing the length of diseased tissue by twice the length of the

stem from seed-piece to soil line. Then taking into account the entire belowground stem, the number of distinct areas of diseased tissue that were separated by non-diseased tissues along that length of stem were also recorded. These distinct areas of disease were called foci. Areas of diseased tissue were considered regions upon which microsclerotia formed in concentration, and the margin between diseased and non-diseased tissue was judged to be where microsclerotia failed to develop after incubation. Microsclerotia were used as a guide to determine the areas of diseased tissue, but not used directly in quantification.

Daughter tubers were evaluated for incidence of infection with *C. coccodes* by sampling two tubers selected from each of the plants dug for assessment of belowground stem colonization. Four tubers from each replication were assessed for presence of *C. coccodes* as described for seed-tubers.

Samples collected at 140 DAP were handled identically, but subsamples consisted only of four entire plants. Therefore, four stems per replication were evaluated for aboveground and belowground *C. coccodes* colonization, and four tubers were selected from each plant providing a 16 tuber subsample per replication for tuber incidence on the final assay date.

Data analysis. All statistical analysis was conducted with SAS 9.1 procglm (SAS Institute, Cary, NC). In all experiments, residual analysis was typical for a binomial outcome in the disease incidence data and the model assumptions of normality and independence were suspect. However, no transformation (ln, $\sqrt{}$, arcsin$\sqrt{}$) improved assumptions so non-transformed data was used for the final analysis (10).

In the fungicide application timing experiments, statistical analysis for the percentage of plants with *C. coccodes* detected in the inoculation court, percentage of plants with *C. coccodes* detected in stem cross-sections in addition to the inoculation court, and the mean radius of the *C.*

coccodes colony that developed from each inoculation court after being placed on nutrient agar was conducted. In the fungicide efficacy experiments, statistical analysis compared the percentage of plants with *C. coccodes* detected in the inoculation court, and also percentage of plants with *C. coccodes* detected in stem cross-sections in addition to the inoculation court. Significance was determined using a 95% confidence interval constructed with the Dunnett method for multiple comparisons to control treatment.

In the application by chemigation study, an ANOVA t-test at the 95% confidence level was conducted to compare treated vs. non-treated wedges. The disease severity score in the aboveground stem, the percentage of plants from which *C. coccodes* could be detected in the belowground stem, the percentage of belowground stem that was diseased, the number of distinct diseased tissue foci on belowground stems and the percentage of progeny tubers infected were analyzed. All comparisons were made only within collection date, and plant organ.

RESULTS

Fungicide application timing. *Colletotrichum coccodes* was isolated from all of the no-fungicide control plants that were inoculated, and never from a non-inoculated control plant (Table 1). Disease incidence for plants treated with the fungicide dicloran was not significantly different from the no-fungicide control, nor was the growth rate of the fungal colony arising from the infection court (Table 1). Incidence of infection in plants treated with azoxystrobin and pyraclostrobin was significantly less than the inoculated control plants only when the fungicides were applied prior to inoculation (Table 1). Incidence of infection did not significantly differ ($P > 0.05$) among any of the post-inoculation fungicide treatments and the inoculated control.

Colonization of the stem tissue outside of the inoculation court occurred in only three plants (Table 1). *Colletotrichum coccodes* was detected 1 cm above the inoculation court in a single plant in the inoculated control in a one trial, and 1 cm above the inoculation court in a single plant in the azoxystrobin 10 day post-inoculation application in both trials. Incidence of *C. coccodes* isolated outside the inoculation court was not statistically different between fungicide treatments and the no-fungicide control (Table 1, $P > 0.05$).

The diameter of the *C. coccodes* colony that developed after inoculation court cross-sections were placed on MPDA significantly differed among treatments (Table 1). Pyraclostrobin applied at the 1 & 10 day sequential treatment significantly reduced colony diameter in both trials. Pyraclostrobin applied at 1 day post and 5 days post, and azoxystrobin applied pre-inoculation reduced colony diameter relative to the no-fungicide control in a single trial. Mean diameter of the colony grown was calculated using only inoculation courts that developed *C. coccodes* colonies in culture.

Fungicide efficacy. *Colletotrichum coccodes* was detected in 100% of the inoculation courts from the no-fungicide, inoculated treatment. The inoculation courts from non-inoculated control plants did not developed *C. coccodes* in culture. Incidence of infection was significantly lower than the no-fungicide control when fluoxastrobin, penthiopyrad, famoxadone, polyoxin, azoxystrobin and mandipropamid + difenoconazole were applied in the first trial. In the second trial statistically significant differences were not detected (Table 2).

Colonization of the stem outside the inoculation court was detected in 4% (2 plants) of inoculated plants in the first trial and 15% (8 plants) in the second trial. Between the two trials, plants treated with famoxadone accounted for 70% of the plants where *C. coccodes* was detected outside of the inoculation court (Table 2). In six of these plants, *C. coccodes* was only detected 1

cm from the inoculation court, however, in one stem *C. coccodes* was detected as far as 7 cm in both directions from the inoculation court. *Colletotrichum coccodes* was not detected outside of the inoculation court in the inoculated, no-fungicide control treatment in either trial.

When famoxadone was applied at the highest rate, incidence of *C. coccodes* outside the inoculation court was significantly greater than the no-fungicide control in the second trial. Incidence of *C. coccodes* detected outside the inoculation court did not differ between the no-fungicide control and any of the other treatments (Table 2).

Application by chemigation. *Colletotrichum coccodes* was detected in 2.5% of the seed planted in 2007 and 5.5% in 2008 (Figure 1). The severity score for *C. coccodes* colonization in the aboveground stems was significantly less on plants collected from fungicide treated wedges on 79-, 102- and 140-DAP in 2007 and at 79 DAP in 2008 (Table 3).

Microsclerotia of *C. coccodes* were not visible at the time of plant collection at 79- and 102-DAP, however, after several days in the "improvised moist chamber bag" they became apparent on belowground stems, and after 12 days the roots could readily be evaluated for the extent of latent colonization by observing microsclerotia. Belowground stems sampled from azoxystrobin treated wedges had a significantly smaller percent area of *C. coccodes* diseased tissue when compared to the non-treated wedges at 79- and 140-days after planting in 2007. A smaller percentage of the belowground stem was colonized at 79 DAP in 2008 ($P < 0.04$, Table 3). On belowground stems, the localized areas of diseased tissue or foci typically appeared to run longitudinally rather than girdle the stem circumference, but this characteristic was not quantified. The number of distinct black dot foci on the belowground stems were only significantly lower in treated wedges during the 79 DAP assay in 2008 (data not collected at 79 DAP in 2007).

Incidence of infected progeny tubers did not differ significantly between tubers collected from fungicide treated wedges and the non-treated wedges in 2007 or at 79- and 102-DAP in 2008. However, incidence of progeny tuber infection was significantly lower than the non-treated wedges at 140 DAP in 2008 ($P < 0.02$, Table 3).

DISCUSSION

Fungicides were only effective when applied before inoculation. In the assessment of fungicide timing, azoxystrobin and pyraclostrobin applied pre-inoculation were the only treatments that reduced incidence of infection by *C. coccodes* ($P < 0.001$). Curative action was not observed with these two fungicides when applied post-inoculation. *Colletotrichum coccodes* ability to infect and remain viable in potato stems after a 21 hour incubation period and subsequent fungicide application is clearly demonstrated in the near 100% incidence of the pathogen in the no-fungicide control and post-inoculation fungicide treatments. This result corresponds with the spray schedule used to control tomato fruit anthracnose, caused by *C. coccodes*, where applications must be made at fruit initiation and continued throughout the season in order to prevent the occurrence of infections that remain latent and are not expressed until fruit maturity (7, 11, 16, 22, 26). Fungicide applications made just prior to tomato ripening when anthracnose fruit rot symptoms are typically expressed does not control this disease. Based on the potato stem inoculation results, the importance of fungicide application prior to infection of potato foliage follows the same pattern.

Colony size of *C. coccodes* grown from the inoculation court after placement on MPDA was significantly affected by fungicide treatment. Differences in colony size were primarily seen in the fungicides applied post-inoculation. Potential explanations for this effect include a

difference in starting inoculum levels in the stem cross-section at the time of placement on agar or the fitness of the surviving inoculum. Persistence of fungicide in the potato stem either due to active ingredient, formulation or application timing may also have resulted in the retarded growth rates observed for some treatments.

Two long time industry standard fungicides, chlorothalonil and mancozeb have previously been evaluated for efficacy against *C. coccodes*. Chlorothalonil applied at 2.5 kg a.i./ha, (maximum label rate for tomato) reduced black dot severity on roots of tomato (13), and reduced fruit anthracnose when applied on a 7- or 14-day interval (7). Chlorothalonil also reduced radial growth and the percent conidia germination of *C. coccodes* in V8 agar culture (12). Mancozeb was shown to reduce black dot severity on tomato roots (13) with foliar application (1.68 kg a.i./ha). However, conidia germination and radial growth in culture were only reduced at the highest rate tested (12). In a separate study, daily growth rate of *C. coccodes* in culture was reduced by mancozeb at 100 mg a.i./liter of media, but not at lower rates (2). Maneb a related EBDC significantly reduced conidia germination at 1 µg a.i./ml but sclerotial germination was not reduced until applied at 10 µg a.i./ml of media (44).

Lower rates of chlorothalonil and mancozeb used in media cultures were not effective in reducing fungal growth, and while higher rates sometimes slowed growth they did not completely inhibit growth in those studies. What the rates identified translate to in terms of labeled rates that can be legally applied to crops is difficult to determine. Using the live plant to screen fungicides in the greenhouse as described in this study provides a framework for comparing products and known rates over time and between different studies and crops. In this study, chlorothalonil and mancozeb grouped with the no-fungicide control, and did not provide a significant reduction in incidence of plant stem infection by *C. coccodes*. The chlorothalonil rate

used in the greenhouse was 42% of the rate used to obtain control in field grown tomato (13), and the mancozeb rate was 62% of the rate used in that study (13). A rate effect might explain the unfavorable result for both chlorothalonil and mancozeb in this study since median label rates were used. Given the possibility that maximum rates are required from chlorothalonil and mancozeb, control of potato black dot is probably better attained by the timely application of one of the superior performing fungicides identified in this study.

Foliar applications of azoxystrobin (Quadris, 12 oz/acre, 218.6 g a.i./ha.) made to potato cultivars 'Russet Norkotah' and 'Russet Burbank' have been shown to reduce black dot severity on belowground stems and reduce incidence of progeny tuber infection in field research plots (32). When azoxystrobin was chemigated onto the commercial potato crop at 50- and 67-DAP, colonization of the belowground and aboveground stem was significantly reduced at the 79 DAP assay. Black dot often occurs on the root system and belowground plant parts where fungicides are more difficult to apply directly. Therefore, even if a fungicide is highly effective against the pathogen, the delivery of the fungicide to the target organ complicates black dot management. During both years of chemigation study, fungicide treatments were applied in a commercial potato field by the cooperator and incorporated the dynamics of the true host-pathogen-environment interaction. This resulted in the first reported reduction in black dot severity in a commercial potato field. However, effects were not season-long and had expired by the time of the 102 DAP assay.

An unidentified factor such as host vigor that is affected by fungicide application may be a feature in reducing black dot severity. Whether reduced disease is due to direct fungistatic action of the fungicide on the pathogen, or an indirect effect resulting from fungicide application to the cropping system is unclear. Reduced black dot severity observed in the fields chemigated

with azoxystrobin cannot be attributed to the direct effects of the fungicide on *C. coccodes* since the non-treated wedges received no fungicide of any type on those dates. An alternative possibility to azoxystrobin migrating in the chemigation water and directly protecting the belowground plant parts, is that predisposition to infection was reduced in treated plants that were protected from other foliar pathogens. In this case, the argument could be made that most foliar fungicides favor the reduction of black dot severity simply by providing an environment where the host is less predisposed to infection. This might offer an alternative explanation to why mancozeb and chlorothalonil performance was low in the greenhouse but provided favorable results in field grown tomato (13). Control plants in that study did not receive any fungicides throughout the entire season, and foliar disease such as early blight was controlled in the treated plants. One explanation that was offered by Dillard for the reduced black dot on roots was that host pre-disposition to colonization by *C. coccodes* was reduced when the stress from foliar disease was reduced (13).

Ethylene levels increase in plants after wounding or during senescence (1), and ethylene has also been demonstrated to stimulate germination of conidia of selected *Colletotrichum* species that infect climacteric fruit (8, 15). Use of any foliar fungicide in order to prevent foliar disease such as early blight and the associated wound response in plants could fit the scenario of reduced host predisposition. Alternatively, a direct inhibition of ethylene biosynthesis in wheat plants has been demonstrated using kresoxim-methyl, a strobilurin fungicide related to azoxystrobin (19, 29). Should the same effect be produced by azoxystrobin application to potato, then reduction of black dot could also be attributed to reduced plant ethylene biosynthesis in the chemigated fields. Whether black dot reduction using fungicides is multi-faceted, with one major component of severity governed by the fungicide active ingredient acting directly on the

pathogen, and a second component from the fungicides effect on plant vigor that in some way mitigates signals that initiate host colonization by *C. coccodes* is unclear.

Colonization of the potato plant stem distal to the inoculation court was not as great or consistent as was anticipated in the greenhouse grown plants. *Colletotrichum coccodes* was not isolated more than 1.5 cm from the margin of the inoculation court in the fungicide application timing trial, however, re-isolation from inoculation courts in the positive control plants was 100%, indicating that a viable pathogen was present. In the fungicide efficacy trial, stem colonization was detected up to 7 cm in both apical and basal directions from the inoculation court (Table 2). 'Russet Burbank' is an indeterminate growth type cultivar and will continue to flower as long as sufficient nitrogen (N) is present (14). In the fungicide efficacy experiments, 86% less N was used when compared to the fungicide application timing experiments. This reduced level of N was intended to, and likely contributed to the earlier plant decline and senescence. This in turn may have lead to a higher degree of stem colonization seen at the time of assay in that study. Plants were still fully green in the fungicide application timing experiments, while plants were senescent and the bottom two to four leaves necrotic at the time of assay in fungicide efficacy trials. The effects of senescence on stem colonization by *C. coccodes* has previously been demonstrated (30, 33, 35), and levels of plant senescence observed in the fungicide efficacy study were comparable to the quantified stage of plant senescence when stem colonization had previously been observed to begin in greenhouse studies (33). This trend supports previous observations that *C. coccodes* can infect aboveground stems but does not colonize them extensively until the onset of senescence or a signal (4, 9, 18, 26, 30, 33, 35, 36, 38), and is perhaps a survival tactic to exclude other organisms or saprophytes from host tissue as the plant dies (6, 23).

Disease progress in the chemigated field was monitored over the course of the season. Mean number of *C. coccodes* foci on belowground stems across treatments and collection dates ranged from 0.17- to 3.08-per plant (Table 3). The number of distinct black dot foci was reduced in treated wedges only in the 79 DAP collection in 2008. Foci data were not collected at 79 DAP in 2007. Since no statistically different effect on foci number were observed in the latter two assay dates, season long effects from azoxystrobin applied exclusively in the early growing season does not seem a likely method for reducing disease from primary infection events that take place later in the season. Also, since the pathogen was readily isolated from all parts of the potato plant 12 days after the second application of azoxystrobin, this would support the greenhouse observations that azoxystrobin has no curative activity on *C. coccodes*.

Latent infections were detected in non-symptomatic plants. All samples collected from the chemigated field at 79- and 102-DAP were considered asymptomatic or free from symptom and sign of *C. coccodes* infection as visually determined at time of sample collection in the field. However, when the same samples were cultured in the laboratory, *C. coccodes* was isolated from roots, stems and tubers. Similar results have also been reported by Otazu (35). Furthermore, by 102 DAP, 100% of belowground stems in both treated and non-treated wedges were infected with *C. coccodes* in 2007 and 83% of plants in treated wedges and 100% of plants in non-treated wedges in 2008 (Table 3). This indicates that diagnosis or assessment of black dot in the field is not practical until senescence when latent infections finally become evident. This conclusion was previously proposed by Nitzan (33) and is supported by these results. Microsclerotia were evident on the drying plant tissues at the final 140 DAP collection in the chemigation study, but that collection occurred after chemical vine desiccation.

The low incidence of infected seed but high disease incidence on stems within the plant population indicate that soilborne inoculum was important in disease development in the fields. Mean severity of belowground stem infection was significantly reduced at 79 DAP in both years. Chemigation with azoxystrobin initially reduced disease severity on belowground plant parts that was likely caused by overwintering inoculum in the soil. However, by 102 DAP or 33 days after the final azoxystrobin application, a high proportion of plants in the treated and non-treated wedges were infected. Even with early season prevention, the time remaining in the growing season for additional infection and colonization apparently makes an important contribution to black dot severity.

This study points to the importance of the timing of application of fungicides to prevent infection of potato by *C. coccodes*, and that there is a range of efficacy in the available potato fungicides. When applied early in the growing season the well-studied application schedules for processing tomato would be mimicked. Ensuring that the fungicide used can control the foliar pathogens of primary concern like early blight and late blight, but also provide a benefit for managing black dot is possible with fungicides now available. Identification of a strategy to maintain season long effect after the initial early season reduction is the next step. Early season fungicide applications to manage infection that remains latent has been successful in tomato. In tomato however, treatments are continued throughout the season rather than discontinued after the two preventative applications in this study, and likely explains why a season long treatment effect was not obtained in the chemigated potato.

Early season protection appears to be a foundation for control of this pathogen, and this strategy has been successful in reducing losses to *C. coccodes* in processing tomato crops. Greenhouse results confirming that fungicides effective against *C. coccodes* were useful only

when applied prior to potato plant infection follows suit. Therefore, use of fungicides effective against the common foliar diseases such as early blight, that have also demonstrated efficacy on *C. coccodes* could be incorporated in the first fungicide application of the season. However, subsequent application throughout the season at yet undetermined intervals are probably needed to further minimize disease, despite the trend that black dot does not become visually evident in the field until late season.

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Table 1. Incidence of infection and mean *Colletotrichum coccodes* colony size that developed from inoculation courts on potato stems that were treated with fungicides at various timings.

Treatment*	Frequency of Infection†		Frequency of Infection Outside Inoculation Court#		Mean Diameter of Colony Grown from Inoculation Court‡ (mm)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Inoculated Control						
No Fungicide	1.00[a]	1.00[a]	0.00[a]	0.17[a]	7.3[a]	6.8[a]
Pre-inoculation						
Dicloran	0.75[a]	1.00[a]	0.00[a]	0.00[a]	6.8[a]	7.2[a]
Pyraclostrobin	0.00[b]	0.25[b]	0.00[a]	0.00[a]	-	4.5[b]
Azoxystrobin	0.50[b]	0.25[b]	0.00[a]	0.00[a]	4.6[b]	0.4[b]
Post-inoculation 1 Day						
Dicloran	1.00[a]	NP	0.00[a]	NP	6.7[a]	NP
Pyraclostrobin	0.75[a]	1.00[a]	0.00[a]	0.00[a]	4.4[b]	3.2[b]
Azoxystrobin	1.00[a]	1.00[a]	0.00[a]	0.00[a]	5.7[a]	5.9[a]
Post-inoculation 5 Days						
Pyraclostrobin	1.00[a]	1.00[a]	0.00[a]	0.00[a]	5.2[a]	2.8[b]
Azoxystrobin	1.00[a]	1.00[a]	0.00[a]	0.00[a]	6.9[a]	5.6[a]
Post-inoculation 10 Days						
Pyraclostrobin	1.00[a]	1.00[a]	0.00[a]	0.00[a]	6.0[a]	4.2[b]
Azoxystrobin	1.00[a]	1.00[a]	0.25[a]	0.25[a]	6.7[a]	6.2[a]
Post-inoculation 1&10 Days						
Pyraclostrobin	1.00[a]	0.75[a]	0.00[a]	0.00[a]	3.9[b]	2.7[b]
Azoxystrobin	1.00[a]	1.00[a]	0.00[a]	0.00[a]	6.2[a]	3.4[b]
Non-inoculated Control	0.00	0.00	0.00	0.00	-	-

* Rate equivalent of Dicloran, 4.2kg a.i./ha (Botran 5F rate 3 qt/acre); azoxystrobin or pyraclostrobin, 164g a.i./ha (Quadris & Headline 9 oz/acre) in 355 liters/ha (38 gallons/acre) water carrier.
† Proportion of infection courts from which *C. coccodes* could be cultured on nutrient agar.
# *C. coccodes* was re-isolated from at least one stem cross-section distal to the inoculation court.
‡ The mean size (mm) of the colony grown from inoculation courts 14 days after being placed on nutrient agar.
[a,b] Treatments with [a] were not different than the no-fungicide control as determined using Dunnett's method for multiple comparisons to control at 95% confidence level.
NP - Treatment not performed.

Table 2. Incidence of infection from inoculation courts on potato stems that were treated with different fungicides and rates and then inoculated with *C. coccodes*.

Treatment	Product Rate / Acre	Active Ingredient (a.i.)	a.i. / ha[*] (grams)	FRAC Code[1]	Frequency of Infection[†]		Frequency of Infection Outside Inoculation Court[#]		Mean Diameter of Colony Grown from Inoculation Court[‡]	
					Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
No fungicide	N/A	N/A	N/A	N/A	1.00[a]	1.00[a]	0.00[a]	0.00[a]	6.43	7.35
Bravo Wx Stick	1.25 pt	Chlorothalonil	1050	M5	0.75[a]	0.75[a]	0.00[a]	0.00[a]	5.97	6.10
Evito 480 SC	2.90 oz	Fluoxastrobin	101	11	0.00[b]	0.75[a]	0.00[a]	0.00[a]	-	5.77
DPX-Lem17 20EC	9.60 oz	Penthiopyrad	140	7	0.25[b]	1.00[a]	0.00[a]	0.00[a]	6.70	6.25
	16.80 oz	Penthiopyrad	245	7	0.00[b]	1.00[a]	0.00[a]	0.25[a]	-	6.90
	24.00 oz	Penthiopyrad	351	7	0.50[a]	0.50[a]	0.00[a]	0.00[a]	6.00	4.85
Famoxate 25SC	4.00 oz	Famoxadone	76	11	0.25[b]	0.75[a]	0.25[a]	0.00[a]	7.20	6.87
	6.00 oz	Famoxadone	114	11	0.75[a]	0.75[a]	0.00[a]	0.50[a]	7.10	6.30
	8.00 oz	Famoxadone	152	11	0.50[a]	0.75[a]	0.25[a]	0.75[b]	6.85	7.03
Penncozeb 75DF	1.25 lb	Mancozeb	1050	M3	0.50[a]	1.00[a]	0.00[a]	0.00[a]	7.20	6.98
Polyoxin	1.00 lb	Polyoxin D zinc salt	201	19	0.00[b]	1.00[a]	0.00[a]	0.25[a]	-	6.98
Quadris	10.75 oz	Azoxystrobin	196	11	0.00[b]	0.75[a]	0.00[a]	0.00[a]	-	5.17
Revus Tops	6.25 oz	Mandipropamid + Difenoconazole	114 114	40 3	0.00[b]	0.75[a]	0.00[a]	0.25[a]	-	4.63
Non-inoculated Control	N/A	N/A	N/A	N/A	0.00	0.00	0.00	0.00	-	-

[1] FRAC Code, resistance management and mode of action (3).

[*] Based on 45,822 plants/ha (18,544/acre). Each plant received 7.8 ml water carrier (355 liters/ha or 38 gallons/acre) that was amended with the listed a.i./ha divided by 45,822.

[†] Proportion of infection courts from which *C. coccodes* could be cultured on nutrient agar.

[#] *C. coccodes* was re-isolated from at least one stem cross-section distal to the inoculation court.

[‡] No statistical comparison was made due to single observations for some treatments. Mean diameter of colony grown from inoculation court is the average size of the colony grown from inoculation courts 14 days after being placed on nutrient agar.

- Could not be calculated for the treatment because no inoculation court developed *C. coccodes* in culture.

[a,b] Treatments with [a] were not different than the no-fungicide control as determined using Dunnett's method for multiple comparisons to control at 95% confidence level.

Table 3. Black dot disease ratings on below- and aboveground potato stems and infected tuber incidence when azoxystrobin was and was not chemigated in a commercial potato pivot.

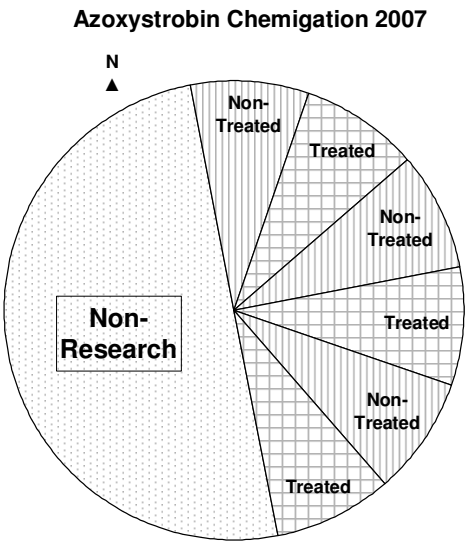
		Collection Date 79 Days After Plant			Collection Date 102 Days After Plant			Collection Date 140 Days After Plant		
	Year	Treated	Non-Treated	lsd†	Treated	Non-Treated	lsd†	Treated	Non-Treated	lsd†
Above Ground										
Severity Index[1]	2007	1.11*	1.83	0.15	0.67*	1.72	0.90	1.50*	2.67	1.09
	2008	0.00*	0.56	0.15	0.00	0.39	0.62	0.58	1.25	1.76
Below Ground										
Proportion of Plants Infected with *C. coccodes*	2007	0.17*	0.83	0.65	1.00	1.00	0.00	0.83	1.00	0.23
	2008	0.17	0.67	0.65	0.83	1.00	0.46	0.67	1.00	0.92
Proportion of Stem Surface Area Diseased	2007	< 0.01*	0.13	0.11	0.16	0.20	0.27	0.23*	0.48	0.14
	2008	< 0.01*	0.06	0.04	0.15	0.39	0.49	0.19	0.46	0.42
Number of Distinct *C. coccodes* Foci[2]	2007	-	-	-	2.33	2.83	1.73	3.08	3.25	2.58
	2008	0.17*	1.33	0.65	1.84	2.34	2.85	1.17	2.08	1.73
Proportion of Progeny Tubers Infected	2007	0.00	0.33	0.61	0.08	0.33	0.65	0.48	0.63	0.23
	2008	0.00	0.00	0.00	0.00	0.33	0.61	0.02*	0.21	0.13

* Disease rating was significantly different from non-treated for the plant part and date of collection.

† LSD at the 95% confidence level within plant part and collection date.

[1] Severity index calculation used stem cross-sections from which *C. coccodes* could or could not be isolated from and were assigned a value equal to 1 or 0. Values were totaled for the six subsamples (stems) collected from each replication and the mean was calculated. Collection date 79 DAP had stem cross-sections taken at 2- & 10-cm above the soil line. Collection Date 102- & 140-DAP had cross-sections taken at 10-, 18- & 26-cm above the soil line.

[2] *C. coccodes* foci were determined visually after latent infections were expressed during incubation in a plastic bag. Foci were considered distinct if the visually assessed tissues surrounding the focus were determined to be non-diseased based on the lack of microsclerotia.



Crop Year	Cropping History	
	2007 Trial	2008 Trial
2008	-	**Potato**
2007	**Potato**	Winter Wheat
2006	Winter Wheat	Winter Wheat
2005	Canola	**Potato**
2004	Winter Wheat	Winter Wheat
2003	**Potato**	Winter Wheat
2002	Winter Wheat	**Potato**
Previous Potato Crops	3	3
% Seed with *C. coccodes*	2.50%	5.50%

Figure 1. Map depicting location of treatments in the field. Field in 2008 was organized identically except for a different orientation vs. North.

The Effect of Seedborn Inoculum of *Colletotrichum coccodes* on Disease Severity and Potato Yield

Jason Ingram

Department of Plant Pathology, Washington State University, Pullman, WA 99164

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J.T. INGRAM. Dept. Plant Pathology, Washington State Univ., Pullman WA 99164.

ABSTRACT

Colletotrichum coccodes is one of several potato pathogens that is seedborne. Seed-tuber selection could be improved by quantifying the effect of seedborne inoculum on the crop. The effect of seedborne inoculum of *C. coccodes* on final disease severity and potato yields was assessed in fields with and without recent potato crops. Yields were affected by seed lot in three of the four fields ($P < 0.05$), but those differences were not related to percentage *C. coccodes* in the seed lot ($P > 0.1$). Quantity of progeny tubers was significantly affected by seed lot in three of four fields ($P < 0.05$), but tuber quantity was only related to incidence of *C. coccodes* in the seed lot in one of those fields ($P < 0.03$). Number of *C. coccodes* foci on belowground stems was significantly different among seed lots in three of four fields ($P < 0.05$). Percentage of seed lot infected and disease on the belowground stems was not related in 2007 ($P > 0.1$), however, they were significantly related in both fields planted in 2008 ($P < 0.03$, $R^2 = 0.73$). Black dot was not a yield limiting factor under the conditions of this study, but differences in yield among seed lots illustrates the importance of selecting the highest quality seed-tubers available to maximize yields.

INTRODUCTION

Potato black dot is caused by *Colletotrichum coccodes* (Wallr.) Hughes. Black dot is aptly named from the microsclerotia that develop on host tissue and appear as black dots. Microsclerotia are the survival structure of *C. coccodes* and are a melanized arrangement of mycelia that are resistant to environmental degradation and survive in soil several years (Blakeman and Hornby, 1966; Dillard and Cobb, 1998; Farley, 1976; Griffiths and Campbell, 1972). Microsclerotia of *C. coccodes* are capable of sporogenic germination where the microsclerotum forms an acervulus and conidia are produced. Alternatively, myceliogenic germination is possible and mycelia develop from the sclerotia and penetrate the host directly (Sanogo and Pennypacker, 1997). Two sources of microsclerotia that serve as initial inoculum to cause disease in potato have been identified. Microsclerotia in soil are one source, the seed potato is the second (Barkdoll and Davis, 1992; Dickson, 1926; Komm and Stevenson, 1978; Stevenson and American Phytopathological Society., 2001; Stevenson et al., 1976). Potatoes are vegetatively propagated and seed-tubers used for planting in commercial fields may have high frequencies of infection with *C. coccodes.* The percentage of seed lots contaminated with *C. coccodes* was as high as 34% in Israel (Tsror (Lahkim) et al., 1999) and 53% in Washington State (Johnson et al., 1997). In Washington State, seed lots that are infected with *C. coccodes* can have as great as 90% of tubers infected. (Johnson et al., 1997). Additionally, the pathogen is common in soils of potato growing regions and by 1926 had been reported on five continents. (Dickson, 1926). Quality (Hunger and McIntyre, 1979) and yield losses due to this pathogen have been reported (Johnson, 1994; Tsror et al., 1999).

Previously, the relative importance of both seedborne and soilborne inoculum has been investigated. Introduction and reintroduction of the pathogen due to seedborne inoculum, along with an increase in disease incidence was considered a threat to potato production by Komm (Komm and Stevenson, 1978). Some work, however, has shown that soilborne inoculum may result in a greater degree of host plant colonization (Dashwood et al., 1992; Denner et al., 1998). Quantifying the effect of *C. coccodes* seedborne inoculum on the potato crop would increase the ability to make management decisions for seed lot and field selection in the Columbia Basin. The purpose of this study was to quantify the effect of seedborne inoculum of *C. coccodes* on final disease severity on aboveground and belowground stems, roots, and tubers, and on yields of potato grown in fields that have recently and not recently grown potatoes.

MATERIALS AND METHODS

Plant material. Collections of seed-tubers were made from certified seed lots of potato (*Solanum tuberosum* L.) that were delivered to various Washington State growers in 2007 and 2008. Ten seed lots of cv. 'Russet Burbank' were collected each year. All seed lots were Generation 3, according to the Washington limited generation scale for seed potatoes and were collected from grower and distributor storages in the spring of each year. Each collection consisted of 45 kg (100 lbs or approximately 225 tubers). The seed lots were assayed to determine the percentage of seed-tubers infected with *C. coccodes* by assaying the basal end of tubers. Prior to assay, 35 seed-tubers were removed from 4 C storage and warmed to 22 C for 12 hours. They were then washed with warm soapy water, gently scrubbed with a sponge, rinsed with distilled water and air-dried. A disk of tissue from the basal end of each tuber was aseptically removed using a scalpel and plated on polygalacturonic acid media (NPX)

(Butterfield and DeVay, 1977). Each disk of tissue was approximately 1.5 cm in diameter, 3 mm thick, centered on the stolon scar and consisted of periderm, cortex and vascular tissue. Tuber disks were incubated in the dark at 22°C for 14 days and then evaluated for presence of *C. coccodes*. A seed-tuber was considered infected if the tuber disk developed microsclerotia of *C. coccodes* in culture. Based on the tuber disk assay, the percentage of tubers in each seed lot that were infected with *C. coccodes* was calculated.

Six seed lots were chosen for additional study from the ten tested seed lots. Seed lots were selected to provide the greatest range of incidence of *C. coccodes*. Percentage of infected tubers ranged from 0% to 60% in 2007 and 9% to 80% in 2008 (Table 1). Intact tubers that had not been used for the assessment of *C. coccodes* in the selected seed lots were removed from 4 C storage, cut to 56- to 113-g (2- to 4-oz) seed-pieces then stored at 10 C and 95% relative humidity until planting.

The study was completed twice, once in 2007 and again in 2008. During each year, two fields located in the Columbia Basin of Washington State were utilized. Both fields were planted with seed-tubers from the same selected seed lots. One field had a history of several four year potato rotations, and the other field had not been planted to potato for 30 years. The fields with recent potato rotations were located at the WSU Othello Research Station (Shano Silt Loam, 46° 47’ 14.46N, 119° 02’ 38.60W), and were considered common rotation. The fields without a potato crop in the past 30 years were designated as long rotation out of potato. The long rotation field in 2007 (Chedahap Fine Sandy Loam, 46° 41’ 18.92N, 118° 51’ 17.09W) had never been planted to potato, and the field in 2008 had not been planted to potato in over 30 years (Shano Silt Loam, 46° 45’ 39.48N, 118° 51’ 04.84W). The plots in the long rotation fields were located within commercial potato crops and were irrigated with a center-pivot system. The fields with

previous potato crops (common rotation) were irrigated with an overhead, linear system. Fields were planted on May 3, 2007 and April 24, 2008. The seed-pieces were planted at a depth of 20 cm (8 in). Seed-piece spacing was approximately 25 cm (10 in) and row spacing was 86 cm (34 in), providing an approximate population of 45,588 plants ha^{-1} (18,449 plants $acre^{-1}$). Within the common rotation and long rotation fields, plots containing one of each of the six seed lots were replicated four times in a completely randomized design in 2007. The six seed lots were planted in a randomized complete block design with four replications in 2008. Each plot in which a seed lot was planted was considered an experimental unit. Plots contained three rows, 4.6 m in length (15 ft). The center row was used for yield evaluation and one of the adjacent rows originating from the same seed lot was used to evaluate extent of plant colonization by *C. coccodes*. All three rows were used in visual determination of canopy necrosis.

The insecticide, thiamethoxam, 140 g a.i. ha^{-1} (Platinum 8 oz $acre^{-1}$) was applied in furrow, but no fungicide or seed treatment was used at planting. Both types of field were otherwise managed according to normal practices for the Columbia Basin, except that the irrigation system in the 2007 long rotation field was inoperative for 10 days.

Disease and yield assessment. The crop canopy was assessed for necrosis at 119 DAP in the common rotation and 125 DAP for the long rotation in 2007, and at 137 DAP for both fields in 2008 (Table 1 & 2). Plant colonization by *C. coccodes* was assessed in a sample of three intact plants that were removed from each replication at 109 DAP in the common rotation field and 125 DAP in the long rotation field in 2007. Plant colonization was assessed in both fields at 109 DAP in 2008. At that time, the severity of plant colonization was measured on roots, belowground stems, and aboveground stems.

The sampled plants were dug and the root system carefully shaken to remove soil, the petioles were removed using pruning shears and the plants were placed in coolers and stored at 4 C overnight. The following day, plants were rinsed in tap water and placed in a 10% bleach water solution for 5 minutes. The three plants from each replication were then rinsed in distilled water, the excess water was shaken from the stem and root system, and the three subsamples (stems) for each replication were placed in a single plastic bag (20- x 10- x 45-cm) with the stems extending from the top of the bag. Black dot severity was assessed in aboveground parts of the stem by determining the presence of the pathogen in 5 mm thick cross-sections of stems that were excised aseptically at 2-, 6-, and 12-cm above the soil line. The cross-sections were placed onto NPX and incubated for 14 days at 21 C and then evaluated for *C. coccodes* incidence based on the presence of microsclerotia. A disease severity score was assigned to each subsample (stem) based on maximum *C. coccodes* colonization height above the soil line at 2-, 6-, and 12-cm. The disease severity score was calculated by evaluating each cross-section within subsample for microsclerotia. A cross-section that developed *C. coccodes* at the 2 cm height was given a value of 1, the 6 cm height a value of 3, and 12 cm height a value of 6. Cross-sections that did not develop *C. coccodes* were given a value of 0. The values for each cross-section were then totaled within each subsample (stem) and the mean value across subsamples was calculated for each replication.

After the aboveground stems were removed, the belowground stems and root system remained in the bag. A twist tie was placed around the bag and stems at the crown of the plant, and the whole unit was stored upright for 12 days. The bag was secured loosely around the three stems so that a pencil could be inserted into the neck. This permitted air exchange and slowed bacterial decay. After 12 days in the "improvised moist chamber bag" the roots were evaluated

for extent of *C. coccodes* colonization by observing the microsclerotia. Each root system received a score of 0 through 4 for the percentage area of the roots that were covered with microsclerotia using the following scale: No sclerotia = 0; 1-25% = 1; 26-50% = 2; 51-75% = 3; and 75-100% = 4. The number of distinct areas of diseased tissue that were separated by non-diseased tissues along the entire length of stem from seed-piece to soil line was also recorded. These distinct areas with microsclerotia were called foci. Areas of diseased tissue were considered regions of tissue upon which microsclerotia formed in concentration, and the margin between diseased and non-diseased tissue was judged to be where microsclerotia failed to develop after incubation. Microsclerotia were used as a guide to determine the areas of diseased tissue, but not used directly in quantification.

Vines were mechanically flailed prior to harvest, and the plots were dug 125 DAP in normal rotation and 131 DAP in the long rotation field in 2007. Both fields were dug at 146 DAP in 2008. Yields were taken using the center row of each plot. The tubers from each replication were mechanically weighed and counted, and total yields ha^{-1} were calculated. The progeny tubers were assessed for presence of *C. coccodes* as described for seed-tubers using 14 tubers randomly selected from each replication. Incidence of *C. coccodes* was then calculated.

Data analysis. Statistical analyses for crop and disease data including canopy necrosis, severity of root infection, number of disease foci on the belowground stem, aboveground stem infection severity, percentage of progeny tubers infected, total yield and progeny tuber quantity were conducted using ANOVA, SAS 9.1 procglm (SAS Institute, Cary, NC). Comparisons were made within field using Fisher's protected LSD, at the 95% confidence level (Tables 1 & 2).

Where significant differences for a particular crop response were observed among the seed lots using ANOVA, the relationship of percentage seed lot infected with *C. coccodes* to

characteristics such as yield, and disease severity were assessed using regression analysis. Simple linear regression was conducted using SAS 9.1 procreg. The relationship of percentage of seed-tubers infected with *C. coccodes* (independent variable) was individually assessed relative to total yield, progeny tuber quantity, number of *C. coccodes* foci on the belowground stem and root severity (dependant variables). These specific analyses were chosen because of significant differences among seed lots that occurred in three of four fields (root severity was only significantly different among seed lots in two of four fields, but was also assessed using simple linear regression). Disease severity of aboveground stems, percentage of progeny tubers infected, and canopy necrosis were not assessed using regression analysis because differences due to seed lot were not consistently detected with ANOVA.

RESULTS

Percentage of necrosis of the crop canopy and incidence of infected progeny tubers were significantly different among seed lots in a single field ($P < 0.05$, Table 1). Aboveground stem severity was significantly different among seed lots in two of four fields ($P < 0.05$), both fields where differences were noted were in common rotation. Disease severity of roots was different among seed lots in two of four fields ($P < 0.05$). One common rotation and one long rotation field had disease severity on roots that was related to percentage of seed lot infected with *C. coccodes* ($P < 0.001$, $R^2 = .88$, Tables 1 & 2). Number of disease foci on belowground stems was different among seed lots in three of four fields ($P < 0.05$) and number of disease foci on belowground stems was related to percentage of seed lot infected with *C. coccodes* in two of those fields ($P < 0.03$, $R^2 = .74$)

Total yield was different among seed lots in three of four fields ($P < 0.05$), but was not related to percentage of seed lot infected with *C. coccodes* ($P > 0.1$). The field devoid of significant yield differences was in common rotation. Progeny tuber quantity was different among seed lots in three of four fields ($P < 0.05$) and progeny tuber quantity was related to percentage of seed lot infected with *C. coccodes* in only one of the common rotation fields ($P < 0.03$, $R^2 = .74$, Tables 1 & 2). Percentage of seed-tubers infected in seed lots was only related to a crop or disease characteristic in fields planted in 2008. No specific crop characteristic or measure of disease was statistically different among seed lots in all four fields (Tables 1 & 2).

DISCUSSION

The percentage of infected seed-tubers in a seed lot was not related to yield in this study. Significant yield differences that were observed among seed lots were likely due to some factor other than *C. coccodes*. Potential explanations for differences among seed lots could include seed maturity and vigor, a yield limiting disease that was transmitted via the seed-tuber, or a more complex set of interactions. Based on the lack of relationship of percentage seed-tubers infected in a seed lot with yield, *C. coccodes* did not appear to be a yield-limiting factor in any of the four fields studied.

Significant relationships between seedborne inoculum and severity of disease on the roots and the belowground stems were observed, but did not result in an associated yield loss. Yield reductions due to *Colletotrichum coccodes* have been reported, but many of the reported yield reductions are the result of foliar inoculations rather than an effect from seedborne inoculum (Johnson, 1994; Mohan et al., 1992; Tsror et al., 1999). In those trials, effects due to seed lot such as physiological age of the seed-tuber were not a source of variance since a single seed lot

was used to compare yield between inoculated and non-inoculated crops. Also, during foliar inoculation studies, blowing sand was often incorporated into the inoculation process. If the sand abrasion incites a wound response, this could lead to increased germination and host penetration by *C. coccodes* (Flaishman and Kolattukudy, 1994). Increased levels of ethylene associated with plant wounding have been shown to cause some *Colletotrichum* species that infect climacteric fruit to increase germination of conidia and sometimes form as many as 4 appressoria from a single conidium (Flaishman and Kolattukudy, 1994).

Common and long rotation ground was not replicated, so direct statistical comparisons between the two types of fields are not appropriate. An informal comparison, however, illustrates some differences between fields that are intuitive. Canopy necrosis always appeared greater in the common rotation fields and exhibited severe necrosis at mechanical vine kill, while the long rotation fields were still green. Reduced canopy necrosis could imply extended season tuber-bulking, which could have management implications with regard to cultivar selection, seed spacing and harvest date contracts. *Colletotrichum coccodes* was detected in the aboveground stems collected from all treatments in the common rotation field, and never aboveground from the long rotation field in 2007.

Yield was not related to the percentage of infected seed-tubers in a seed lot in this study. However, for both fields in 2008, disease severity was related to increased percentage of infected seed-tubers in a seed lot. Increased disease in the crop with an increase in seedborne inoculum has previously been reported (Komm and Stevenson, 1978). In this study, neither increased percentage of infected seed-tubers in a seed lot, nor the increased levels of disease in the plants were related to yield.

The yield differences among the seed lots does emphasize the importance of seed lot selection. In 2008 the same seed lot produced the best yield in both fields and another seed lot produced the lowest yield in both fields. The cause of the differences is unknown, but the recommended best practices and guidelines for potato seed lot selection are well established (Bohl et al., 2003; Secor and Johnson, 2008) and should be used in order to choose and then handle the best available seed. These recommendations are likely to be helpful in avoiding seed lots with other yield limiting factors more serious than *C. coccodes*. Given the frequency with which *C. coccodes* can be detected in existing potato fields, the choice to not purchase a seed lot with *C. coccodes* is a probably critical only if the field has not previously been planted to potato.

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Table 1. Characteristics of potato crop and extent of host plant colonization by *C. coccodes* when seed lots with different levels of *C. coccodes* incidence were planted into fields with common potato rotations.

		Common Rotation Ground[1]						
	Percent Seed Infected[2]	Percent Field Necrosis[3]	Root Severity	Above Ground Stem Severity	Foci on Below Ground Stem	Incidence of Infected Progeny Tubers	Total Yield (metric ton ha^{-1})	Progeny Tuber Quantity
2007	0	47	0.6	3.0 a	2.1 a	0.02	87.4 a	175 abc
	25	45	0.4	1.1 b	0.9 b	0.02	72.2 b	179 abc
	31	45	0.3	0.7 b	1.5 ab	0.02	83.4 a	184 ab
	54	60	0.5	2.3 ab	1.8 ab	0.04	74.9 b	193 a
	57	48	0.6	0.5 b	1.5 ab	0.04	74.2 b	158 c
	60	60	0.6	0.8 b	1.7 ab	0.09	78.7 ab	169 bc
	Regression	*NA*	$P > 0.1$	*NA*	$P > 0.1$	*NA*	$P > 0.1$	$P > 0.1$
	Collection Date (DAP)	119	109	109	109	125	125	125
2008	9	91 ab	0.7 b	3.0 b	0.8 b	0.32 ab	61.4	128 b
	11	85 b	0.8 ab	4.4 a	0.7 b	0.18 b	60.3	128 b
	23	95 a	1.0 ab	3.3 ab	0.6 b	0.19 b	60.1	125 b
	43	90 ab	1.1 ab	2.8 b	1.3 b	0.21 ab	67.7	130 b
	57	89 ab	1.2 ab	2.4 b	1.1 b	0.31 ab	75.1	155 a
	80	84 b	1.4 a	3.3 ab	2.8 a	0.38 a	70.8	153 a
	Regression	*NA*	$P < 0.001$ $R^2=.94$	*NA*	$P < 0.03$ $R^2=.74$	*NA*	$P > 0.1$	$P < 0.03$ $R^2=.74$
	Collection Date (DAP)	137	119	119	119	146	146	146

[1] Common rotation field has several four year potato rotations; Long rotation field had not been planted to potato in 30 years.

[2] Percentage of seed tubers in which *C. coccodes* was detected in each seed lot.

[3] Mean percentage of dead canopy per plot for each seed lot.

[NA] No difference among seed lots was detected, or only in two or fewer years so regression analysis was not performed.

[a,b] Significant differences among seed lots were detected ($P < 0.05$).

Table 2. Characteristics of potato crop and extent of host plant colonization by *C. coccodes* when seed lots with different levels of *C. coccodes* incidence were planted into fields that had never been planted to potato (2007) or in the last 30 years (2008).

	Long Rotation Ground[1]							
	Percent Seed Infected[2]	Percent Field Necrosis[3]	Root Severity	Above Ground Stem Severity	Foci on Below Ground Stem	Incidence of Infected Progeny Tubers	Total Yield (metric ton ha^{-1})	Progeny Tuber Quantity
2007	0	11	0.2	0.0	0.4	0.00	79.6 ab	206 ab
	25	15	0.0	0.0	0.0	0.00	85.0 a	205 ab
	31	10	0.3	0.0	0.5	0.00	63.0 b	166 b
	54	13	0.1	0.0	0.1	0.02	78.2 ab	194 ab
	57	11	0.0	0.0	0.0	0.00	76.2 ab	190 ab
	60	11	0.4	0.0	0.3	0.00	85.4 a	221 a
	Regression	*NA*	$P > 0.1$	*NA*	$P > 0.1$	*NA*	$P > 0.1$	$P > 0.1$
	Collection Date (DAP)	125	125	125	125	131	131	131
2008	9	9	0.2 b	0.6	0.2 c	0.07	86.3 b	145
	11	10	0.3 b	0.1	0.5 abc	0.09	91.2 ab	181
	23	7	0.2 b	0.0	0.3 bc	0.02	83.4 b	145
	43	9	0.3 ab	0.0	0.5 abc	0.04	83.4 b	147
	57	5	0.5 ab	0.0	1.3 ab	0.05	97.5 a	172
	80	6	0.8 a	0.3	1.4 a	0.09	85.2 b	169
	Regression	*NA*	$P < 0.005$ $R^2=.88$	*NA*	$P < 0.008$ $R^2=.85$	*NA*	$P > 0.1$	$P > 0.1$
	Collection Date (DAP)	137	119	119	119	146	146	146

[1] Common rotation field has several four year potato rotations; Long rotation field had not been planted to potato in 30 years.

[2] Percentage of seed tubers in which *C. coccodes* was detected in each seed lot.

[3] Mean percentage of dead canopy per plot for each seed lot.

[NA] No difference among seed lots was detected, or only in two or fewer years so regression analysis was not performed.

[a,b] Significant differences among seed lots were detected ($P < 0.05$).

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