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Response of *Colletotrichum Coccodes* to Selected Fungicides Using a Plant Inoculation Assay and Efficacy of Azoxystrobin Applied by Chemigation

Jason Ingram · Thomas F. Cummings · Dennis A. Johnson

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Abstract A plant inoculation assay was developed and used to evaluate fungicide efficacy and application timing for reduction of infection of potato stems by *Colletotrichum coccodes*. Incidence of infected stems treated with azoxystrobin, pyraclostrobin, fluoxastrobin, and mandipropamid + difenoconazole was significantly less than the non-treated control plants when fungicides were applied prior to inoculation. However, fungicide application after inoculation did not significantly reduce infection. Chlorothalonil and mancozeb were not effective in preventing infection by *C. coccodes*. Additionally, early season fungicide applications by center pivot chemigation in commercial potato fields were evaluated as a potential black dot management tactic. Below-ground stems sampled from replicated plots in commercial fields where azoxystrobin was applied by chemigation at 50 and 67 days after planting (DAP) had significantly less stem surface area covered with sclerotia at 79 DAP than the non-treated control in two of 2 years. However, the effect was not observed both years at a subsequent collection at 102 DAP and only one of 2 years at 140 DAP. Latent infections were detected in non-symptomatic plants collected from the field.

Resumen Se desarrolló y utilizó un ensayo de inoculación de plantas para evaluar la eficacia y tiempo de aplicación de fungicida para la reducción de la infección en tallos de papa por *Colletotrichum coccodes*. La incidencia de tallos infectados tratados con azoxystrobin, pyraclostrobin, fluoxastrobin y mandipropamid + difenoconazole, fue significativamente menor que las plantas testigo no tratadas cuando se aplicaron los fungicidas antes de la inoculación. No obstante, la aplicación del fungicida después de la inoculación no redujo significativamente la infección. Clorotalonil y mancozeb no fueron efectivos en prevenir la infección por *C. coccodes*. Adicionalmente, se evaluaron aplicaciones de fungicidas al principio del ciclo de cultivo por quimirrigación de pivote central en campos comerciales de papa, como táctica potencial de manejo de la mancha negra. Los tallos subterráneos muestreados de lotes con repeticiones en campos comerciales donde se aplicó azoxistrobin por quimirrigación a 50 y 67 días después de la siembra (DAP), tuvieron significativamente menos área superficial del tallo cubierta con esclerocios a 79 DAP, que los testigos no tratados en dos años. No obstante, no se observó el efecto en ambos años en una colecta posterior a 102 DAP, y solo en uno de los dos años a 140 DAP. Se detectaron infecciones latentes en plantas asintomáticas colectadas en el campo.

Keywords Black dot · Strobilurin · Chemigation · Quinone outside inhibitor

J. Ingram · T. F. Cummings · D. A. Johnson (✉)
Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA
e-mail: dajohn@wsu.edu

Introduction

Black dot of potato (*Solanum tuberosum* L.) is caused by the soil- and tuberborne fungus *Colletotrichum coccodes* (Wallr.) Hughes. Small (150 to 230 um in diameter), black sclerotia of the fungus develop on infected roots, stolons, stems and tubers. *C. coccodes* can colonize below ground stems, stolons, roots and tubers from infected seed pieces at a rate of 1 mm/day (Ingram and Johnson 2010) however, soilborne inoculum of *C. coccodes* has the potential to cause more severe disease than tuber borne

inoculum (Nitzan et al. 2005; Nitzan et al. 2008). Infections of the tuber periderm result in gray to brown blemishes in which sclerotia may develop. Tuber shrinkage from water loss (Hunger and McIntyre 1979) and deep sunken lesions (Glais and Andrivon 2004) may occur on tubers in storage.

In addition to potato, *C. coccodes* can cause severe economic losses in processing tomato (*Solanum lycopersicum* L.) in which infected fruit develop black sunken lesions, a disease known as tomato fruit anthracnose (Byrne 1997; Dillard 1988; Dillard and Cobb 1997). Other hosts include pepper and a wide range of plant species, many in the Solanaceae as well as other plant families (Nitzan et al. 2006b; Raid and Pennypacker 1987).

C. coccodes frequently infects potato stems and other tissues early in the growing season but symptoms which include foliar chlorosis and necrosis, and signs of the pathogen in the form of sclerotia are often not expressed until relatively late in the growing season (Andrivon et al. 1998; Johnson and Miliczky 1993; Pasche et al. 2010). Potato yield and tuber quality can be reduced by *C. coccodes* (Hunger and McIntyre 1979; Johnson 1994; Mohan et al. 1992; Pasche et al. 2010; Stevenson et al. 1976; Tsror et al. 1999); however, the effect on yield is variable and yield losses are not always evident (Kotcon et al. 1985; Read and Hide 1995; Pasche et al. 2010; Scholte et al. 1985). Even though yield losses are not always observed, *C. coccodes* is a serious threat to potato production (Pasche et al. 2010). This is because there is the persistent threat for direct yield losses from *C. coccodes,* interaction with *Verticillium dahliae* to cause greater yield reductions (Tsror and Hazanovsky 2001) and reduced crop value from tuber blemish and shrinkage during storage.

The quinone outside inhibitor (QoI) fungicide azoxystrobin is efficacious against black dot on potato (Nitzan et al. 2005). Foliar applications of azoxystrobin either before or near row closure reduced black dot severity and incidence on stems and progeny tubers in the Columbia Basin of Washington; whereas, later applications appeared not to affect the disease (Cummings and Johnson 2008; Nitzan et al. 2005). However, a significant yield increase, when compared with a non-fungicide treated control, only occurred 1 of 3 years when azoxystrobin was applied multiple times to foliage during the growing season (Nitzan et al. 2005). In furrow applications at planting had no measurable effect on black dot severity (Cummings and Johnson 2008) and seed treatments have not been effective when soil borne inoculum is present (Denner et al. 1997; Read and Hide 1995).

Fungicides offer the potential of an effective disease control tactic for potato black dot. However, an increased understanding of the disease on yield and how specific fungicides and fungicide application timings affect disease development is needed to gain a consistent benefit. Timing of fungicide application is important for reducing black dot severity (Cummings and Johnson 2008) and more information is needed about when and how to effectively apply fungicides. Early season application of fungicide was suggested as a potential timing for black dot control from a study where infections of various plant organs, disease development, symptoms expression and potato yield were all compared in epidemics in commercial fields in the Midwestern U.S. (Pasche et al. 2010).

Application of fungicides through overhead sprinkler irrigation systems (chemigation) is commonly used for potatoes grown in the Columbia Basin (Johnson et al. 2000). With chemigation, fungicides are distributed in a high volume of water. Volumes over 18,708 l/ha (2000 gal/acre) are common, which are much greater than a ground sprayer at 327 l/ha (35 gal/acre) or aerial application at 47 l/ha (5 gal/acre). Fungicides applied by chemigation are deposited differently in the crop canopy than those made by air and ground applicators (Geary et al. 1999; Hamm et al. 2006). Foliar fungicides applied by air may not directly reach the lower plant canopy after row closure; whereas, fungicides applied by chemigation are deposited through out the canopy and on the soil.

The purposes of this research were to (i) Develop an inoculated plant assay to evaluate fungicide efficacy against *C. coccodes*. Such an assay under controlled conditions would be helpful in identifying fungicides effective against *C. coccodes*. An inoculated plant assay may be preferred in some situations over assessing fungal growth on fungicide amended media as has been previously used (Uribe and Loria 1994), and the two techniques used together could be useful in selection fungicides for further evaluation in the field. (ii) Evaluate the hypothesis that strobilurin fungicides act curatively against *C. coccodes* using the inoculated plant assay. Curative activity with strobilurins has been suggested and would be useful for black dot control since infections can occur relatively early in the growing season. (iii) Evaluate selected fungicides for efficacy in preventing infection of potato foliage against *C. coccodes*. (iv) Evaluate fungicide application by center pivot chemigation before closure between rows for management of black dot in commercial potato fields.

Materials and Methods

An inoculated potato plant assay was used in two experiments conducted in the greenhouse and a strobilurin was applied by chemigation in commercial fields in a third experiment. In the first greenhouse study (*application timing*), the fungicides azoxystrobin, pyraclostrobin and dicloran were evaluated for protective and curative proper-

ties by applying them before and after plant inoculation, respectively. In the second greenhouse study (*fungicide efficacy*), the fungicides chlorothalonil; fluoxastrobin; famoxadone; mancozeb; polyoxin D zinc salt; azoxystrobin; and mandipropamid + difenoconazole were evaluated as protectants with application being made prior to inoculation. In the field study (*application by chemigation*), azoxystrobin was applied.

Greenhouse Pre-nuclear generation seed-tubers (whole mini-tubers, 9 to 35 g in weight) of cultivar Russet Burbank from a certification program were used in the first experiment. Certified Generation 1 seed tubers, cut into 42 to 85 g seed-pieces, were used in the second experiment. Seed tubers or pieces were planted in the greenhouse in the spring of 2007 and 2008, first and second experiments, respectively. Prior to planting, seed tubers were evaluated for *C. coccodes* by plating the basal end of the tubers (Johnson et al. 1997) on modified PDA (mPDA) (Nitzan et al. 2006a) in Petri dishes and incubating in the dark at 22°C for 14 days. Tubers were considered infected if *C. coccodes* was detected from the plated tissue and produced characteristic sclerotia and conidia.

Only seed-tubers in which *C. coccodes* had not been detected were planted in the greenhouse. Seed-pieces were planted 5.5 cm deep in a potting mix (LC1 Soil Mix, Sun Gro Horticulture, Canada Ltd.) in 15 cm diameter x 18 cm tall, 3 l pots. The potting mix was amended with 3 g of 16-16-16 NPK fertilizer before planting. Greenhouse conditions were maintained under natural light with mean day/night temperature of 22/14C and relative humidity of 38/65%. Plant inoculation was carried out once 25% of plants reached primary bloom, which corresponded with tuber initiation. This occurred approximately 67 days after planting for both trials in the first greenhouse experiment and at 55 and 48 days, respectively, in the first and second trials of the second greenhouse experiment.

The most dominant stem of each plant was lightly wounded at a point on the stem halfway between the soil line and stem apex. This was done with a small piece of 120 grit sandpaper that was wrapped around an 8 mm diameter rod that was then dragged perpendicular to the stem axis with light hand pressure. Only 3 cm of the total sandpaper length was dragged across the stem resulting in wounds approximately 4- x 3- x 1-mm deep. This wound served as the inoculation court. After wounding, any pre-inoculation fungicide treatments were applied and permitted to air dry on the foliage.

Colonies of *C. coccodes,* isolate CR-102 (NA-VCG 2) were grown on V-8 agar (Porter et al. 2006) under constant florescent light (10 W/m^2) at 22C. Isolate CR-102 was originally isolated from a seed potato obtained from a commercial seed lot and was stored on rye kernels at room temperature until culture on V8 media. This isolate is aggressive on potato (Heilmann et al. 2006; Nitzan et al. 2006c). A conidial suspension of 6×10^4 conidia ml^{-1} was prepared as described in Nitzan et al. (2006a).

Inoculation was done by placing 40 µl of the *C. coccodes* conidial suspension onto a 1 cm^2 piece of sterilized Whatman #1 filter paper and placing the paper over the wound (Nitzan et al. 2006a). 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. Plants were placed in a mist chamber for 21 h at 21C. Plants were then removed from the chamber, permitted to air dry, and the filter paper squares removed before any post-inoculation fungicide applications. Fungicide treatments were arranged in a randomized complete block design with four replications per treatment.

Fungicides were applied within the manufacturers labeled rate per acre for potato in 355 l ha^{-1} (38 gal acre^{-1}) water carrier without a surfactant 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^{-1} (18,544 plants acre^{-1}).

Application Timing Three fungicides, azoxystrobin at 164 g a.i. ha^{-1} (Syngenta, Wilmington, DE, Quadris, 9 oz acre^{-1}), pyraclostrobin at 165 g a.i. ha^{-1} (BASF, Research Triangle Park, NC, Headline, 9 oz acre^{-1}) and dicloran at 4.2 kg a.i. ha^{-1} (Gowan, Yuma, AZ, Botran 5F, 3 quarts acre^{-1}) were used. The applications were made: prior to inoculation, 1 day after inoculation (DAI), 5 DAI, 10 DAI, and a sequential application made at 1 & 10 DAI (Table 1). Control of black dot is listed on the labels of azoxystrobin and pyraclostrobin and both were applied using all five application timings. The fungicide dicloran was applied at only two times, pre-inoculation and 1 day post-inoculation. Dicloran is not labeled for black dot but is for white mold control. Two additional treatments included a non-fungicide control on inoculated and non-inoculated plants.

Fungicide activity against *C. coccodes* was evaluated when stems were collected for assay at 20 days and 35 days after the last fungicide application in the first and second trials, respectively. All plants at the time of assay in both trials, were in the tuber bulking stage (growth stage IV) (Miller and Hopkins 2008) and were not yet senescent. Stems for disease assessment were excised just above the soil line using flame-disinfested pruning shears, and petioles were removed using flame disinfested scissors. The stems were soaked in a 10% bleach water solution for 3 min, rinsed with distilled water and permitted to air dry

Table 1 Incidence of inoculation courts that *Colletotrichum coccodes* was isolated on potato stems of cultivar Russet Burbank that were treated with fungicides at various timings pre and post inoculation

Fungicide application treatments [y]	Incidence of infected inoculation courts (%)[z]	
	Trial 1	Trial 2
Inoculated Control		
No Fungicide	100 a	100 a
Pre-inoculation		
Dicloran	75 a	100 a
Pyraclostrobin	0 b	25 b
Azoxystrobin	50 b	25 b
Post-inoculation 1 Day		
Dicloran	100 a	–
Pyraclostrobin	75 a	100 a
Azoxystrobin	100 a	100 a
Post-inoculation 5 Days		
Pyraclostrobin	100 a	100 a
Azoxystrobin	100 a	100 a
Post-inoculation 10 Days		
Pyraclostrobin	100 a	100 a
Azoxystrobin	100 a	100 a
Post-inoculation 1&10 Days		
Pyraclostrobin	100 a	75 a
Azoxystrobin	100 a	100 a
Non-inoculated Control	0	0

[y] Rate equivalent of dicloran, 4.2 kg a.i. ha^{-1} (Botran 5 F rate 3 qt $acre^{-1}$); azoxystrobin or pyraclostrobin, 164 g a.i. ha^{-1} (Quadris & Headline 9 oz $acre^{-1}$) in 355 l ha^{-1} (38 gal $acre^{-1}$) water carrier

[z] Percentage of infection courts from which *C. coccodes* was detected on nutrient agar

Values with the same letters are not significantly different as by determined by Dunnett's method for multiple comparisons at the 95% confidence level

– = Treatment not performed

on a laboratory bench top. After drying, a 1 cm long stem cross-section centered on the inoculation court was aseptically removed from each stem, plated on MPDA and incubated at 21C for 14 days to determine if *C. coccodes* was present (Nitzan et al. 2006a). Colonization of the stem segments was considered positive if sclerotia and conidia of *C. coccodes* were observed. The frequency of detection was the proportion of plated stem segments from which *C. coccodes* was detected. To confirm that *C. coccodes* detected in the stem was not due to colonization arising from infected seed-tubers, a stem cross-section was taken near the soil line of inoculated stems and placed onto MPDA. *C .coccodes* was not detected in any of the soil line cross-sections.

Fungicide Efficacy Fungicide application was carried out once, immediately after stem wounding but prior to inoculation with *C. coccodes*. Fungicide treatments included: chlorothalonil at 1.05 kg a.i./ha (Bravo Weather Stick, Syngenta, Wilmington, DE); fluoxastrobin at 101 g a.i./ha (Evito 480SC, Arysta Life Science, Cary, NC); famoxadone at 76 g a.i./ha, (DuPont, Wilmington, DE); mancozeb at 1.05 kg a.i./ha, (Penncozeb 75DF, Cerexagri, King of Prussia, PA); polyoxin D zinc salt at 201 g a.i./ha, (Kaken Pharmaceutical, Tokyo, Japan); azoxystrobin at 196 g a.i./ha (Syngenta); and mandipropamid + difenoconazole at 114 g + 114 g a.i./ha (Syngenta).

Fungicide activity against *C. coccodes* was evaluated when stems were collected for assay for *C. coccodes* 35 and 45 days after inoculation in the first and second trials, respectively. All plants at time of assay were chlorotic and the bottom two to four leaves were necrotic. Stem tissue pieces were prepared and plated as described for the application timing experiment.

Application by Chemigation Azoxystrobin was evaluated for efficacy against black dot when applied by chemigation in replicated plots in commercial fields in 2007 and 2008. Separate fields were used each year and were located east of Moses Lake. Fields were planted with certified seed tubers (Generation 3) of Russet Burbank. A sample of 60 seed tubers was assayed for *C. coccodes* each year. Both fields had a total of three previous potato crops (Table 3). The field used in 2007 was in a 4-year potato rotation and planted April 30. The field used in 2008 was in a 3-year rotation and was planted on May 1. The soil types in the two fields were Shano and Burke silt loams. Seed pieces were treated with thiophanate-methyl, mancozeb and imidacloprid (Tops-MZ-Gaucho, 1:100 weight seed) and the fields were managed according standard regional practices.

Azoxystrobin at a rate of 113 g a.i. ha^{-1} delivered in 35,544 l ha^{-1} water via center pivot chemigation (Quadris 6.2 oz $acre^{-1}$ in 3800 gal $acre^{-1}$ or 0.14 acre-inches water

carrier) and a non-treated control were arranged as randomized complete blocks replicated three times. Plots were wedge-shaped sections of the field. Non-treated plots received water but no fungicide. The fungicide was applied at 50 and 67 days after planting (DAP) in 2007 and 50 and 69 DAP in 2008. Crop maturity at 50 DAP corresponded to tuber initiation or potato growth stage III (Miller and Hopkins 2008) and within row closure of plants. At 67 and 69 DAP the two respective years, maximum tuber size was 4.25 cm in diameter and between row closure was 90%.

Black dot was quantified in the crop when plants were sampled for *C. coccodes* at 79, 102 and 140 days after planting. Plants were collected from the center of each plot between the two outer most pivot wheel tracks along a transect between the outer edge of the field and the center of the pivot. Above-ground stem samples were obtained by clipping six stems per plot 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 and transported to the laboratory. The following day the remaining petiole stubs were removed using scissors that were flame-disinfested between each cut. The stems were then rinsed in tap water, placed in 10% bleach water solution for 5 min, 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 plated on MPDA, incubated for 14 days at 21 C, and then evaluated for *C. coccodes* incidence. 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 summed for each subsample (stem) and the mean value of subsamples was calculated for each replication.

Below-ground stems were obtained by digging two plants per plot. The top of each plant stem was removed at approximately 30 cm above the soil line. Petioles were trimmed and the below-ground stems were transported in a cooler to the laboratory. The below-ground stems were rinsed in tap water and placed in a 10% bleach water solution for 5 min. 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 plot were placed in a single plastic bag (20- × 10- × 45-cm) with the aerial 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 samples were incubated upright for 12 days at 21C. The bags were secured loosely around the stems to permitted air exchange and allow slow drying. Sclerotia developed on plant tissues and the extent of below-ground stem colonization was determined by measuring the length of the stem that was covered with sclerotia and comparing it to the total length of the stem. The percentage of the linear length of the below-ground stems with sclerotia was calculated. The below ground stems were further assessed by counting the number of distinct areas of tissue with dense concentrations of sclerotia that were separated by areas with no sclerotia along that length of stem. These distinct areas of disease were called foci.

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 below-ground stem colonization. Four tubers from each replication were assessed for presence of *C. coccodes* as described for seed-tubers.

Data Analysis All trials were repeated and data was subjected to statistical analysis using the GLM procedure in SAS (SAS 9.1 SAS Institute, Cary, NC) In the greenhouse experiments the main fixed effects were fungicides and application timing where treatments were compared to the inoculated control with $\alpha=0.05$, adjusted for multiple comparisons using Dunnett's method (1955). In the field study, ANOVA was conducted to compare plant colonization in treated vs. non-treated plots. The responses of interest were disease severity for the above-ground stems, the incidence of plants with below-ground stems that were diseased, the proportion of below-ground stem surface area that was diseased, the number of diseased tissue foci on below-ground stems, and the incidence of infected progeny tubers. Comparisons were made within collection date.

Results

Application Timing Colletotrichum coccodes was isolated from all of the inoculated stems that were not treated with fungicides, but not from the non-inoculated control plants. When the fungicides azoxystrobin and pyraclostrobin were applied prior to inoculation, incidence of infected stems was significantly less ($P<0.05$) than in the inoculated, non-fungicide control plants (Table 1). Incidence of infected stems did not significantly differ among the post-inoculation fungicide treatments and the inoculated control. Incidence of infected stems was not significantly different between the dicloran fungicide treatment and the inoculated control (Table 1). *C. coccodes* was not detected in any of the soil line cross sections.

Fungicide Efficacy *C. coccodes* was isolated from all of the inoculated plants that were not treated with fungicides, but not from non-inoculated control plants. When fluoxastrobin, and mandipropamid + difenoconazole were applied, incidence of infected stems was significantly less ($P<0.05$) in both trials than the inoculated, non-fungicide control plants (Table 2). Incidence of infected stems was significantly less ($P<0.05$) than the inoculated, non-fungicide control when azoxystrobin was applied in the first but not the second trial. Colonies of *C. coccodes* that were isolated and grew from the azoxystrobin treated infection court in the second trial were severely restricted in growth and about 30% of the size as colonies from the inoculated control.

Application by Chemigation *C. coccodes* was detected in 2.5% of the seed tubers in 2007 and 5.5% in 2008. The severity index of *C. coccodes* colonization in above-ground stems was significantly less on plants collected from fungicide treated than from non-treated plots at 79, 102 and 140 DAP in 2007 and at 79 DAP in 2008 (Table 3). Below-ground stems sampled from azoxystrobin treated plots had significantly less surface area with sclerotia of *C. coccodes* than the non-treated plots at 79 and 140 DAP in 2007 and at 79 DAP in 2008 ($P<0.04$, Table 3). Mean number of *C. coccodes* foci on below-ground stems ranged from 0.17 to 3.08 per plant. The number of distinct black dot foci on the below-ground stems was significantly less in treated plots than non-treated only during the 79 DAP assay in 2008 (foci data were not collected at 79 DAP in 2007).

Sclerotia of *C. coccodes* were not immediately observed on host tissue collected at 79 and 102 DAP. However, sclerotia developed on below-ground stems after 12 days of incubation in the humid environment provided by the loosely tied plastic bags and *C. coccodes* colonization could then be determined by observing the regions of sclerotia that developed on tissue that was previously asymptomatic. On below-ground stems, the localized areas of diseased tissue or foci typically ran longitudinally rather than girdle the stem circumference.

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

Discussion

The plant inoculation assay was effective in evaluating fungicides for efficacy against *C. coccodes*. The use of plant stems to screen fungicides in the greenhouse provided a framework for comparing fungicides, rates of fungicides and application timings. Specifically, it demonstrated that curative action was not obtained when azoxystrobin or pyraclostrobin were applied post-inoculation. The plant inoculation assay also demonstrated that conidia of *C. coccodes* could successfully infect the potato stems during a 21 h mist period and survive subsequent fungicide applications. This observation corresponds with the management practice currently used for tomato fruit anthracnose where fungicide applications are required to be made

Table 2 Incidence of infected inoculation courts on Russet Burbank potato stems that were wounded, treated with fungicide and then inoculated with *C. coccodes* in the greenhouse

Treatment	Formulation & Rate acre^{-1}[a]	a.i./ha	Incidence of infection at inoculation court (%)[b]	
			Trial 1	Trial 2
Inoculated control		0	100 a	100 a
chlorothalonil	Bravo Wx Stick 1.25 pt	1.05 kg	75 a	75 ab
mancozeb	Penncozeb 75DF 1.25 lb	1.05 kg	50 a	75 ab
famoxadone	Famoxate 25SC 4.0 fl.oz	76 g	25 b	75 ab
Oxin D zinc salt	Polyoxin 1.00 lb	201 g	0 b	75 ab
fluoxastrobin	Evito 480SC 2.9 fl.oz	101 g	0 b	0 c
Mandipropamid difenoconazole	Revus Top 6.25 fl.oz	114 g 114 g	0 b	0 c
azoxystrobin	Quadris 10.75 fl.oz	196 g	0 b	50 abc
Non-inoculated control		0	0	0

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

[b] Percentage of infection at inoculation court based on infection courts from which *C. coccodes* could be cultured on nutrient agar. Within trial, values followed by the same letters are not significantly different at $P=0.05$ according to Dunnett's method for multiple comparisons

Table 3 Severity of black dot on above- and below-ground stems of Russet Burbank and incidence of infected tubers when azoxystrobin was and was not applied by chemigation to replicated wedge shaped plots in a commercial potato field irrigated by a center pivot system

	Collection date						
	79 days after plant			102 days after plant		140 days after plant	
	Year	Treated	Non-Treated	Treated	Non-Treated	Treated	Non-Treated
Above Ground							
Severity index[y]	2007	1.11*	1.83	0.67*	1.72	1.50*	2.67
	2008	0.00*	0.56	0.00	0.39	0.58	1.25
Below Ground							
Incidence of plants with *C. coccodes*	2007	0.17*	0.83	1.00	1.00	0.83	1.00
	2008	0.17	0.67	0.83	1.00	0.67	1.00
Proportion of stem surface with sclerotia	2007	0.01*	0.13	0.16	0.20	0.23*	0.48
	2008	0.01*	0.06	0.15	0.39	0.19	0.46
Number of foci[z]	2007	–	–	2.33	2.83	3.08	3.25
	2008	0.17*	1.33	1.84	2.34	1.17	2.08
Incidence of progeny tubers infection	2007	0.00	0.33	0.08	0.33	0.48	0.63
	2008	0.00	0.00	0.00	0.33	0.02*	0.21

*Value is significantly different than the non-treated at $P=0.05$

[y] Mean of sums of cross section in which *C. coccodes* were detected for six subsamples (stems) collected per replication. 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

[z] *C. coccodes* foci on the roots were determined visually after infections were expressed during incubation in plastic bags

at fruit initiation and continued regularly throughout the season to prevent infections that remain latent until the fruit ripen (Byrne 1997; Dillard 1992; Fulton 1948; Illman et al. 1959; Kendrick and Walker 1948). Timing of fungicide applications is critical to prevent infection of potato by *C. coccodes*. Early season protection appears to be a foundation for managing this pathogen and has previously been suggested (Cummings and Johnson 2008; Pasche et al. 2010).

The fungicides azoxystrobin, fluoxastrobin, mandipropamid + difenoconazole, and pyraclostrobin were effective in reducing infection in the inoculated plant assay when applied before potato plants were inoculated with *C. coccodes*. These fungicides also have activity against *Phytophthora infestans* and may be cost effective when integrated in disease management programs where both late blight and black dot are concerns. Chlorothalonil and mancozeb were not effective in preventing infection by *C. coccodes*. Dicloran was not expected to reduce infections and was included in the experiment as a potential non-effective standard.

Foliar applications of azoxystrobin on potato reduced severity of black dot on stems and incidence of progeny tuber infection in previous field studies (Cummings and Johnson 2008; Nitzan et al. 2005). Below-ground organs are often infected by *C. coccodes* where fungicides are difficult to directly apply, and chemigation may be a method to deliver water soluble fungicides to the rhizosphere. When azoxystrobin was applied by chemigation at 50 and 67 DAP in this study, colonization of the below-ground and above-ground stems was significantly reduced at the 79 DAP assay. However, the effects of the azoxystrobin applications did not endure and had mostly expired by the time of the 102 DAP collection. This indicates that azoxystrobin applied at 50 and 67 DAP did not prevent infections that took place later in the season. Additionally, a lack of curative activity is demonstrated in that the pathogen was isolated from all sampled parts of the potato plant 12 days after the second application of azoxystrobin at 67 to 69 DAP.

The low incidence of infected seed-tubers but high incidence of disease on stems indicate that soilborne inoculum played a major role in the development of black dot in the test fields. Black dot development from soil borne inoculum is especially difficult to manage because infection can take place through out the growing season, the soil environment generally favors infection in moist, irrigated soils, and newly developed below-ground plant tissues are difficult to protect with fungicide. In comparison, foliar diseases such as late blight often require repeated fungicide applications to protect new above-ground plant foliage during disease favoring weather (Stevenson et al. 2008).

Latent infections were detected in non-symptomatic plants. Samples collected from field plots at 79 and 102 DAP were asymptomatic as visually determined at time of

sample collection. However, when the same samples were cultured in the laboratory, *C. coccodes* was isolated from roots, stems and tubers. Similar results have been previously reported (Johnson and Miliczky 1993; Otazu et al. 1978; Pasche et al. 2010). Furthermore, by 102 DAP, 100% of below-ground stems in both treated and non-treated plots were infected but asymptomatic in 2007 and 83% of plants in treated plots and 100% of plants in non-treated plots in 2008. This indicates that visible assessment of black dot in the field is not practical until senescence when latent infections finally become evident as previously proposed by Nitzan et al. (2006a). Sclerotia were evident on drying plant tissue in the field at the final 140 DAP collection, but that collection occurred after chemical vine desiccation.

Severity of black dot was reduced when applications of azoxystrobin were made by chemigation at row closure within rows and just before closure between rows; however, protection did not endure. Fungicides should not be expected to be a primary disease management tactic for black dot because of the cost of multiple fungicide applications through out the season and an inability to apply fungicides directly to below-ground plant organs, where the effects of black dot are a major concern. Fungicides targeting other foliar disease that also have activity against *C. coccodes* and early season fungicide applications may give an incremental improvement to an integrated management program. However, efforts to manage black dot should emphasize black dot resistant cultivars (Nitzan et al. 2009) and cultural practices that reduce soil borne inoculum and that suppress disease development during the season.

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