Effect of Seed-Tuber Generation, Soilborne Inoculum, and Azoxystrubin Application on Development of Potato Black Dot Caused by Colletotrichum coccodes

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ABSTRACT

The effect of azoxystrubin on potato black dot and the role of seed- and soilborne inocula of Colletotrichum coccodes in the development of black dot were evaluated in the field using two potato seed generations (generation 1 and 3) of the susceptible cvs. Norkotah Russet and Russet Burbank over 3 years (2002 to 2004). Plants of Norkotah Russet and Russet Burbank treated with azoxystrubin had 13 and 23% higher yields, respectively, than nontreated plants in 2003. Disease severity on both cultivars was reduced to 19% and 22% in 2003 on above- and belowground stem sections, respectively, when plants were treated with azoxystrubin. Plants of both cultivars that were treated with azoxystrubin had 9 to 26% less infected progeny tubers than the nontreated. These results indicated the efficacy of azoxystrubin to reduce black dot severity on both stems and progeny tubers. The roles of seed- and soilborne inocula in disease development were evaluated in 2003 and 2004 using generation 1 and 3 seed tubers. The incidence of C. coccodes in generation 1 mother tubers of Norkotah Russet and Russet Burbank were 2 and 16% in 2003, respectively, and 0 and 30% in 2004, respectively. The incidence of C. coccodes in generation 3 mother tubers of Norkotah Russet and Russet Burbank were 14 and 49% in 2003, respectively, and 12 and 38% in 2004, respectively. Generation 1 plants of Norkotah Russet had 36 and 13% greater yield than generation 3 plants in 2003 and 2004, respectively. In 2004, generation 1 plants of Norkotah Russet had 26 and 15% greater disease severity, respectively, on belowground stem than generation 3 plants. Generation 1 plants of Norkotah Russet had 7.5 and 11% more infected progeny tubers in 2003 and 2004, respectively, than generation 3. Significant differences for yield reduction and incidence of infected progeny tubers between the two seed generations were not recorded for Russet Burbank, suggesting that the effect of inoculum source of C. coccodes on black dot severity may be cultivar specific.

Additional keywords: seed-tuber generation, seedborne inoculum, soilborne inoculum

Potato black dot, caused by Colletotrichum coccodes (Wallr.) S. J. Hughes, is a concern in commercial potato production areas worldwide. The disease may cause up to 30% yield reduction on susceptible cultivars (24) and may damage tuber quality by causing skin blemishes, reducing tuber weight, and affecting tuber specific gravity (4).

Inoculum sources for infection of plants in the field by C. coccodes may be either one or a combination of soil-, seed-, and airborne inocula. Potato seed tubers that carry the pathogen internally in the vascular tissue, externally on the skin, or in a combination of both are one of the major inoculum sources. Seed tubers may spread the pathogen among and within potato production areas. Once introduced to the soil via infected seed tubers, the fungus may establish itself and become soilborne (13,14,16,19).

Potato seed tubers that are used commercially in Washington State are the progeny of plants produced in tissue culture from potato meristem cells. These pathogen-free plants are grown in greenhouses to produce pathogen-free progeny tubers that are referred to as “nuclear seed tubers”. The latter are planted in the field and their progeny, which are the first potato tuber generation produced in the field, are referred to as generation 1 seed tubers. The progeny of generation 1 is referred to as generation 2, which will later produce the third generation of potato seed tubers in the field, known as generation 3. Generation 3 seed tubers are the most commonly used by commercial potato growers. In the field, each passing generation of progeny tubers accumulate seedborne pathogens; therefore, generation 3 seed tubers tend to have higher incidence of infection with C. coccodes than generations 1 and 2 seed tubers (14).

To date, no specific fungicide has been demonstrated to control potato black dot. Among the fungicides recently made available against black dot are the strobilurins. These are antifungal compounds, also known as Qo inhibitors (QoIs), which inhibit the respiratory cytochrome bc1 complex in the mitochondria by blocking electron transport at the quinol-oxidizing (Qo) site (2,12,17,26,28). Fungicides of the strobilurin class are used commercially to control diseases caused by a wide range of fungal and fungal-like pathogens from the divisions Oomycota, Ascomycota, and Basidiomycota (1,3,7,10,11,15,18,27). In the present study, we tested two hypotheses: (i) that azoxystrubin may reduce black dot under field conditions and (ii) that seed- and soilborne inocula of C. coccodes affect black dot development and severity differently. We hypothesized that exposing naturally infected certified potato seed tuber generations 1 and 3, which differ in incidence of initial inoculum, to soil naturally infested with C. coccodes would allow this evaluation.

MATERIALS AND METHODS
General. Trials were conducted in 2002, 2003, and 2004 at the Washington State University experimental station in Othello, WA. Trials were carried out on a Shano silt loam soil. Each year, trials were conducted in a different location of the station in fields where potatoes were grown previously and demonstrated high severities of black dot. All fields had 4 years of potato rotations (potato was grown every fourth year) with barley, mustard, wheat, or bean prior to planting. Experimental plots consisted of three rows, 4.5 m long and 86 cm wide, with seed pieces planted 25 cm apart within a row. Treatments were replicated four times in a randomized complete block design. The trial in 2002 was planted on 18 April, canopy was cut by mechanical flailing on 6 September, and trial was harvested on 16 September. The trial in 2003 was planted on 15 April, canopy was cut by mechanical flailing on 1 October, and trial was harvested on 3 October. The trial in 2004 was planted on 15 April, canopy was cut by mechanical flailing on 20 September, and trial was harvested on 22 September.

Plant material. Generation 1 and 3 seed tubers of the black dot-susceptible cvs. Norkotah Russet (short-season cultivar), and Russet Burbank (long-season cultivar)
were used. Generation 1 seed tubers were grown in fields in which potato had not been grown previously, or had not been grown for at least 10 years and which, therefore, were either disease free or carried low incidence of seedborne inoculum. Generation 3 seed tubers were grown in fields in which potato had not been grown for 4 to 5 years and carried a higher incidence of seedborne inoculum than generation 1 seed tubers. In 2002, generation 1 and 3 seed tubers of Norkotah Russet and Russet Burbank, respectively, were used. The Russet Burbank seed tubers in 2002 originated from two different seed sources (indicated as lot 1 and lot 2). In 2003 and 2004, seed-tuber generations 1 and 3 of both cultivars were used. Seed tubers were cut (60 g on average) and stored at 10°C 3 days prior to planting.

**Fungicide applications.** Azoxystrobin (Quadris 2.08 SC; Syngenta) was applied with a CO2 backpack boom sprayer (TXVS-18 ConeJet nozzle; R&D Sprayers, Inc., Opelousas, LA) at a rate of 218.6 g/a.i./ha. Four applications were carried out: in furrow at planting time at a rate of 140 liters/ha, and 60, 83, and 93 days after planting to the foliage at a rate of 372 liters/ha.

**Treatments.** The trial in 2002 tested generation 1 seed tubers of Norkotah Russet with and without azoxystrobin application and generation 3 seed tubers of Russet Burbank (lots 1 and 2) with and without azoxystrobin application. The trial in 2003 tested generation 1 seed tubers of Norkotah Russet without azoxystrobin application and generation 3 of Norkotah Russet and generations 1 and 3 of Russet Burbank with and without azoxystrobin application. The trial in 2004 tested generations 1 and 3 of both Norkotah Russet and Russet Burbank with and without azoxystrobin application.

**Evaluation of initial inoculum.** Soil assays in 2004 did not detect *C. coccodes* in any of the sampled plots. Infection of mother tubers with *C. coccodes* of generation 1 Norkotah Russet and generation 3 Russet Burbank (lots 1 and 2) in 2002 was 0 and 41%, respectively. Generation 1 mother tubers of Russet Burbank were less (P < 0.05) infected than generation 3 in 2003 (16 and 49%, respectively), but not in 2004 (30 and 38%, respectively). Generation 1 mother tubers of Norkotah Russet were less (P < 0.05) infected than generation 3 in 2003 (2.3 and 14.3%, respectively) and in 2004 (0 and 12%, respectively). Azoxystrobin application resulted in decreased disease severity on both aboveground and belowground stem sections all years (Table 1). In 2002, Norkotah Russet plants treated with azoxystrobin had 59% less disease severity (P = 0.07) on belowground stems than non-treated plants (Table 1). Russet Burbank plants treated with azoxystrobin in 2002 had 24% less disease severity (P = 0.09) on aboveground stem sections and 34% less disease severity (P = 0.02) on belowground stems than non-treated plants (Table 1). In 2003, Norkotah Russet and Russet Burbank plants treated with azoxystrobin had 19 and 25% less (P < 0.05) disease severity, respectively, on aboveground stem sections than non-treated control plants. Russet Burbank plants treated with azoxystrobin had 29% less (P < 0.05) disease severity on belowground stem sections than non-treated control plants (Table 1). In 2004, Norkotah Russet and Russet Burbank plants treated with azoxystrobin had 49 and 81% less (P < 0.05) disease severity, respectively, on aboveground stem sections and 69 and 81% less (P < 0.05) disease severity on belowground stem section than non-treated control plants (Table 1).

Application of azoxystrobin generally decreased infection of progeny tubers of Russet Burbank and Norkotah Russet significantly (P < 0.05) (Table 1). In 2002, azoxystrobin nontreated plants of Norkotah Russet had 13.7% more (P = 0.09) infected progeny tubers than treated plants (Table 1). In 2003, Russet Burbank plants treated with azoxystrobin had 8.3% less infected progeny tubers than non-treated plants (Table 1). In 2004, azoxystrobin-treated plants of Norkotah Russet and Russet Burbank had 10.6 and 26% less (P<0.05) infected progeny tubers, respectively than nontreated plants.

**Effect of seed generation.** Yield of Norkotah Russet plants from generation 1
were 36 and 13% higher (P < 0.05) than generation 3 plants in 2003 and 2004, respectively (Table 2). Disease severity on aboveground stem sections did not differ significantly (P > 0.05) between the seed generations in either year for either cultivar (Table 2). However, significant differences for disease severity were recorded on belowground stems in 2004, with 25.6 and 15.2% more infection on generation 1 plants of Norkotah Russet and Russet Burbank, respectively, than on generation 3 (Table 2).

The incidence of infected progeny tubers differed significantly between seed generations in both 2003 and 2004 only for plants of Norkotah Russet (Table 2). Generation 1 plants had 7.5 and 11.2% more infected progeny tubers than generation 3 plants in 2003 and 2004, respectively (Table 2).

DISCUSSION

Application of azoxystrobin to potato plants of cvs. Russet Burbank and Norkotah Russet resulted in reduced black dot severity on stems and progeny tubers compared with nontreated control plants in all 3 years of the study. Plants treated with azoxystrobin had fewer infected progeny tubers and lower stem colonization than nontreated plants, demonstrating the ability of the fungicide to inhibit both stems and progeny tuber colonization.

Yield was increased with fungicide application on both cultivars in 2003, but not in 2002 and 2004. The lack of yield differences between azoxystrobin-treated and nontreated plants in 2002 and 2004 may have been due to the presence of Verticillium dahliae rather than azoxystrobin inability to control black dot. Furthermore, black dot effect on yield previously has been reported to be inconsistent and infrequent (21,23–25). Tsror et al. (24) indicated that, even in seasons in which high disease severity was recorded on dry stems at or just before harvest, yield reduction did not occur consistently. The effect of black dot on yield was reported to be associated mostly with abiotically stressed plants. In Israel, yield loss was recorded when plants infected with C. coccodes were exposed to high day temperatures and drought during the spring growing season (25) and in the autumn season when plants were exposed to a shorter day photoperiod (23,24). In the United States, Norkotah Russet and Russet Burbank plants grown in growth chambers in soil infested with C. coccodes at 23 and 28°C had lower yield than plants grown at 18°C (9). In the Columbia basin of Washington State, potato plants generally were grown from March to October, when day length is relatively long, until the end of the season, when disease symptoms and pathogen signs often appear. Potato plants in the Columbia basin of Washington State are kept relatively well hydrated and well fertilized. Therefore, under these growing conditions, plants are kept relatively stress free, which may explain the infrequent yield reduction in the Columbia basin and in our trials.

However, because black dot is a monocyclic disease, building inoculum over seasons, a threshold of inoculum eventually may be reached, in which case consistent reduction in yield would become apparent. Therefore, we advise not dismissing the importance of black dot on the basis of infrequent yield reduction.

Table 1. Main effect contrasts between azoxystrobin-treated and nontreated plants of Norkotah Russet and Russet Burbank potato in 2002, 2003, and 2004 field trials conducted at the Washington State University station, Othello

<table>
<thead>
<tr>
<th>Year, cultivar</th>
<th>Yield (t/ha)</th>
<th>Disease severity (stem colonization) (%)</th>
<th>Infected progeny tubers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non</td>
<td>Treated</td>
<td>P</td>
</tr>
<tr>
<td>2002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>70.2</td>
<td>69.4</td>
<td>0.21</td>
</tr>
<tr>
<td>RB (lots 1 &amp; 2)</td>
<td>80.3</td>
<td>87.3</td>
<td>0.12</td>
</tr>
<tr>
<td>2003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK-G3</td>
<td>42.0</td>
<td>55.0</td>
<td>0.04</td>
</tr>
<tr>
<td>RB</td>
<td>84.2</td>
<td>96.5</td>
<td>0.02</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>88.7</td>
<td>93.1</td>
<td>0.37</td>
</tr>
<tr>
<td>RB</td>
<td>122.5</td>
<td>129.7</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2. Main effect contrasts between azoxystrobin-nontreated plants of generation 1 and generation 3 Norkotah Russet and Russet Burbank potato in 2003 and 2004 field trials conducted at the Washington State University station, Othello

<table>
<thead>
<tr>
<th>Year, cultivar</th>
<th>Yield (t/ha)</th>
<th>Disease severity (stem colonization) (%)</th>
<th>Infected progeny tubers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G3</td>
<td>P</td>
</tr>
<tr>
<td>2003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>65.9</td>
<td>42.0</td>
<td>0.0078</td>
</tr>
<tr>
<td>RB</td>
<td>82.0</td>
<td>86.4</td>
<td>0.5</td>
</tr>
<tr>
<td>2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>95.0</td>
<td>82.5</td>
<td>0.02</td>
</tr>
<tr>
<td>RB</td>
<td>122.6</td>
<td>122.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

- **A:** Aboveground and belowground parts of the stem (5 cm each, 12 stems per treatment) were cut into six to eight segments and placed onto potato dextrose agar (PDA; 1 g/liter). The incidence of infected segments was evaluated. Non = nontreated.
- **B:** Total yield of progeny tubers in metric tons per hectare.
- **C:** The stolon end of the tuber (80 tubers per treatment) was cut and placed onto PDA (1 g/liter). The incidence of infected tubers was evaluated.
- **D:** Total yield of progeny tubers in metric tons per hectare.
- **A:** The stolon end of the tuber (80 tubers per treatment) was cut, and placed onto PDA (1 g/liter). The incidence of infected tubers was evaluated.
- **B:** The stolon end of the tuber (80 tubers per treatment) was cut and placed onto PDA (1 g/liter). The incidence of infected tubers was evaluated.
- **C:** The stolon end of the tuber (80 tubers per treatment) was cut, and placed onto PDA (1 g/liter). The incidence of infected tubers was evaluated.
- **D:** The stolon end of the tuber (80 tubers per treatment) was cut, and placed onto PDA (1 g/liter). The incidence of infected tubers was evaluated.
To determine which inoculum source, seed- or soilborne, is more dominant in black dot development and severity, naturally infected certified potato seed genera-
tions differing in incidence of initial inocu-
rum were exposed to soil naturally infested with C. coccodes. Assays to quantify in-
oculum levels of soilborne pathogens, in which soil extracts were plated on agar
media, were found to be inaccurate (22); and molecular-based techniques, such as real-time PCR, although sensitive (5), are unable to distinguish between viable and nonviable inoculum. Furthermore, in the
case of black dot, the relationship between C. coccodes soilborne inoculum threshold
and disease severity is still unknown. Therefore, in order to overcome the soil-
borne inoculum obstacle, we decided to expose the seed tubers to fields with a history of severe black dot that had similar crop rotations (potato was grown every
fourth year). C. coccodes sclerotia were reported as viable up to 8 years (8); therefore,
we assumed similar viable inoculum levels in the soil. Lower yields, higher
disease severity, and a higher incidence of infected progeny tubers were expected on
Generation 3 plants than generation 1 plants due to the higher initial disease
incidence in the mother tubers.

Differences between the seed genera-
tions were recorded for Norkotha Russel in 2003 and 2004. Generation 1 Norkotha
Russel plants, which had lower seedborne infection than generation 3, had higher yields under a similar soilborne inoculum level, pointing to the additive effect of seedborne inoculum, which is in agree-
ment with the results of Dashwood et al. (6). The incidence of infected progeny tubers
was significantly higher for Norko-
thia Russel generation 1 than for generation 3 in 2003 and 2004, which suggested that
progeny tubers developing from less-
fected mother tubers (generation 1) were more prone to infection from soilborne inoculum than those developing from
more-infected mother tubers (generation 3). These results are in agreement with
Read and Hide (19,20) and Danner (7),
who pointed out that soilborne inoculum may be more dominant than seedborne.
However, the results recorded for Russel Burbank did not indicate a different effect
of inoculum source on black dot severity. These results were in agreement with those of Dashwood et al. (6), who did not record a difference in disease severity when plants of cv. Maris Piper were in-
fected from either seed- or soilborne in-
oculum. The inconsistencies obtained among the different studies may be due to
different sampling methodology; how-
ever, it is also probable that the effect of inoculum source on black dot severity is
cultivar specific.

Black dot studies to date evaluated seedborne inoculum based on external skin
blemishes (7,19,20), overlooking internal
infection in the vascular tissues at the stolon end. The russet-skinned potato cultivars
used in the present study were the most commonly produced by the potato industry in
the Columbia basin of Washington State and infrequently demonstrate external skin
blemishes. Therefore, seedborne infection in this study was determined by isolations from
the vascular tissue at the stolon end. Isolation of C. coccodes from the stolon end is a valid way to evaluate the incidence of infected seed tubers. However, it is pos-
sible that latent (14,24) microscopic skin
blemishes, for which no test was done, were present on mother and progeny tu-
bbers. Further research should look into the importance and contribution of the two
types of seedborne inoculum to black dot severity.

In conclusion, azoxystrobin-based fun-
gicides are a promising means for black
dot management. In the future, azox-
ystrobins-based fungicides should be used in combination with practices that mini-
imize plant stress and possibly reduce high inoculum levels in soil. Strategies should
be employed that reduce the risk of select-
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