THE EFFECT OF TEMPERATURE ON RHIZOCTONIA DISEASE DEVELOPMENT AND FUNGICIDE EFFICACY IN CONTROLLING RHIZOCTONIA ROOT ROT ON SUGARBEET

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Rhizoctonia solani is a soil-borne pathogen that causes Rhizoctonia root and crown rot of sugarbeet. The disease occurs world-wide and is the most common root disease of sugarbeet in the United States (11). Strains of the fungus are traditionally grouped into genetically isolated anastomosis groups (AGs) based primarily on hyphal anastomosis reactions, and our further sub-divided into intraspecific groups or ISGs. Although a number of R. solani AGs are able to infect sugarbeet, AG 2-2 IV is considered the primary cause of Rhizoctonia root and crown rot in most sugarbeet growing regions of the USA (6, 10). AG 2-2 IIIB is perhaps better known for causing diseases on mat rush, rice, soybean, and maize (5, 9, 10), but recent studies have shown IIIB to be more aggressive than IV in greenhouse testing of susceptible sugarbeet (7). A recent survey of AG 2-2 IIIB and AG 2-2 IV distribution has shown that both ISGs are found throughout the sugarbeet production areas in the Red River Valley (1).

The use of resistant germplasm is an effective and environmentally friendly way to manage plant disease. However, commercial sugarbeet varieties with resistance to Rhizoctonia crown and root rot have been associated with a significant loss in yield potential or lack resistance characteristics to other diseases (2), underlying the need for an integrated control strategy (3). A single application of azoxystrobin fungicide at the four-leaf stage can offer sufficient disease control in some regions (3), but several applications during sugarbeet development are often necessary in the Red River Valley (12). The application of azoxystrobin more than three day after R. solani infection does not contain the disease. In contrast, azoxystrobin application prior to infection can offer long-term disease protection (3, 12). Taken together, the timing of fungicide application is a critical component in Rhizoctonia root and crown rot prevention. However, such applications may not be necessary if environmental conditions are not conducive for disease development.

OBJECTIVES

The objectives of this study were to 1) determine the temperatures at which R. solani AG 2-2 IIIB infect a susceptible sugarbeet variety under controlled conditions and 2) test the efficacy of three fungicides to control infection by R. solani AG 2-2 IIIB.

MATERIALS AND METHODS

Pelleted sugarbeet seed of the cultivar Beta-1305 (Betaseed, USA) was used in this study. The R. solani AG 2-2 IIIB isolates were isolated from artificially-inoculated sugarbeet plants by placing diseased tissue on potato dextrose agar (PDA) and allowing the fungus to grow onto the media. Pure PDA cultures of IIIB were used to produce inoculum in bulk as described by Kirk et al. (4) except that sterilized barley was used in place of millet seed. Sugarbeet seeds were planted three per 10 cm square plastic pot (T.O. Plastics, Minneapolis, MN) filled with sunshine mix potting soil mix number 1 (Sun Gro Horticulture, Seba Beach, Canada). Pots were placed in a greenhouse with an average day time temperature of 24°C, an average night time temperature of 16°C, and a 16 h photoperiod. After 2.5 wks, plants were thinned to one plant per pot and were subsequently placed in one of four growth chambers (PGR15, Conviron, Canada) all set with a 16 h day/8 h night light regime and a photon flux of 283 μE. Growth chamber temperatures were set for day/night temperatures of 10/4.4°C, 15.6/10°C, 21.1/15.6°C, or 26.7/21.1°C. In all cases, pots were placed in the growth chambers 1.5 days before pathogen inoculations and/or fungicide application to allows soil to adjust to ambient conditions in the growth chamber.

In each growth chamber there were four replications of each treatment (R. solani AG 2-2 IIIB or mock-inoculated) with three pots per replication. For pathogen inoculations, two R. solani-infected barley seeds were placed approximately 1.5 cm below the soil surface on either side of the sugarbeet hypocotyl. Mock-inoculations were the same as above except autoclaved barley seeds were used as inoculum. Pots were watered on an as-needed basis. The entire experiment was repeated once.

Disease progress was determined at three day intervals beginning three days after inoculation (DAI). For disease incidence (DI) ratings, plants were considered diseased if a brownish-black sunken lesion was visible on the crown
area at the soil level, if leaves were wilted, or if the plant was stunted compared to non-inoculated controls. For leaf disease severity (LDS) ratings, the numbers of wilted leaves or blackened petioles were counted on each plant. Plants were considered dead with a LDS of eight. Once a leaf was scored as diseased, it was not considered in subsequent LDS evaluations.

For fungicide application experiments, root disease severity (RDS) was conducted at 14 DAI using the method of (8). Briefly, plants were removed from the pots and gently washed under tap water to remove any remaining soil particles adhering to the roots. Plant roots were then rated using a scale of 0 to 7 where 0 = healthy plants with no visible lesions; 1 = < 1% of the root surface with visible lesions; 2 = 1 to 5% of the root surface with visible lesions; 3 = 5 to 25% of the root surface with dry root canker; 4 = 25 to 50% of the root surface with dry root canker; 5 = 50-75% of the root surface with dry root canker; 6 = 75% of the root surface with dry root canker; 7 = plant is considered dead where leaves have senesced and root is rotten (8).

Since the 26.7/21.1°C growth chamber described above provided conditions that were optimum for \textit{R. solani} AG 2-2 IIIB infection, this temperature was used for all further experiments. There were 4 replications of each treatment (\textit{R. solani} AG 2-2 IIIB with fungicide application, \textit{R. solani} AG 2-2 IIIB without fungicide application (untreated control), or no pathogen with fungicide application (treated control)) with three pots per replication in each growth chamber set at 26.7°C day/21.1°C. Pathogen inoculations were as described above. Fungicides used in this study were an azoxyxstrobim (Quadris®, Syngenta, USA); the triazolinthione class fungicide prothioconazole (Proline®; Bayer Crop Science, USA); and an azole class fungicide containing difenoconazole (Inspire®, Syngenta, USA). Experiments utilized full label rates of azoxyxstrobim (0.672 L ha\(^{-1}\)), prothioconazole (0.365 L ha\(^{-1}\)), and difenoconazole (0.511 L ha\(^{-1}\)). Fungicide treatments were carried out by adding 1.8 ml of fungicide to the soil near the crown of the plant immediately after inoculation. Plants were subsequently put back into growth chambers and monitored for two weeks. The entire experiment was repeated once.

Each treatment (fungicide application or temperature) had four replications and each replicate had three plants. Means for DI, LDS, and RDS were calculated per replicate. Considering each growth chamber as a one block, the means were analyzed by using completely randomized block design with a factorial arrangement using SAS (SAS Institute, Cary, NC) program.

**RESULTS**

**Disease progress of \textit{R. solani} AG 2-2 IIIB.** During the evaluation period, no disease was found on any AG 2-2 IIIB-inoculated plants growing in growth chambers set at 10/4.4°C or 15.6/10°C. However, significant disease occurred in AG 2-2 IIIB-inoculated plants growing in growth chambers at 21.1/15.6°C, or 26.7/21.1°C. By 6 DAI, all AG 2-2 IIIB-inoculated plants growing at 26.7/21.1°C were diseased while AG 2-2 IIIB-inoculated plants growing at 21.1/15.6°C were not all diseased until 12 DAI (Table 1). Although there were significant differences in DI between these temperatures at 3 DAI, there was no significant difference between these temperatures when looking at LDS (data not shown).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Disease Incidence (%)</th>
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<tbody>
<tr>
<td></td>
<td>3 DAI</td>
</tr>
<tr>
<td>21.1</td>
<td>25.0(^a)</td>
</tr>
<tr>
<td>26.7</td>
<td>83.3(^b)</td>
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<tr>
<td>LSD</td>
<td>61.2</td>
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**Fungicide trials.** The application of azoxyxstrobim and prothioconazole at full label rate (0.672 and 0.365 L ha\(^{-1}\), respectively) completely inhibited disease progression during the evaluation period while application of difenoconazole allowed for disease severity similar to untreated controls.

**DISCUSSION**

In this study, we utilized growth chambers set at biologically relevant temperatures to determine the temperatures at which \textit{R. solani} 2-2 IIIB is able to infect sugarbeet. Under the two lowest temperatures tested (10 and 15.6 °C), \textit{R. solani} 2-2 IIIB caused no significant disease (Table 1). However, at the two highest temperatures tested (21.1 and...
26.7 °C), *R. solani* 2-2 IIIB caused significant DS and DI (Table 1). A second focus of this study was to determine whether fungicide application made at the seedling stage protected the plant from infection of *R. solani* 2-2 IIIB. Since we were interested in the efficacy of fungicides when conditions were optimal for fungal growth, fungicide testing was carried out at the 26.7 °C temperature. The application of the azoxystrobin and prothioconazole completely contained fungal growth when applied at the full recommended rate under the conditions tested. Difenconazole at 0.511 L ha⁻¹ (full label rate) was not effective at controlling Rhizoctonia root rot.

**References**