A new root rot of watermelon in Arizona caused by a binucleate *Rhizoctonia* sp. (*Ceratobasidium* sp.)

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Abstract

A new root rot disease of watermelon has been observed sporadically in commercial fields in central Arizona since 2002. Affected plants were often wilted and slower growing than healthy plants. Numerous light brown lesions were observed on all roots. A *Rhizoctonia* species, initially identified by morphological characteristics of hyphae in culture, was isolated consistently from the lesions. Isolates were further identified by DNA sequencing as a species of binucleate *Rhizoctonia* sp. Koch’s postulates were fulfilled and confirmed that the isolates from watermelon were pathogenic, producing lesions similar to those observed on plants in the field. DNA sequencing analysis of isolates from inoculated plants were genetically identical to the original isolate. Although this disease has been documented only occasionally in Arizona, it should be recognized as a potential problem in early season watermelon.

Introduction

It is unusual to have root rot diseases of watermelon in early spring in the low desert agricultural areas of Arizona. From early summer and through the fall when soil temperatures are high, mature watermelons are susceptible to Monosporascus vine decline, and *Pythium aphanidermatum* may cause root rot and collapse, especially in furrow irrigated fields where soils may remain saturated for hours. *Rhizoctonia* root rot is usually not considered a problem, and there are no reports of *Rhizoctonia* root rot of watermelon in the University of Arizona disease databases. With the exception of *Rhizoctonia* damping off of seedlings, to our knowledge *Rhizoctonia* root rot has not been reported as a disease of established vining watermelon plants in the United States. However, a binucleate *Rhizoctonia* sp. was shown to cause root rot on watermelon in Italy where it was isolated among other root rot pathogens from collapsed watermelon plants (Aiello et al., 2012).

In April and May 2002, young wilting watermelon plants with reduced growth occurred sporadically in patches in several fields near Coolidge, Arizona (Fig.1), and symptomatic plants had numerous light brown lesions on roots (Fig. 2). A *Rhizoctonia* species was consistently isolated from lesions, and no other pathogens were observed or isolated. Subsequently, a similar *Rhizoctonia* species was isolated from symptomatic plants in watermelon fields near Eloy, AZ in 2006, 2008, 2010 and in Litchfield, AZ and Goodyear, AZ in 2011. Because of the sporadic and unusual occurrence of this disease, laboratory studies were conducted to determine the identity of the *Rhizoctonia* species isolated and to confirm its pathogenicity.
Materials and Methods

Isolation and morphological identification. Roots of symptomatic watermelon plants were washed under running tap water for at least 15 minutes. Sections of roots with brown lesions were cut aseptically to about one cm, placed in water or 5% bleach solution up to 1 min, and rinsed in sterile tap water. Sections were cut from the leading margin of lesions, placed on 1.5% water agar and incubated at 25°C. Within 24-48 hr, emerging hyphae were identified as a *Rhizoctonia* species by their growth pattern, hyphal size, hyphal branching and septation (Sneh, et al., 1991). Isolates were transferred to ¼ strength potato dextrose agar (PDA) and incubated at 25°C.

Hyphal anastomosis was used to find relatedness of two isolates from different locations, Rh1534 from Eloy and Rh1542 from Litchfield (Table 1). A known isolate of *Thanatephorus cucumeris* (*Rhizoctonia solani*) from diseased cotton seedlings in a field near the Eloy watermelon field was used as a tester strain (Rh1549). It was previously isolated and identified by DNA sequencing (Table 2). Using the slide technique of Kronland and Stanghellini (1988), isolates Rh1534 and Rh1542 anastomosed with themselves and with each other but not with isolate Rh1549.

The nuclear condition of the watermelon isolates Rh1534 and Rh1542, and the cotton isolate Rh1549 was determined microscopically using the staining technique of Bandoni (1979). Hyphae from 3 day old ¼ PDA cultures were placed in 0.5-1.0% Safranin O stain solution on a glass slide and a cover slip applied immediately. Hyphae were observed under 400X and/or 1000X magnification, and the number of nuclei counted in at least 10 cells per isolate.

Pathogenicity. Watermelon ‘Crimson Sweet’ seed was germinated and grown to 1-2- true leaf stage in a commercial pasteurized potting mix in seedling trays. For pathogenicity trials, seedlings were transplanted individually into 6 inch pots in a mixture of 50% pasteurized field soil, 25% perlite and 25% vermiculate. At transplanting, soil was infested with isolates Rh1534 and Rh1542 that had been grown for 3 weeks on sterile barley in flasks in the laboratory. About 10 g colonized seed was used as inoculum and mixed into soil in each of 5 pots. Sterile barley seed or no barley seed were used in 5 pots each as controls. Plants were grown for three weeks or until symptoms were observed. Roots were removed from pots and washed for 15 min in running tap water; lesions were assayed by plating on 1.5% water agar. Emerging hyphae were transferred to ¼ PDA and potato dextrose broth and incubated for 3-5 days. Hyphal morphology and DNA sequencing were used to verify identity of the isolates used for inoculation.

Identification by DNA sequencing. For sequencing analyses, pure cultures from symptomatic watermelon in Litchfield (Rh1542) and Eloy (Rh1534, Rh1534Eloy1 and Rh1534Eloy2) were grown in ¼ strength potato dextrose broth (PDB) liquid culture at room temperature for a week, and DNA was extracted from mycelia using the FastDNA extraction kit (MP Biomedicals, Solon, OH). Polymerase chain reaction (PCR) amplification was performed in Bio-Rad DNA Engine thermocycler (Bio-Rad Laboratories, Hercules, CA). PCR was conducted using the QIAGEN Taq PCR Core kit (QIAGEN, Valencia, CA). The reaction mixture per 50 μl reaction was: 5 μl 10x PCR buffer, 5 μl Q-solution, 1 μl dNTPs, 1 μl ITS 4 (100 pmol/μl), 1 μl ITS 5 (100 pmol/μl) (White et al. 1990), 2 μl DNA template (2 ng/μl) and 0.25 μl Taq polymerase. Nuclease-free water was added to bring the final reaction volume to 50 μl. The thermocycler program was: 94°C for 3 min, followed by 40 cycles: 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with final extension at 72°C for 10 min. The PCR products were separated on 1% agarose gel by electrophoresis, stained with ethidium bromide and visualized under UV trans-illuminator. The PCR products were cleaned using exonuclease I and rAPid (alkaline phosphatase) by following the manufacturer protocol (USB products, Cleveland, Ohio). The cleaned PCR products were sequenced in both directions at the University of Arizona Genetics Core facility. The sequences were queried against the NCBI GenBank sequences using blastn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the top two matches are listed in Table 2. The reference sequence was deposited in the NCBI (accession number: KC140583). The NCBI query search revealed the isolates from watermelon were *Ceratobasidium* sp.

*Ceratobasidium* spp. are considered the teleomorph stage of binucleate *Rhizoctonia* species (Sneh, et al., 1991; Yang and Li, 2012). Therefore, to further confirm the binucleate nature of the *Ceratobasidium* sp. (*binucleate Rhizoctonia* species) from watermelon, we performed two additional PCRs; one with primer specific for binucleate pathogenic *Rhizoctonia* sp. (Cubeta et. al., 1991) and another one with primer pair specific for binucleate non-pathogenic
Rhizoctonia sp. (Potvin et al., 1999). The PCR conditions were followed as described in the reference. The PCR products were separated on 1% agarose gel by electrophoresis and stained with ethidium bromide to confirm the specific amplification under UV-trans-illuminator.

Results and Discussion

Rhizoctonia sp. was consistently isolated from young watermelon plants showing root rot in Arizona. In pathogenicity trials isolates Rh1534 and Rh1542 caused light brown lesions on main roots and numerous secondary roots. No lesions developed on control plants with or without sterile seed. A Rhizoctonia sp. was isolated from at least one lesion on each of the five inoculated plants. They were identified further by DNA sequencing to be 100% match to isolates Rh1534 and Rh1542 used for inoculations. All isolates were confirmed to be binucleate by hyphal staining as described previously. In cells where nuclei were distinct, there were always 2 nuclei in Rh1534 and Rh1542 while the Rh1549 from cotton, a known Thanatephorus cucumeris isolate used for comparison, consistently contained 3-5 nuclei.

Using the BLAST database of NCBI, the ITS (internal transcribed spacer) sequences of watermelon isolates were all 99-99.8% identical to sequences of Ceratobasidium sp. (Table 2). PCR amplification with the primer pair specific for identification of pathogenic binucleate Rhizoctonia sp. produced the expected 1.8 kb band (Fig 4) for all four isolates tested. On the contrary, there was no amplification with primers specific for binucleate non-pathogenic Rhizoctonia sp. (data not shown). Taken together these results suggested that the isolates from watermelon were binucleate and pathogenic.

Arizona isolates are closest to Ceratobasidium AG-F and Ceratobasidium AG-Fa, both reported from strawberry (Sharon, et al., 2007). Recent findings from Aiello, et al., 2012 show that a root rot of watermelon in Italy is caused by binucleate Rhizoctonia AG-F. They report a binucleate Rhizoctonia causing rot of secondary and feeder roots of watermelon that was 99% homologous to BNR AG-F isolate (accession no. AB 219144), one of the isolates reported from diseased strawberry in Israel (Sharon, et al., 2007). Arizona isolate Rh1534 was 99.8% homologous to the same BNR isolate, so it was concluded that the binucleate Rhizoctonia reported to cause root rot of watermelon in Italy is identical to the isolates in Arizona.

To our knowledge, no Ceratobasidium species has been previously reported as a pathogen of watermelon in the United States. The first observation of this disease on watermelon was in May 2002 near Coolidge, Arizona. The field was irrigated by buried drip and had been minimally tilled after barley. In other occurrences in Eloy and Litchfield, the fields also were irrigated by buried drip but were conventionally tilled and rotated from cotton or potatoes. Pollinator and seedless varieties in the same areas of any one field were symptomatic. This disease has been observed sporadically in different locations over the past 10 years in Arizona, and its impact on yield is unknown. In most cases, plants either remained smaller but with no further symptom development or they recovered as temperatures increased in late May and early June. However severe losses were reported in Italy from genetically identical isolates, increasing concern about impact of the disease in Arizona. Because of the recent reports in Italy and confirmed incidences in major watermelon production areas in Arizona, this disease should be recognized as a potential problem in watermelon.

Literature cited


Table 1. Identifier, host, location and year of isolation of *Rhizoctonia* isolates used in this study.

<table>
<thead>
<tr>
<th>Isolate identifier</th>
<th>Host</th>
<th>Location and year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh1534Eloy1</td>
<td>Watermelon</td>
<td>Eloy, AZ, 2008</td>
</tr>
<tr>
<td>Rh1534Eloy2</td>
<td>Watermelon</td>
<td>Eloy, AZ, 2010</td>
</tr>
<tr>
<td>Rh1534</td>
<td>Watermelon</td>
<td>Eloy, AZ, 2006</td>
</tr>
<tr>
<td>Rh1542</td>
<td>Watermelon</td>
<td>Litchfield, AZ, 2011</td>
</tr>
<tr>
<td>Rh1549</td>
<td>Cotton</td>
<td>Eloy, AZ, 2011</td>
</tr>
</tbody>
</table>

Table 2. GenBank accession numbers for ITS regions and their corresponding top blastn matches in GenBank, NCBI, for isolates used in this study.

<table>
<thead>
<tr>
<th>Isolate identifier</th>
<th>GenBank accession number</th>
<th>Genus</th>
<th>Top blastn match in GenBank</th>
<th>% identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh1534Eloy1</td>
<td>KC140583a</td>
<td><em>Rhizoctonia</em> sp. AG-Fa</td>
<td>AB219144.1</td>
<td>99.8</td>
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<tr>
<td>Rh1534Eloy2</td>
<td></td>
<td><em>Ceratobasidium</em> sp. AG-F</td>
<td>DQ102422.1</td>
<td>99.0</td>
</tr>
<tr>
<td>Rh1534</td>
<td>KC140584</td>
<td><em>Thanatephorus cucumeris</em> isolate CR-2</td>
<td>JF699277.1</td>
<td>99.0</td>
</tr>
<tr>
<td>Rh1542</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh1549</td>
<td></td>
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</tbody>
</table>

* Isolates Rh1534, Rh1534Eloy1, Rh1534Eloy2 and Rh1542 are 100% sequentially identical, and only the representative sequence has been submitted to GenBank.
Figure 1. Plant symptoms in field. Infected plants are smaller and wilting at mid-day.
Figure 2. Roots with light brown lesions over main and secondary roots.

Figures 3A and 3B. Nuclear staining of binucleate isolate Rh1534 from watermelon (A) and of multinucleate isolate Rh1549 from cotton (B).
Figure 4. Polymerase chain reaction amplification (PCR) based confirmation of binucleate pathogenic *Rhizoctonia* sp. from watermelon. PCR was performed with primer specific for binucleate pathogenic *Rhizoctonia* sp. On the gel from left to right: 1. 1kb-DNA marker, 2. Rh1534, 3. Rh1534Eloy1, 4. Rh1534Eloy2, 5. Rh1542, and 6. Negative water control.