A NEW OCCURRENCE OF DRY ROT CANKER?

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ABSTRACT

In mid-September, 2011 a field in Morrill County near Bridgeport NE was noted with wilting and yellowing symptoms suggestive of Rhizoctonia root rot. Root symptoms consisted of localized, dry sunken lesions covering brown spongy material penetrating deeply into taproots sharply delimited from healthy beet tissue. The surface tissues of the cankers produced a series of concentric circles. These symptoms are inconsistent with Rhizoctonia root and crown rot disease, but are suggestive of the rarely occurring dry rot canker (DRC). DRC is root disease first identified from Utah in 1921, and has since been reported from California, Colorado, Minnesota, Montana, Nebraska, North Dakota, and Wyoming. The disease is caused by an uncharacterized strain of *Rhizoctonia solani*, but little else is known about the pathogen or disease due to its rare appearances. To initiate characterization of the pathogen, the internal transcribed spacer (ITS) was sequenced from four strains isolated from separate DRC lesions. Sequence analysis suggests that these isolates are distinct from *R. solani* anastomosis groups (AGs) that typically cause disease in sugar beet.

Introduction

In 1921, B. L. Richards with the Utah Agricultural Experiment Station reported an apparently previously undescribed root disease of sugar beet, caused by *Rhizoctonia solani*, calling it dry rot canker (DRC) (3). He first observed the disease near Cornish, Utah in August, 1920. Another similar sugar beet root disease was then reported widely distributed throughout Minnesota and Colorado in the summers of 1936-1938 (2). It has since been additionally reported from California, Montana, Nebraska, North Dakota, and Wyoming (6).

Symptoms

Foliar symptoms were suggestive of Rhizoctonia root and crown rot consisting of yellowing and abnormal wilting in the day with partial recovery at night. However, root symptoms were different from those normally associated with root and crown rot, characterized by localized, dry sunken lesions scattered over the root surface (2,3,6). The surface tissues of the cankers also produce a distinctive series of concentric circles, like a target board.

Richards also described another distinctive feature of the disease that further distinguished it from crown rot. The pathogen infected plants underground through the roots and spread upward. As infection progressed, the rot beneath the lesions penetrated deeply into the interior of the taproot, causing the decaying tissue to rapidly dry out as infection continued inward (3). This activity left a cavity filled with a dry pithy material consisting of both fungal hyphae and decayed host materials. The contents could then be removed from the cavity of the canker like a plug (3).

Richards mentioned that cracks frequently merged, resulting in large fissures which in severe cases could achieve lengths of 2.5 to 3 inches within lesions. As canker numbers increased on the root surface, the root became dry, brittle and completely rotted through the entire taproot, thereby destroying the entire root and crown. This resulted in a dry rot of the beet thus illustrating the origin of the disease name (3).

Little else is known about this form of *R. solani*, or its root rot, primarily due to its rare appearances. However, it was recently identified from Nebraska again in 2011, and all the symptoms previously described by Richards and LeClerg (2, 3) were additionally observed from this recent Nebraska outbreak. We began this study in 2012 to preliminarily characterize several presumed dry canker isolates in comparison with "typical" crown rot isolates using both traditional biological and molecular methods.

Materials and Methods

Radial growth of 4 DRC isolates was compared to 4 crown rot isolates on one half strength potato dextrose agar (PDA) at 10, 15, 20, 25, 30, and 35C. Further comparisons were made with radial growth of the same 8 isolates on water agar at room temperature (28-30 C). Greenhouse-grown sugar beet plants of two ages (2 months and 1 month) were each inoculated by placing three mycelial plugs (8 mm diameter) taken from the leading edge of one half strength PDA plates on the surface of each pot from 12 isolates (6 DRC and 6 crown rot). The nuclear condition for both DRC and crown rot isolates was then evaluated as described by Burpee et al. (1). Briefly, a drop of lactophenol blue placed on the edge of a 60 hour-old culture of DRC and crown rot isolates, each grown on water agar and viewed under the microscope.

Liquid cultures were initiated by placing an agar plug taken from the leading edge of one half strength PDA plates into a 250 ml flask containing 50 ml potato dextrose broth. After 7 day's incubation, mycelia were harvested using a Büchner funnel and DNA was isolated using the CTAB method (4). Amplification of the ribosomal DNA internal transcribed spacer (ITS) region was achieved using the ITS1 and ITS4 primers (5) using standard PCR conditions. PCR amplicons were sequenced (MCLAB, South San Francisco, CA, USA). Consensus sequences were aligned with ClustalW and analyzed using Vector NTI software (Invitrogen, Carlsbad, CA, USA). Sequences were subjected to BLASTn and bl2seq analyses at NCBI.

Results

Very few differences in radial growth were observed on PDA, however growth of all isolates were optimal at 25-30 C (Table 1). Additionally 2 of the four suspected DRC isolates (409 and 410) were inhibited at the two extreme temperatures utilized (10 and 35 C) compared to the crown rot isolates. We cannot explain why the other 2 DRC isolates (411 and 412) grew so much better at 35 C than even the crown ort isolates (Table 1), but this will be repeated for further confirmation. Furthermore, growth of dry rot canker isolates on water agar at 30 C after 48 hours was substantially less than the growth of crown rot isolates (Figure 1). Binucleate cells were observed from the suspected DRC isolates while only multi-nucleate cells were seen from the crown rot isoaltes. Koch's postulates were completed with both groups of isolates. The crown rot isolates were appeared to be more virulent, killing higher numbers of plants than the DRC, particularly in the younger plants (one month-old). Few of the DRC-inoculated plants died, but wilting and root lesions were observed on sugar beet plants.

The ITS sequences from all isolates were identical. The derived sequence had significant homology (*E*-value = 0.0, 96% identity) to ITS sequences from *Rhizoctonia solani* AG-Fb (a binucleate *R. solani*).

Discussion

In mid-September, 2011 a field near Bridgeport, in Morrill County, NE was observed with numerous plants (10-15%) exhibiting wilting and yellowing symptoms suggestive of Rhizoctonia root rot. However, resulting examination of roots found lesions inconsistent with disease caused by root and crown rot (6). *Rhizoctonia*-like isolates were readily recovered from all parts of lesions distributed throughout taproots.

The original investigators suspected that the *Rhizoctonia* isolates they found inducing the dry rot canker disease were different than typical *R. solani* isolates based on different symptoms (2,3). This was a prescient observation, being proposed long before the availability of our current molecular tools. Our preliminary data confirms those suspicions and also strongly suggests the identity of a new binucleate *Rhizoctonia* species, pathogenic on sugar beets.

Richards further mentions that he learned through correspondence with G.L. Peltier that a similar disease was also observed from Nebraska in 1920 (3). Furthermore, based on preserved herbarium specimens, the disease was known to be present in Utah as early as 1915. To our knowledge, this brief communication represents the first new report of DRC on sugar beets in 75 years. Based on different symptoms, and biological and pathogenicity characteristics compared with crown rot isolates these isolates may warrant a new species assignment to the DRC pathogen.

Literature Cited

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Table 1. Radial growth of *R. solani* isolates on ½ PDA after 96 hours.

Temperature (centigrade)						
Isolate*	*10*	*15*	*20*	*25*	*30*	*35*
409	**15.1**	**34.1**	**74.8**	**88**	**88**	**30**
410	**15.9**	**34.3**	**71.3**	**80**	**80**	**36.3**
411	**16.5**	**31.8**	**67.3**	**86**	**88**	**70.8**
412	**11.5**	**26.3**	**57.3**	**81.3**	**88**	**675**
276	**20.3**	**37.8**	**71.8**	**86.8**	**86.3**	**61.5**
69	**21.4**	**43.5**	**81.8**	**88**	**86.8**	**56.5**
67	**19.5**	**43.5**	**81**	**88**	**88**	**62**
420	**26.3**	**54.5**	**88**	**88**	**88**	**50**

*Isolates 410-412 = DRC isolates
Isolates 276, 69, 67, and 420 = crown rot isolates

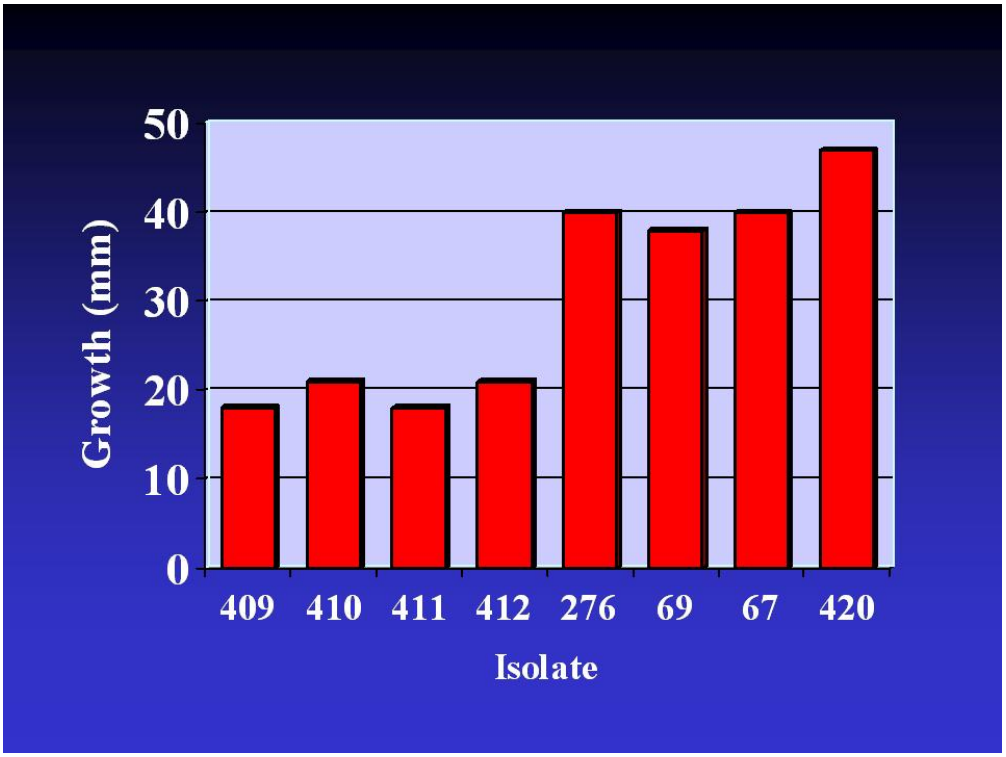

Figure 1. Radial growth (mm) of 4 DRC isolates (409-412) and 4 crown rot isolates (276, 69, 67, and 420) for 48 hours at room temperature on water agar.