CONTINUING STUDIES ON DRY ROT CANKER DISEASE OF SUGAR BEET

Robert M. Harveson[1] and Melvin D. Bolton[2]
Panhandle Research and Extension Center, [1]Univeristy of Nebraska, 4502 Ave I, Scottsbluff, NE 69361 and [2] USDA-ARS, Northern Crop Science Laboratory, 1307 18TH St N Fargo, ND, 58102

Abstract

Dry rot canker (DRC) is a root disease of sugar beet that was first identified from Utah in 1921, and since that time has been identified from California, Colorado, Minnesota, Montana, Nebraska, North Dakota, and Wyoming. Until recently, the disease was thought to be caused by a strain of *Rhizoctonia solani*, causal agent of the universally familiar Rhizoctonia root and crown rot (RRCR) disease. DRC has now been proven by sequence analysis of the internal transcribed spacer region to be initiated by a binucleate species of *Rhizoctonia*, anastomosis group (AG) F. Foliar symptoms of DRC are similar to those of RRCR, consisting of yellowing and wilting. However, root symptoms are distinct and serve as the major method for distinguishing between the two diseases. Lesions on roots are dry, sunken, and circular to oblong in shape. Beneath surface lesions is a brown spongy material that penetrates deeply into taproots and is sharply demarcated from healthy tissue. The root lesions additionally produced a distinctive series of concentric circles. Further biological and molecular analyses of isolates infesting more than a dozen fields throughout western Nebraska suggest that DRC is a distinct disease caused by a pathogen unmistakably divergent from *R. solani*. Historically, this disease has been considered to be very rare in occurrence however our continuing investigations are revealing a more prevalent presence in sugar beet production than previously thought.

Disease History and Background

Introduction

In August 1920, B. L. Richards with the Utah Agricultural Experiment Station, first observed a previously undescribed root disease of sugar beet near Cornish, Utah (3). Another similar sugar beet root disease was then reported widely distributed throughout Minnesota and Colorado in the summers of 1936-1938 (2), and has since been additionally reported from several western and central U.S. sugar beet-growing states, including California, Montana, Nebraska, North Dakota, and Wyoming (6).

Disease Symptoms

Foliar symptoms are somewhat suggestive of Rhizoctonia root and crown rot consisting of yellowing and abnormal wilting. However, root symptoms were quite different from those normally associated with root and crown rot, characterized by localized, dry sunken lesions scattered over the root surface that sometimes coalesced to form large rotted areas (2,3,6). The

surface tissues of the cankers also produced a distinctive series of concentric circles, like a target board.

Another distinctive feature of the disease that further distinguished it from crown rot is the mechanism of infection. 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 dry out as infection continued inward (3). This activity left cavities filled with a dry pithy material consisting of both fungal hyphae and decayed host materials (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, illustrating the origin of the disease name (3).

Little else is known about the pathogen or this form of root rot, primarily due to its rare appearances. However, it has been recently identified from Nebraska in three of the last four years (2011-2014), based on the symptoms previously described by Richards and LeClerg (2,3). 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 multiple DRC isolates (4) obtained from multiple fields over this four-year period was compared to two known root and crown rot isolates (*R. solani*) on one half strength potato dextrose agar (PDA). A single 8mm mycelial plug taken from 48 hour-old cultures, placed in the center of each plate and incubated at 6 temperatures: 10, 15, 20, 25, 30, and 35°C. Further comparisons were made with radial growth of the same 4 DRC and 2 crown rot isolates on water agar after 48 at room temperature.

Sugar beet seedlings (3 days post-emergence) were inoculated by placing three mycelial plugs from representatives of both isolate types using an 8 mm diameter cork borer on the soil surface of each pot. Greenhouse-grown plants of two ages (2 months and 1 month) were additionally inoculated by the same method.

After 7 day's growth in potato dextrose broth, DNA was isolated from mycelia using the CTAB method (4). Amplification of the ribosomal DNA internal transcribed spacer (ITS) region was achieved with the ITS1 and ITS4 primers (5) using standard PCR conditions. Afterward, amplicons were sequenced, aligned, and analyzed using Vector NTI software (Invitrogen, Carlsbad, CA, USA). Sequences were then subjected to BLASTn and bl2seq analyses at NCBI.

Results

No major differences were observed among both types of isolates in terms of radial growth on PDA. It was determined that the growth of all isolates were optimal at 25-30° C. This same relationship has been noted for all isolates tested over the last two years, including 8 DRC isolates and 6 *R. solani* isolates. Surprisingly, we obtained different results utilizing water agar incubated at room temperature. Radial mycelial growth of dry rot canker isolates on water agar at 25° C after 48 hours was significantly less than the growth of root and crown rot isolates (Figure 1).

Koch's postulates were completed with both groups of isolates for both seedling and young roots, providing evidence of pathogencity for all isolates. However, the crown rot isolates, in general, were more virulent on the one and two month-old plants. They were more likely to kill some plants while causing more severe lesions (larger and more destructive) than the DRC isolates. Conversely, two specific DRC isolates (410 and 2013-8) were more aggressive than the two crown rot isolates (RZ1 and RZ69) by causing seedling disease and mortality within 5 days after inoculation. In comparison, it was 8 days before damping-off and death of seedlings occurred with the two *R. solani* isolates.

The ITS sequences from all suspected tested DRC isolates to date were identical. The derived sequence had significant homology (*E*-value = 0.0, 96% identity) to ITS sequences from a binucleate *Rhizoctonia* spp. (AG-Fb) (1). Thus we have provided very strong evidence to show that these isolates are quite different from the "typical" *R. solani* isolates causing root and crown rot, causing a distinct root disease of sugar beets.

Discussion

In mid-September of 2011, 2013, and 2014, we observed with numerous plants exhibiting wilting and yellowing symptoms somewhat reminiscent of Rhizoctonia root and crown rot from more than a dozen fields throughout Morrill and Scotts Bluff Counties, NE. However, resulting examination of roots found distinct lesions inconsistent with disease caused by root and crown rot (1,6). *Rhizoctonia*-like isolates were readily recovered from all parts of lesions distributed throughout taproots.

Incidence of symptomatic plants varied (estimated at 10 to 15% in some fields), but affected plants were found occurring in definitive clusters, distributed randomly throughout fields. Furthermore, it was also noted that DRC-infected roots were easily removed from soil with little resistance and foliage intact, as opposed to *Rhizoctonia*-infected roots that are still often difficult to remove from soil without digging.

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). Our recent findings from multiple isolates confirm those suspicions and indicate that the DRC isolates are genetically distinct, binucleate *Rhizoctonia* species that are additionally pathogenic on sugar beets (1). It has also become evident that the appearance and distribution of this disease is more than just an isolated incident from one field (as assumed after the initial discovery in 2011). We have now identified and collected more than thirty isolates from

multiple fields (12+) within Nebraska from three of the last four seasons. These findings now warrant continued studies of these pathogenic isolates genetically and biologically to establish their relationships with disease-resistant cultivars and sensitivity to currently available fungicides that are being successfully utilized for managing Rhizoctonia root and crown rot.

Literature Cited

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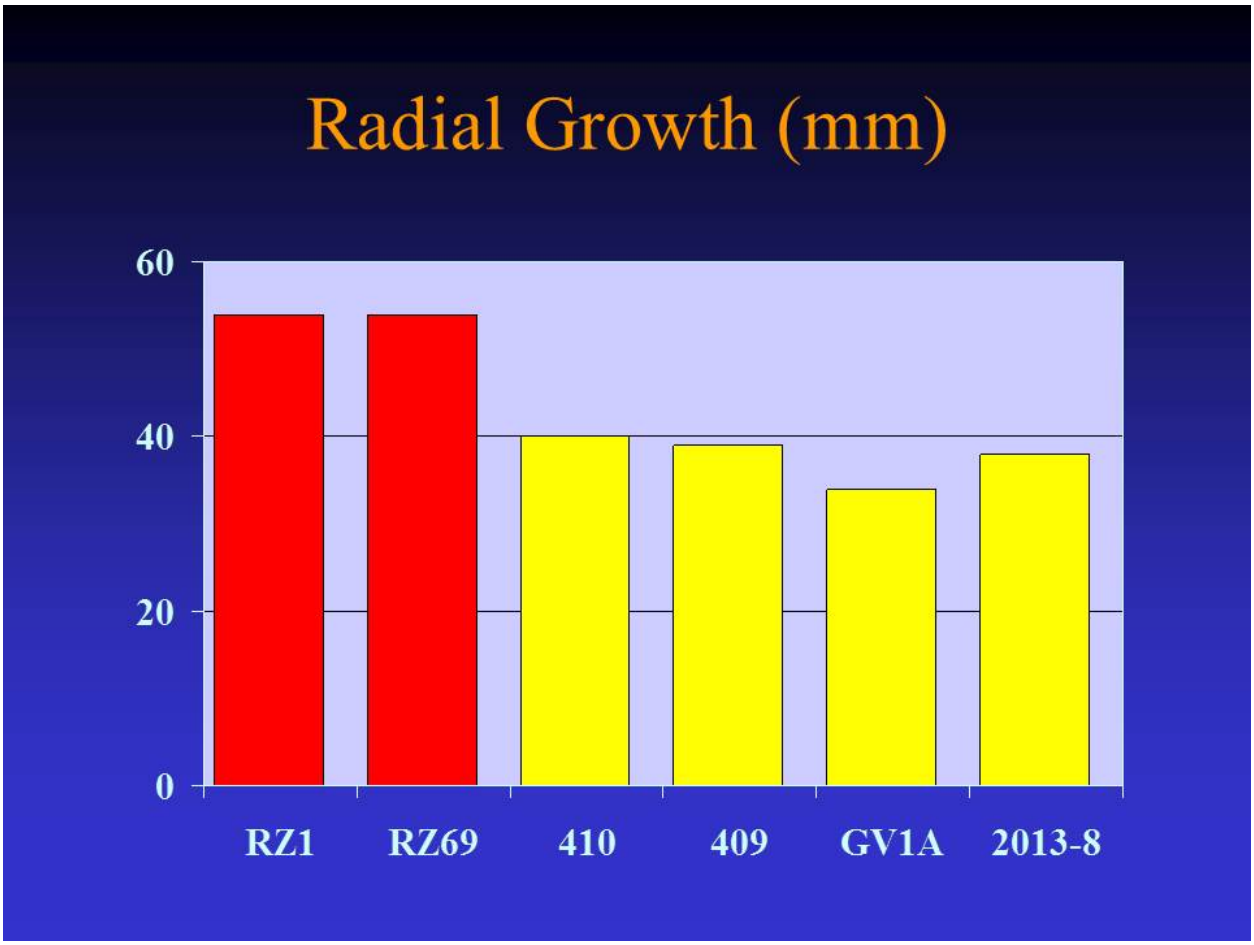

Figure 1. Graph demonstrating the radial growth of DRC (yellow bars) and *R. solani* (red bars) isolates at room temperature after 48 hours. The DRC isolates were statistically slower in growth than the *R. solani* isolates after this period.