Leptosphaerulina Leaf Blight of Turfgrasses... Lion or Lamb?

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Leptosphaerulina spp. incite several diseases of legumes and are often found colonizing necrotic creeping bentgrass, perennial ryegrass, and Kentucky bluegrass plants during humid periods of the late spring through fall. The fungus produces conspicuous fruiting bodies (pseudothecia) which release spores in packets referred to as asci (Figures 1 and 2). Controversy exists regarding the ecological relationship between *Leptosphaerulina* and the turfgrass hosts. The fungus has been classified as a saprophyte (colonizes only dead tissue), senectophyte (colonizes dying or senescent tissue), weak pathogen, and pathogen of turfgrasses by various plant pathologists. To complicate matters, there have been three differing classification schemes published in the literature for determining the species within the genus. Because of the frequency with which *Leptosphaerulina* spp. are found on turfgrasses and because of the uncertainty regarding the virulence and classification of the fungus on turfgrasses, this disease was chosen for my Masters Degree project at Virginia Tech (Abler, 2003).

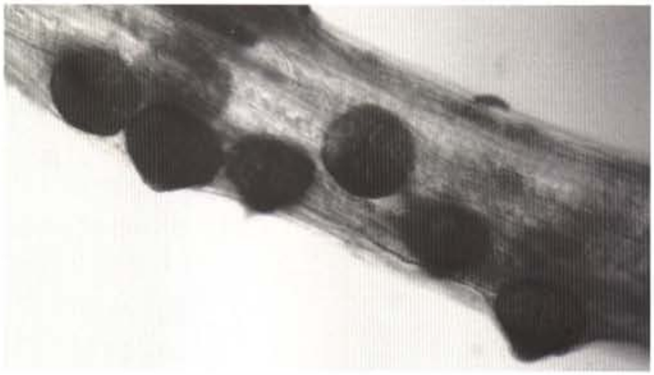

Figure 1. Pseudothecia of *Leptosphaerulina australis* on a necrotic creeping bentgrass leaf

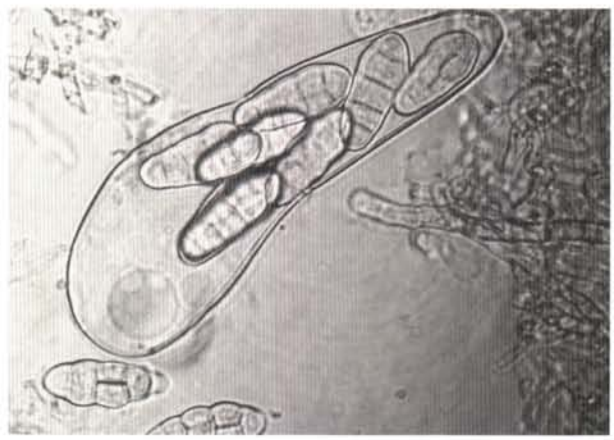

Figure 2. Spores and ascus (pl. asci) of *Leptosphaerulina australis*

Delineation of *Leptosphaerulina* species

The previous three descriptions for the classification of species in the genus were based mostly on the size and septation of spores. The most thorough work was performed by Graham and Luttrell (1961), who in addition to spore morphology, described ecology, host specificity, and growth characteristics on artificial media of a number of isolates. They described six species of *Leptosphaerulina* that occur on forage plants. The number of species in the genus was later modified to two by Booth and Pirozynski (1967) and back to four by Irwin and Davis (1985) on the basis of spore morphology alone. Because the delineation of the species was so controversial when based on spore characteristics, more recent molecular approaches and spore morphology were utilized for this study.

As many samples of *Leptosphaerulina* as possible were collected from turfgrasses and other hosts (including original isolates and herbarium material from previous taxonomic studies). Isolates were characterized based on spore morphology and placed into categories based on the six species key of Graham and Luttrell (1961). DNA was extracted from the samples and two different pieces of the fungal genome were amplified and sequenced using the polymerase chain reaction. By using powerful computer software, the sequences of the DNA were compared and the evolutionary relatedness of each isolate to each other was determined. When the analyses were completed, the molecular and morphological data best supported the six species classification of Graham and Luttrell (1961). Therefore, the species of *Leptosphaerulina* that occur on turfgrasses include *L. australis* (most common) and *L. argentinensis*. Once it was determined which species of *Leptosphaerulina* are found on turfgrasses, experiments to determine their level of virulence could be performed.

Figure 3. Germinated spore with appressoria (rounded tip on lowest germ tube) on a creeping bentgrass leaf

Virulence of *Leptosphaerulina* to turfgrasses

To test the virulence of *Leptosphaerulina* to turfgrasses, healthy and stressed perennial ryegrass (cultivars Palmer III and Fiesta II) and creeping bentgrass (cultivar Crenshaw) were inoculated with six turfgrass isolates of *L. australis*. In order to stress the plants and induce senescence, perennial ryegrass plants and creeping bentgrass plants were incubated at 38°C (100°F) with 100% relative humidity in the dark for 48 and 24 hours respectively. These plants were then placed back in the greenhouse for three days before inoculation. The senescent status of the leaves was measured and verified by the significant reduction in the chlorophyll content of the leaves at time of inoculation. After inoculation, both healthy (unstressed) plants and stressed plants were incubated under humidity domes for three days at 22°C (72°F), the optimum temperature for infection and colonization of legume hosts by *Leptosphaerulina* spp. The plants were inspected for symptoms daily for eight days following the inoculation.

None of the inoculated plants showed any noticeable symptoms of disease at any time during six inoculation experiments. Even the stressed plants which showed mean chlorophyll losses of 30-45% were indistinguishable from control plants. Leaves of inoculated plants were cleared and stained to verify the presence of spores on the leaves. Multiple spores were observed on each leaf, many of which germinated and produced germ tubes and appressoria (specialized structures for penetrating the epidermal cells of plants). These appressoria failed to penetrate the epidermis of the plant (Figure 3). Since *L. australis* was unable to infect and colonize non-stressed and stressed turfgrasses under conditions favorable for disease development on legume hosts, it was determined that *Leptosphaerulina* species are saprophytes of necrotic turfgrasses. The practical implication of these results is that when *Leptosphaerulina* is found on dead turfgrasses, the turfgrass manager must determine the primary cause of the declining turfgrass which could be a multitude of biotic and/or abiotic factors. Determining this underlying cause of the problem may be complicated by the ability of *Leptosphaerulina* spp. to rapidly colonize the moribund turfgrasses.

Although *Leptosphaerulina* leaf blight is no longer even considered a pathogen of stressed grasses, several turfgrass diseases only appear when abiotic stresses predispose the plants to infection and colonization by fungi that are unable to harm healthy plants. Therefore, it is important for superintendents as well as researchers to use sound cultural techniques in order to maintain healthy turf and reduce the possibility of problems from these stress-induced pathogens.

References

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