Colonization of unwounded and wounded creeping bentgrass (*Agrostis stolonifera*) by virulent and hypovirulent isolates of *Sclerotinia homoeocarpa*

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INTRODUCTION

Dollar spot, caused by *Sclerotinia homoeocarpa* (F.T. Bennett), is the most common disease of turf grass (Walsh *et al*., 1999). Symptoms of dollar spot disease include straw-coloured patches about 2 cm in diameter. Previous studies describe infection by *S. homoeocarpa* including the formation of appressoria and direct hyphal penetration through wounds and stomata (Endo, 1966). However, a detailed description of *S. homoeocarpa* colonization of leaf tissue is still required.

Hypovirulent isolates of *S. homoeocarpa* have been shown to reduce dollar spot symptoms by up to 80% and 58% on artificially and naturally infested field plots, respectively (Zhou and Boland, 1998). Hypovirulent isolates contain *Ophiostoma Mitovirus 3a* (OMV3a).

The objectives of this study were to provide a detailed description of the infection and colonization process of *S. homoeocarpa* and to compare the growth of virulent and hypovirulent isolates on creeping bentgra*ss* to determine the impact of OMV3a on *S. homoeocarpa* disease processes.

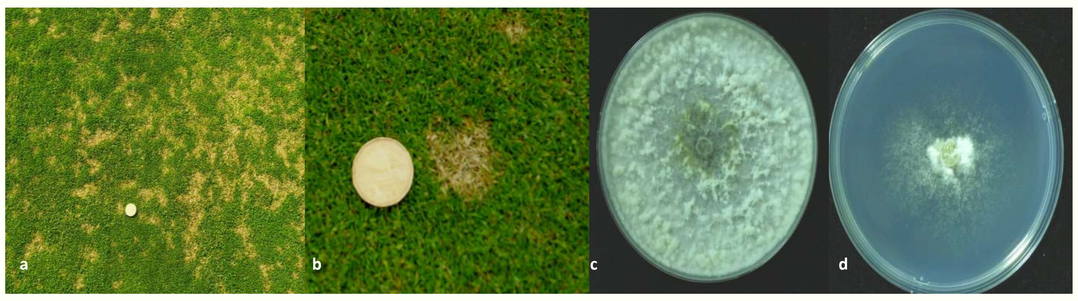

Figure 1: Straw-colored patches of dollar spot disease at the Guelph Turfgrass Institute, Ontario, Canada (a, b). Virulent (c) and hypovirulent (d) isolates of *S. homoeocarpa*

MATERIALS & METHODS

Virulent and hypovirulent isolates used in this study were Sh80 and Sh12B, respectively. Mycelial plugs from active culture were used to inoculate deteached creeping bentgrass leaves. Noninoculated leaves were incubated under the same conditions and remained green and healthy in appearance for over 200 h. All experiments were conducted 3 times.

Inoculation of non-wounded leaf tissue: Leaves were cut to 6 cm in length and the culture plug was placed in the centre of the leaf. Leaves were incubated on moistened filter paper for 48, 96, 144, 192, and 240 h.

Inoculation of wounded leaf blades: Leaves were cut to 4 cm and the cut end of the leaf was put in contact with the culture plug. Leaves were incubated for 8, 16, 24, 32, 40, 48, 56, 64, 72, 84, 96, 108, 120 h. Additional hypovirulent samples were incubated for 132, 144, 156, and 168 h.

Microscopic Observation: Leaves were cleared in a solution of 25 % acetic acid and 75 % ethanol for 48 – 72 h and washed in water for 24 h. Samples were stained with 0.05 % trypan blue in water for 24 h, destained in water for 24 h, and placed in 100 % glycerol overnight prior to mounting the leaves in glycerol on glass slides. Additional leaf samples were cleared and stained with a solution of 0.1 % aniline blue in K_2PO_4, pH 8.0 prior to observation using both brightfield and fluorescence microscopy. Mycelial length of stained hyphae were measured, and the formation of infection structures was monitored under 200 x, 400 x and 1000 x magnification.

COLONIZATION OF WOUND AND NON-WOUND INOCULATED LEAVES OVER TIME

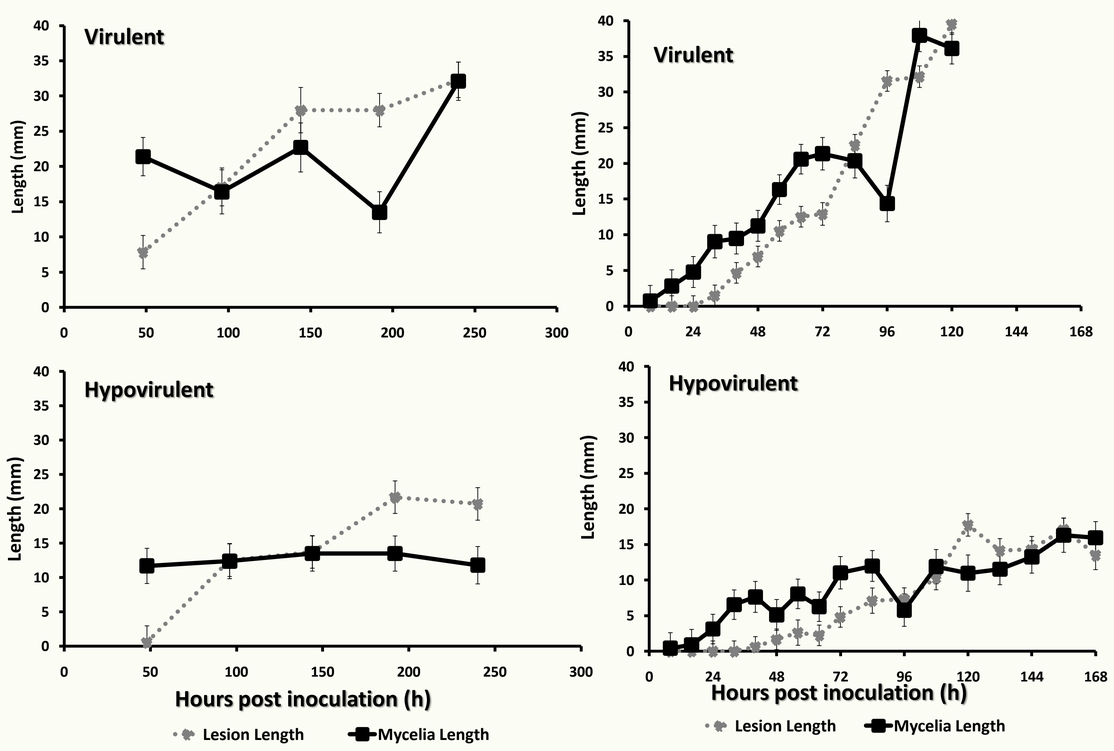

Figure 2: Mycelial length (solid line) and lesion length (dotted line) of virulent and hypovirulent isolates of *S. homoeocarpa* inoculated either on nonwounded (right) or wounded (left) creeping bentgrass leaves.

Fungal mycelia colonized the leaf slightly ahead of lesion development until 80 hours post inoculation (hpi) on both wound and nonwound inoculated leaves. On nonwounded leaves, lesions formed at about 50 hpi for both isolates. On wounded leaves, lesions formed at 24 hpi for the virulent and 40 hpi for the hypovirulent isolate. Incubation of the hypovirulent isolate for 168 h or 240 h for wounded leaves or nonwounded leaves, respectively, seldom resulted in colonization or lesion development past 25 mm.

LESION DEVELOPMENT

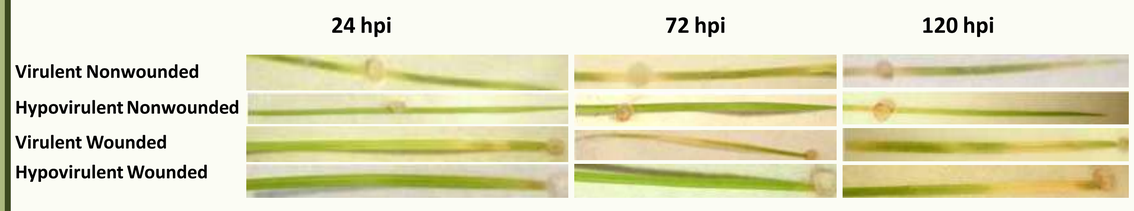

Figure 3: At 24 hpi, wounded leaves inoculated with the virulent isolate had developed dmall, water-soaked lesions. At 72 hpi, the virulent wounded and nonwounded treatments demonstrated water-soaked lesions as well as chlorosis. The hypovirulent isolate did not develop lesions on the nonwounded leaves, but produced small chlorotic lesions with brown necrotic spots on wounded leaves. At 120 hpi, nonwounded and wounded leaves inoculated with the virulent isolate were 100 % diseased. Leaves inoculated with the hypovirulent isolate had only small chlorotic lesions at 120 hpi.

INFECTION AND COLONIZATION OF CREEPING BENTGRASS

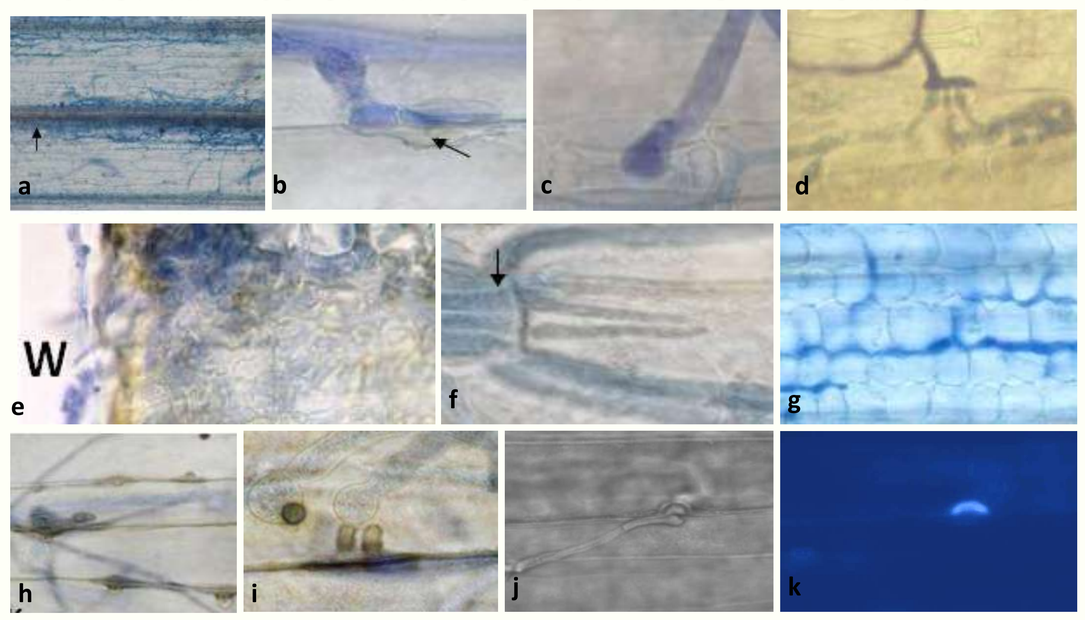

Figure 4: Initial infection of nonwounded grass occurred through the formation of appressoria (arrow) along cell walls (a, b, arrows) and over stomates (c). Multiple infection fronts occurred on nonwounded leaves as appressoria formed at intervals along the leaf from external hyphae. Appressoria produced penetration pegs, infection vesicles (d, arrow) and primary infection hyphae (d). Up to three infection vesicles per appressorium were observed (d). Initial infection of wounded grass tissue occurred as intra- and intercellular hyphae that grew directly into th ewounded cells (e, g). The intracellular hyphae infecting the wound site (W) advanced and formed an infection front (f). Intracellular hyphae demonstrated some restriction while advancing through adjacent cells (f.g) and constriction of advancing hyphae at the cell walls was evident (f, arrow).

Structures involved in the colonization of leaves were the same for both virulent and hypovirulent isolates. In some cases, penetration pegs of hypovirulent isolates were long and had a yellow coloration (h). Long, discolored penetration pegs were still able to produce infection vesicles (i). Callose deposition was detected around some of the penetration pegs of both isolates using fluorescent microscopy of aniline blue stained tissues.

DISCUSSION

This study has provided a detailed description of the infection and colonization of creeping bentgrass by *S. homoeocarpa*. The colonization structures and the ability of the intracellular hyphae to grow through the leaf ahead of lesion development suggest that *S. homoeocarpa* is a hemibiotrophic organism. Additionally, *S. homoeocarpa* caused disease symptoms more rapidly on wounded grass than nonwounded grass which is an important consideration for turf grass management.

This study demonstrated that the etiology of virulent and hypovirulent isolates are similar, and that reduced disease smptoms caused by hypovirulent isolates are likely the result of slower, less vigorous growth. The slow, less vigorous growth of hypovirulent isolates makes them more likely to be impeded by plant defenses such as papillae. Callose deposition observed around the infection structures was seen with both isolates and is likely associated with papillae formation. Since papilla formation has been associate with induced resistance responses (Hammerschmidt, 1999), future studies on the suppression of dollar spot symptoms by hypovirulent isolate-induced resistance in grass are warranted.

Acknowledgements & References

We would like to thank Melody Melzer and Mark Habash for providing assistance with this project. Funding for this project and was provided by the National Science and Engineering Research Council of Canada (NSERC).

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