INTERACTION OF ANAEROBIC SOIL DISINFESTATION AND INTRODUCED BIOCONTROL AGENTS ON *SCLEROTIUM ROLFSII* GERMINATION AND PARASITISM

Utsala Shrestha[1], Mary Dee[2], Bonnie H. Ownley[2], David M. Butler*[1]
[1]Department of Plant Sciences, [2]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, USA

Sclerotium rolfsii causes damping off, root rot, and stem rot of more than 500 plant species. It is also an active saprophyte, surviving two to three years in a wide range of soil properties. Infection from sclerotia of *S. rolfsii* can cause large losses in horticultural production systems. Studies on anaerobic soil disinfestation (ASD), which is a promising non-chemical alternative to soil fumigants for controlling many soilborne diseases, have been reported to reduce the sclerotial germination of *S. rolfsii* (Shrestha et al., 2016). Similarly, biocontrol agents (e.g., *Trichoderma* spp.) are also reported to affect the ability of sclerotia to cause plant disease (Elad et al., 1980; Errakhi et al., 2007). However, the combined effect of ASD and microbial biocontrols has not been reported. Our study compared the effect of ASD and introduced biocontrols separately or in combination, at the initiation of ASD treatment, for impact on sclerotial germination and parasitism.

We evaluated three biological controls, separately, and in combination with ASD. Our biocontrols were *T. asperellum*, which we isolated as a parasite from sclerotia of *S. rolfsii* recovered from field trials of ASD, and two commercially available biofungicides, *Trichoderma harzianum* (RootShield®) and *Streptomyces riseoviridis* (Mycostop®). Our treatments were: i) ASD alone with dry molasses (carbon rates of 4 mg C g^{-1} at C:N ratio 30:1), and ASD in combination with ii) *Trichoderma asperellum*, iii) RootShield®, iv) Mycostop®, and v) RootShield® + Mycostop®. Additional treatments were vi) *Trichoderma asperellum,* vii) RootShield®, viii) Mycostop® and ix) RootShield® + Mycostop®. Two non-amended, negative controls were included: a polyethylene-covered control and a non-covered control. Repeated growth chamber experiments (15-25 °C) including pots (with a soil: sand mixture) with the above mentioned treatments were arranged in a completely randomized block design. Three packets, with ten sclerotia of *S. rolfsii* each, were buried at 5-, 10- and 15-cm depths in each pot. Oxidation-reduction electrodes were inserted at 15-cm depths to measure cumulative redox potential. Pots were saturated with deionized water, with/without biocontrol agents. All pots were covered with black polyethylene (0.03 mm) and secured with a heavy-duty rubber band for 3 weeks, except the non-amended control pots. Packets containing sclerotia were retrieved from 5-, 10- and 15-cm depths, and sclerotia were plated onto 24-well plates containing either antibiotic-amended potato dextrose agar (PDA), actinomycete isolation agar (AIA), or *Trichoderma*-selective medium to assess germination and parasitism of sclerotia.

We observed that anaerobic conditions during ASD treatment, with or without biocontrol agents, were higher than all other treatments (Figure 1). Germination of sclerotia retrieved from 5-, 10- and 15-cm depths (data not shown) was significantly lower in ASD treatments, with or without biocontrol agents, than biocontrol treatments alone. There were no significant differences in sclerotial germination among the ASD and ASD combined with biocontrol treatments. At 5-cm, mortality of sclerotia was lower than greater depths (10- or 15-cm), and use of biocontrol agents alone did not reduce sclerotial germination compared to the covered or non-covered controls. At all depths, regardless of added biocontrol agents, sclerotial parasitism by *Trichoderma*, zygomycetes, and bacteria was apparent, but parasitism of sclerotia by actinomycetes was only observed in AIA medium (Figure 2). Compared to biocontrol agents alone, parasitism of sclerotia by zygomycetes and bacteria was higher in ASD and ASD combined with biocontrol agents. A higher percentage of *Trichoderma* parasitism of sclerotia was observed in sclerotia retrieved from the 5-cm depth plated on PDA than greater depths (data not shown). Our results showed that the use of biocontrol agents such as Mycostop® or RootShield®, or field-isolated *T. asperellum* did not consistently reduce sclerotial germination under the covered condition. Addition of these biocontrol agents at ASD treatment did not promote further suppression of sclerotial germination, compared to ASD treatment alone.

References:

Shrestha U, Augé R. M. and Butler D. M. (2016). A meta-analysis of impact of anaerobic soil disinfestation on pest suppression and yield of horticultural crops. *Frontiers in Plant Sciences* **7**:1254. doi:10.3389/fpls.2016.01254

Elad, Y., Chet, I. and Katan, J., 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology*, *70*(2), pp.119-121.

Errakhi, R., Bouteau, F., Lebrihi, A. and Barakate, M., 2007. Evidences of biological control capacities of *Streptomyces spp.* against *Sclerotium rolfsii* responsible for damping-off disease in sugar beet (Beta vulgaris L.). *World Journal of Microbiology and Biotechnology*, *23*(11), pp.1503-1509.

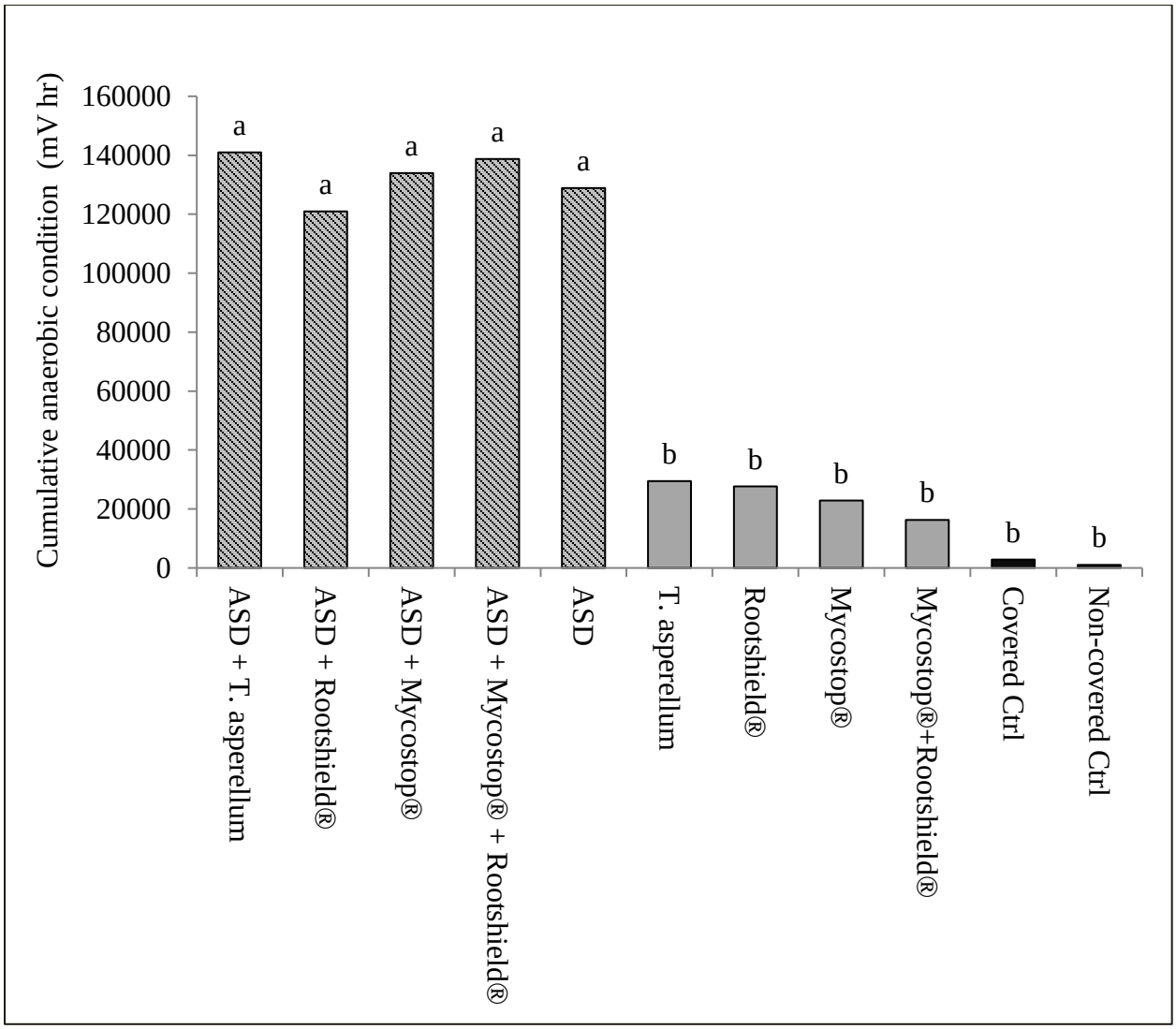

Figure 1. Effect of ASD (with/without biocontrol agents) and biocontrol agents on cumulative anaerobic condition. Bars indicated by different letters are significantly different at $p<0.05$ according to Fisher's PLSD test.

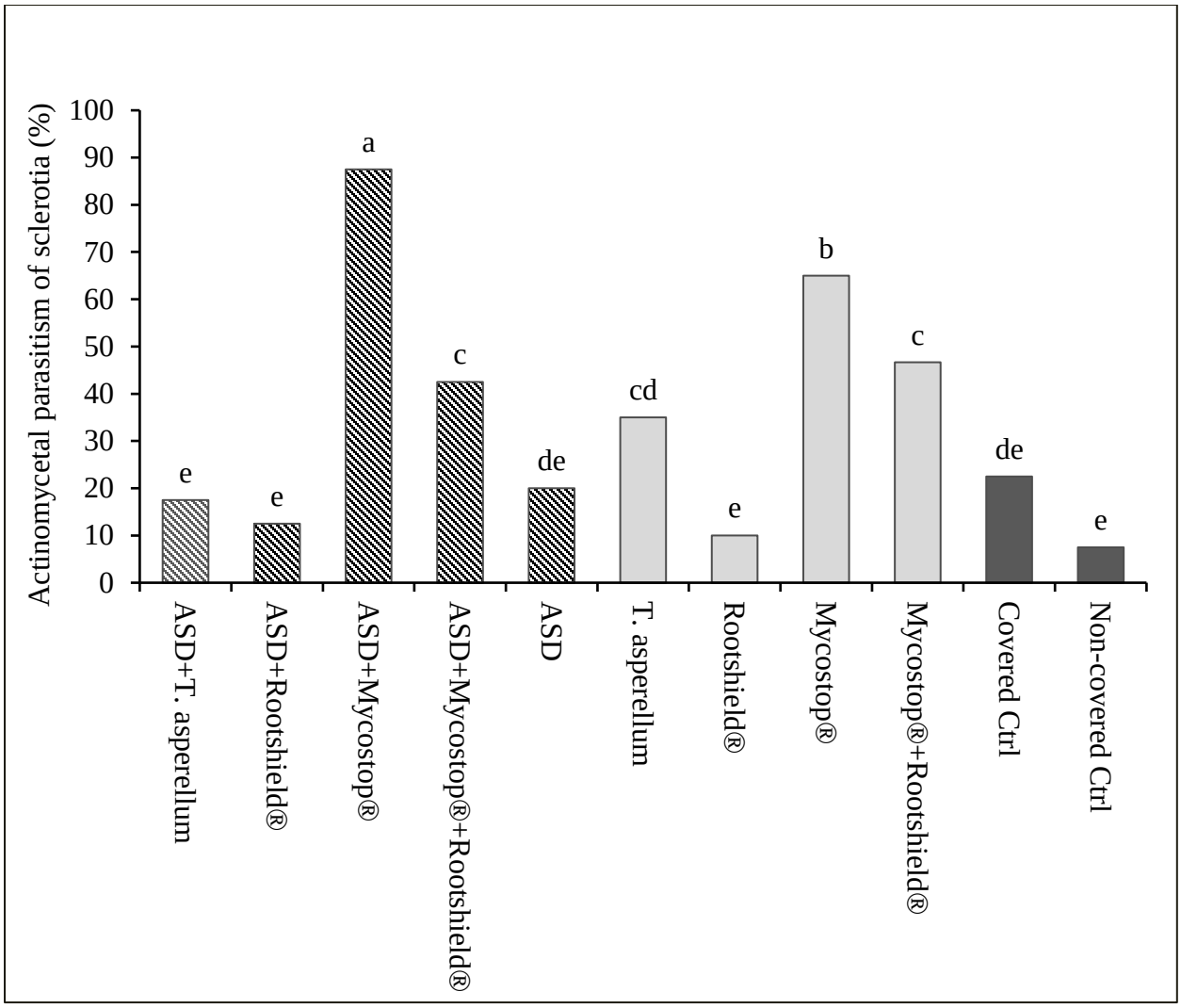

Figure 2. Effect of anaerobic soil disinfestation, biocontrol agents, or their combination on actinomycetal parasitism of sclerotia of *Sclerotium rolfsii* recovered from 10-cm examined on actinomycete isolation agar. Bars indicated by different letters are significantly different at $p<0.05$ according to Fisher's PLSD test.