Allyl Isothiocyanate Released From *Brassica Juncea* Suppresses Mycelial Growth of *Sclerotium Rolfsii.*

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The need for an alternative to methyl bromide for controlling soilborne plant diseases has prompted an increase in research, much of which involves manipulation and application of biological control mechanisms. Glucosinolates (GSL), found in *Brassica* species, are of interest due to the potential for using their degradation products as fumigants. When hydrolyzed by the enzyme myrosinase, GLS produce D-glucose, sulfate, isothiocyanates (volatile mustard oils), thiocyanates and nitriles (Larsen, 1981; Poulton and Moller, 1993). Isothiocyanates (ITC) and nitriles have been demonstrated to control fungi (Charron and Sams, 1999; Mayton *et al.*, 1996; Sarwar *et al.*, 1998) bacteria (Delaquis and Mazza, 1995), nematodes (Mojtahedi *et al.*, 1993 and 1991), insects (Noble et al., 1999) and some weed seeds in laboratory experiments (Al-Khatib *et al.*, 1997). Allyl isothiocyanate (AITC) is the predominant ITC produced by Indian mustard *(B. juncea*).

The objective of this experiment was to determine the effectiveness of biofumigation with Indian mustard and AITC for control of *Sclerotium rolfsii* Sacc., the causative agent of Southern blight of tomato.

Materials and Methods.

To determine the effectiveness of Indian mustard for control of *S. rolfsii*, the following three experiments were conducted: 1) inhibition of mycelial growth by Indian mustard, 2) determination of AITC released by Indian mustard, and 3) suppression of sclerotia germination by AITC. Freeze-dried Indian mustard (FDM) was utilized to increase homogeneity of the treatments. Volatiles produced from FDM are similar to those produced by Indian mustard (Price, 1999).

Treatments of 0, 0.1, 0.2, 0.6, 1.0, 2.0, 4.1, 5.1, 10.2, 20.4, 40.8, 81.6, and 163.3 $g \cdot L^{-1}$ (fresh weight per headspace volume) were selected. FDM was weighed and placed into airtight containers. Deionized water was added to reconstitute FDM to its fresh weight. Plugs (4.6 mm diameter) were cut from margins of an actively growing *S. rolfsii* culture and placed in the center of fresh PDA plates (100 x 15 mm). FDM and water were mixed in a 490-mL glass jar. Plates with hyphal plugs were inverted over the mouth of the jars and sealed with wax film. Jars were then incubated at 30ºC. Radial growth of mycelia was measured after 42 h. To determine the amount of inhibition that was contributable to the AITC released by the mustard, hyphal plugs were fumigated with different concentrations of AITC standard. AITC standard (Sigma-Aldrich Corp, St. Louis, MO) was diluted in ethanol.

To determine the amount of AITC released by Indian mustard, treatments of 0, 0.05, 0.10, 0.30, 0.50, 1.0, 2.0, 2.5, and 5.0 g fresh weight were weighed as stated above. Jars containing the reconstituted mustard were incubated at 30°C for 15 min and sampling was performed using a 100 µm polydimethylsiloxane Solid Phase MicroExtraction (SPME) fiber (Supelco, St. Louis, MO) for 1 min. The fiber was placed in the injection port of an HP 5890 gas chromatograph equipped with an HP 5972 mass selective detector (GC-MS) to desorb for 1min.

For suppression of sclerotia germination, five sclerotia were randomly selected and placed on a 2-cm square of 0.2-mm polyester mesh. The mesh was then tied up around the five sclerotia. Mesh bags were placed in culture tubes containing sterilized clay loam soil moistened to 60% field capacity. The treatments, AITC per headspace volume at 0.0, 4.0, 5.6, 11.2, 16.0, 22.4, 44.8, 64.0, 256.0, 1024.0 and 4096.0 $F mole \cdot L^{-1}$, were applied. Sclerotia were removed and placed on PDA. Radial growth of mycelia from sclerotia was measured.

Results and Conclusions

Volatiles released from Indian mustard were effective in controlling *S. rolfsii* mycelial growth with LC_{50} and LC_{90} (lethal concentration with 50 and 90% kill) at 0.6 and 2.1 $g \cdot L^{-1}$, respectively. AITC also demonstrated effective control of mycelial growth with LC_{50} and LC_{90} at 1.6 and 4.6 $\mu moles \cdot L^{-1}$, respectively. The use of AITC directly as a soil fumigant has been suggested. A similar compound, methyl isothiocyanate is currently under commercial production.

Indian mustard treatments were more effective than equivalent AITC treatments, based on equations describing the relationships of AITC to mycelial inhibition and Indian mustard to AITC release. This suggests that other chemicals released by the Indian mustard may also play a role in its toxicity. A synergistic reaction might explain these results. AITC and other compounds could be working together for greater inhibition than either can accomplish individually (cooperative inhibition).

Inhibition of *S. rolfsii* sclerotia germination will be more difficult than the inhibition of the actively growing mycelia. Sclerotia were only suppressed and germinated in 7 d even with the highest AITC treatment. The IC_{50} and IC_{90} (concentration yielding 50 and 90% inhibition) were 244.9 and 1010.1 $\mu moles \cdot L^{-1}$, respectively. While a higher concentration may prove toxic, it may be economically infeasible. However, if sclerotia can be triggered to germinate prior to treatment, lower concentrations of Indian mustard could provide adequate control of *S. rolfsii.*

Indian mustard biofumigation could be integrated into raised bed production of tomato and strawberry on plastic. The *Brassica* crop could be grown, plowed under and covered with plastic to trap the ITC released in the raised beds. Biofumigation with Indian mustard and other *Brassica* sp. may provide growers with an affordable, environmentally safe control for *S. rolfsii* mycelia if used in an integrated management system.

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