Macerated *Brassica* leaves suppress *Pythium ultimum* and *Rhizoctonia solani* mycelial growth

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The United States tomato industry has relied on methyl bromide for control of soilborne pathogens such as *Pythium ultimum* and *Rhizoctonia solani*. Several studies have suggested the possibility that some plant diseases could be controlled by fumigating soil with natural plant chemicals, a process termed Abiofumigation.@ Generally biofumigation is achieved by incorporating macerated plant tissue from *Brassica* spp. into soil contaminated with pest organisms. When plant tissue of *Brassica* spp. is disrupted by tearing or shredding, fungitoxic compounds called isothiocyanates (ITCs) are produced. These compounds play a major role in the suppression of fungal pathogens and thus are integral to biofumigation.

Since *P. ultimum* and *R. solani* are important soilborne pathogens of tomato, we evaluated the extent to which mycelial growth would be inhibited by volatiles from six macerated *Brassica* spp. After identifying the volatiles, we tested the suppressive potential of pure allyl isothiocyanate (AITC), the predominant compound produced by *B. juncea*.

Materials and Methods

Brassicas were seeded into 1" ASpeedling@ trays in a greenhouse and then transplanted when they were 4 weeks old at the University of Tennessee plant science farm in Knoxville, Tennessee. Leaves from ›Premium Crop= broccoli, ›Charmant= cabbage, ›Michihili Jade Pagoda= Chinese cabbage, ›Blue Scotch Curled= kale, Indian mustard (cultivar undetermined), or ›Florida Broadleaf= mustard were shredded in a food processor and 10 g of the residue was placed in a 500-ml jar. Immediately a petri dish with potato dextrose agar was inverted and placed over the mouth of the jar. Prior to macerating the leaves, a 5-mm plug of *P. ultimum* or *R. solani* was inserted in the center of the petri dish. The radial growth of each fungus was measured at 24-h intervals. Volatile compounds were measured from plant residues placed in duplicate jars. A solid phase microextraction (SPME) device was inserted through a septum in the jar lid 30 minutes after maceration of the leaves. Volatiles adsorbed onto the SPME device were measured by gas chromatography-mass spectrometry (GS-MS).

To test the suppressive potential of AITC, pure AITC was prepared at three concentrations in methanol such that complete volatilization of 1 µl would yield a headspace concentration in the 500-ml jar of 1.1, 2.2, or 3.3 mmol L^{-1}. A petri dish with a *P. ultimum* or *R. solani* plug was placed over jars at each concentration.

Results and Discussion

Indian mustard was the most suppressive plant for both fungi, and was fungicidal to *P. ultimum* (Table 1). This was the same result we obtained in an earlier study when we tested *Brassica* spp. against *B. cinerea*. ›Florida Broadleaf: mustard was the next most suppressive. ›Michihili Jade Pagoda: Chinese cabbage was the least suppressive for both fungi.

Mustard leaves are often high in AITC. We found that AITC was the predominant volatile compound produced by both mustards tested in this study, comprising about 95 % of the total volatiles. Other ITCs detected from the mustards were 3-butenyl ITC and sec-butyl ITC. All plants produced (Z)-3-hexenyl acetate, a lipoxygenase pathway product. (Z)-3-hexenol was detected from all plants but the mustards. The presence of (Z)-3-hexenol is notable since other studies have found it to be fungitoxic. No ITCs were found from ›Premium Crop: broccoli, ›Michihili Jade Pagoda: Chinese cabbage, or ›Blue Scotch Curled: kale, possibly because the ITCs were present at levels too low to detect. The lower detection limit for AITC was about 0.2 mmol L^{-1}.

Indian mustard, which was fungicidal to *P. ultimum*, produced about 2.2 mmol L^{-1} AITC. When pure AITC was used to assay the growth response of *P. ultimum*, a concentration of 2.2 mmol L^{-1} AITC was fungicidal (Table 2). This result demonstrates that AITC was a major factor in the suppression of *P. ultimum* by the mustards. ›Florida Broadleaf: mustard did not prevent mycelial growth of *R. solani*, but slowed the growth compared to the control jars without plant material. ›Florida Broadleaf: mustard produced about 1.1 mmol L^{-1} AITC. When pure AITC at a concentration of 1.1 mmol L^{-1} was injected into the jars, growth of *R. solani* was reduced but not stopped. This shows that AITC was an important factor in the suppression of *R. solani* by the mustards.

Conclusions

The potential of mustard leaves to suppress mycelial growth of *P. ultimum* and *R. solani* was derived largely from AITC production. Consequently, control of these two pathogens under field conditions by mustard cultivars would partly depend on the amount of AITC they can generate. Compared to the other *Brassicas* tested, the superiority of the Indian mustard cultivar for inhibiting *P. ultimum and R. solani* was clear, and demonstrates the importance of careful crop selection for biofumigation. Additionally, factors such as planting time, climactic conditions, and the presence of other target pathogens must be considered when selecting a biofumigation crop.

Table 1. Radial growth (% of control) of *P. ultimum* and *R. solani* after 48 h in a 500-ml jar with 10 g of *Brassica* leaf tissue.

	Fungus radial growth (% of control)^{z}	
Brassica sp.	*P. ultimum*	*R. solani*
›Premium Crop꞉ broccoli	90.5c	73.8bc
›Charmant꞉ cabbage	85.9c	59.6b
›Michihili Jade Pagoda꞉ Chinese cabbage	95.7c	79.6c
›Blue Scotch Curled꞉ kale	94.4c	62.3bc
›Florida Broadleaf꞉ mustard	64.4b	64.9bc
Indian mustard (cultivar undetermined)	0.0a	27.4a

^{z}Radial growth measurements with the same letter within a column are not different based on Duncan multiple range test at *P* = 0.05.

Table 2. Radial growth (% of control) of *P. ultimum* and *R. solani* after 48 h in a 500-ml jar containing AITC at concentrations of 1.1, 2.2, or 3.3 mmol L^{-1}.

	Fungus radial growth (% of control)^{z}	
AITC (mmol L^{-1})	*P. ultimum*	*R. solani*
1.1	19.7 6.4	86.9 4.6
2.2	0.0 0.0	73.1 1.7
3.3	0.0 0.0	45.3 0.6

^{z}Mean of three replications SE.