Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by Shredded Leaves of *Brassica* Species

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ABSTRACT. The U.S. Clean Air Act bans the use of methyl bromide after 2005. Consequently, the development of alternative methods for control of soilborne pathogens is imperative. One alternative is to exploit the pesticidal properties of *Brassica* L. species. Macerated leaves (10 g) from 'Premium Crop' broccoli [*B. oleracea* L. (Botrytis Group)], 'Charmant' cabbage [*B. oleracea* L. (Capitata Group)], 'Michihili Jade Pagoda' Chinese cabbage [*B. rapa* L. (Pekinensis Group)], 'Blue Scotch Curled' kale [*B. oleracea* L. (Acephala Group)], Indian mustard [*B. juncea* (L.) Czerniak, unknown cultivar] or 'Florida Broadleaf' mustard [*B. juncea* (L.) Czerniak] were placed in 500-mL glass jars. Petri dishes with either *Pythium ultimum* Trow or *Rhizoctonia solani* Kühn plugs on potato-dextrose agar were placed over the jar mouths. Radial growth of both fungi was suppressed most by Indian mustard. Volatiles were collected by solid-phase microextraction (SPME) and analyzed by gas chromatography–mass spectrometry. Allyl isothiocyanate (AITC) comprised >90% of the volatiles measured from 'Florida Broadleaf' mustard and Indian mustard whereas (Z)-3-hexenyl acetate was the predominant compound emitted by the other species. Isothiocyanates were not detected by SPME from 'Premium Crop' broccoli and 'Blue Scotch Curled' kale although glucosinolates were found in freeze-dried leaves of all species. When exposed to AITC standard, *P. ultimum* growth was partially suppressed by 1.1 $\mu\text{mol}\cdot\text{L}^{-1}$ (μmol AITC/headspace volume) and completely suppressed by 2.2 $\mu\text{mol}\cdot\text{L}^{-1}$. *R. solani* was partially suppressed by 1.1, 2.2, and 3.3 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC. Use of *Brassica* species for control of fungal pathogens is promising; the presence of AITC in both lines of *B. juncea* suppressed *P. ultimum* and *R. solani* but some *Brassic*as were inhibitory even when isothiocyanates were not detected.

Tomato (*Lycopersicon esculentum* Mill.) production systems in the United States depend on the use of methyl bromide soil fumigation for control of soilborne pests. In accordance with the U.S. Clean Air Act, the use of methyl bromide as a fumigant will be banned in the United States by 2005 (U.S. Dept. of Agriculture, 1999). This regulation and public concern with environmental and health effects of other synthetic chemical pesticides have prompted increased interest in the development of alternative pest control strategies.

One alternative approach has been the use of macerated tissues of *Brassica* species to reduce disease incidence. The incidence of *Aphanomyces euteiches* Drechs. root rot of peas (*Pisum sativum* L.) was decreased when soil was amended with residues from cabbage [*Brassica oleracea* (Botrytis Group)], kale [*B. oleracea* (Acephala Group)], and rapeseed (*B. napus* L.) (Chan and Close, 1987; Muehlchen, 1990). When chopped broccoli [*B. oleracea* (Italica Group)] was incorporated into soil, the number of *Verticillium dahliae* Kleb propagules was lower than in nontreated control plots and was similar in plots fumigated with methyl bromide + chloropicrin (Subbarao et al., 1994).

Pesticidal activity associated with *Brassica* residues is often attributed to release of isothiocyanates (ITCs) from the plant tissue. ITCs are breakdown products of glucosinolates (GLs), a class of plant compounds containing a thioglucose moiety, a sulphonated oxime, and a side chain (R-group). In laboratory studies, canola and Indian mustard (*B. juncea*) roots released methyl ITC and phenylethyl ITC respectively, both of which were inhibitory to *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var. *tritici* Walker grown in pure culture (Angus et al., 1994). Another in vitro study found that allyl ITC (AITC), p-hydroxybenzyl ITC, and 4-methylsulphinybut-3-enyl ITC completely inhibited conidia germination of *Botrytis cinerea* Pers.: Fr., *Monilinia laxa* (Aderhold & Ruhland) Honey, *Mucor piriformis* E. Fisch., *Penicillium expansum* Link, and *Rhizo-*

pus stolonifer (Ehrenb.: Fr.) Vuill. (Mari et al., 1993).

Gamliel and Stapelton (1993) determined that *Pythium ultimum* propagules were reduced by >95% when exposed to volatiles from heated cabbage-amended soil. *Rhizoctonia solani* was also suppressed by cabbage volatiles (Lewis and Papavizas, 1974). Mayton et al. (1996) found that in a closed jar, volatiles from 25 g of *B. juncea* 'Cutlass' completely suppressed growth of *P. ultimum* and *R. solani* whereas 25 g of *B. napus* 'Midas' did not inhibit either fungus.

Since *P. ultimum* and *R. solani* are important tomato pathogens, and since the number of *Brassica* species tested for their control is very limited, one objective was to evaluate the potential of volatiles from macerated leaf tissue from six commonly available *Brassica* species to inhibit *P. ultimum* and *R. solani*. In addition, since fungal suppression by volatile emissions depends on the particular compounds produced, a second objective was to identify these compounds. The third objective was to measure GL concentration to determine qualitatively how it correlated to the ITC profile since GL concentration is often used to predict the suppressive potential of a plant (Sarwar and Kirkegaard, 1998) yet it has not been demonstrated that this is a sound basis for such prediction. Our final objective was to measure the growth responses of *P. ultimum* and *R. solani* to three headspace concentrations of AITC, the predominant compound detected, to determine the extent of its role in suppressing the fungi exposed to *Brassica* volatiles.

Materials and Methods

PATHOGEN CULTURE. *Rhizoctonia solani* was cultured on potato dextrose agar (PDA) supplemented with a penicillin–streptomycin–neomycin solution at 100,000 units/L, 100 mg·L⁻¹, and 200 mg·L⁻¹ respectively. The antibiotic was used to eliminate bacterial contamination. Sensitivity to this antibiotic precluded its use with *P. ultimum*, which also was cultured on PDA. The fungi were produced at 22 °C in darkness. Both were obtained from the Dept. of Entomology and Plant Pathology at Univ. of Tennessee, Knoxville.

FUNGAL RESPONSE TO GROUND *Brassica* LEAVES. Response of *R. solani* and *P. ultimum* to macerated leaves of *Brassica* species was

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tested with the youngest fully expanded leaves from field-grown 'Premium Crop' broccoli, 'Charmant' cabbage, 'Michihili Jade Pagoda' Chinese cabbage [*B. rapa* (Pekinensis Group)], 'Blue Scotch Curled' kale, Indian mustard (unknown cultivar; V and J Seed Service, Woodstock, Ill.) and 'Florida Broadleaf' mustard. We adapted a method described by Mayton et al. (1996). A 5-mm plug of *R. solani* or *P. ultimum* was removed from 5- to 7-d-old cultures and placed in the center of a 100-mm petri dish with fresh PDA. The dish was inverted and placed over the mouth of a 500-mL glass jar containing 10 g of leaf tissue that had been macerated in a food processor for 30 s. Petri dishes were sealed onto the jar with two layers each of Parafilm and labeling tape. The radial mycelial growth of the fungi was recorded at 24-h intervals for 48 h as the mean of two perpendicular diameters. Fungal growth in the *Brassica* treatments was expressed as a percentage of radial growth in control jars without plant material. There were three replications of each combination of fungus and plant species or control.

VOLATILE COMPOUND MEASUREMENT. Volatile organics emitted by macerated *Brassica* species were measured in 500-mL glass jars. Youngest, fully expanded leaves [harvested 27 Oct. 1997, 54 d after planting (DAP)] were macerated in a food processor for 30 s. Ten grams of the tissue was placed immediately in a jar and covered by a metal lid with a 6-mm rubber septum in each center. After 30 min, a solid-phase microextraction (SPME) device (Supelco Inc., Bellefonte, Pa.) with a 100-m polydimethylsiloxane coating was inserted through the septum and exposed to the jar atmosphere. After 1 min, the SPME device was removed from the jar and inserted into the injector of a gas chromatograph [5890 Series II splitless mode; Hewlett-Packard (HP), Palo Alto, Calif.] connected to a mass selective detector (MSD) (5972; HP). Helium was used as the carrier gas and flowed at 1.0 mL·min⁻¹ through a HP retention gap (uncoated, deactivated 5.0 m × 0.25 mm ID) followed by a fused silica DB-1 capillary column (30.0 m × 0.25 mm ID, 0.1 µm film thickness (J & W Scientific, Folsom, Calif.). Analytical setpoints were the following: injector temperature, 200 °C; detector temperature, 280 °C; oven temperature, 40 °C for 1 min., then increased at 4 °C·min⁻¹ to 100 °C. Compounds were identified by comparing their mass spectra to those of authentic standards or previously published spectra (Kjaer et al., 1963; MacLeod and Islam, 1976; Spencer and Daxenbichler, 1980).

TIME COURSE OF VOLATILES PRODUCTION. To determine whether the quantity of individual volatiles emitted by Indian mustard leaves changed over time, 10 g of macerated leaves (harvested 12 Nov. 1997, 70 DAP) were placed in a 500-mL jar covered by a metal lid with a septum. For each of three replications, the same jar atmosphere was sampled by SPME for 1 min at 1/30, 1/4, 1/2, 1, 24, and 48

h after shredding the leaves in a food processor. Volatiles collected by SPME were measured by the GC-MSD procedures described above.

DETERMINING GL CONTENT IN LEAVES. Youngest, fully expanded leaves (harvested 22 May 1998, 53 DAP) were divided along the midrib. Three or four half-leaves from each species were shredded in a food processor. Volatile compounds were collected and analyzed by SPME and GC-MSD as described previously. The opposite half-leaves were lyophilized. Six milliliters of methanol, 1 mL benzyl GL solution (1 mM), and 0.3 mL barium-lead acetate (0.6 M) were added to 500 mg of lyophilized tissue in a 16 × 100-mm culture tube and shaken at 60 rpm for 1 h. Each tube was then centrifuged at 739 g_n for 10 min. The supernatant was added to Sephadex columns and desulfated and derivatized by the procedure of Raney and McGregor (1990). Samples were analyzed by GC-MSD as described previously (Brown and Morra, 1995) and identifications completed based on comparisons of spectra with those from pure standards or published from prior research (Carlson et al., 1987; Chistensen et al., 1982; Olsson et al., 1977). Response factors for allyl, 3-butenyl, 2-hydroxy-3-butenyl, 2-hydroxy-2-phenylethyl, and 4-methylthiobutyl GLs were determined with desulfonated standards provided by Sandro Palmieri of the Istituto Sperimentale Industriale, Bologna, Italy. Other response factors were arbitrarily set to 1.0. Three replications were completed for both the GL extraction and the SPME procedure.

FUNGAL RESPONSE TO AITC. AITC was the predominant compound in Indian mustard, the plant most suppressive to growth of *P. ultimum* and *R. solani*. The radial growth of *P. ultimum* and *R. solani* was measured when exposed to volatilized AITC. Methanol was added to 0.15 mL (volume of compound after subtracting volume of impurities) AITC to achieve 1 mL total volume. One microliter of this mixture in a 500-mL jar resulted in a concentration of 3.3 µmol·L⁻¹ (µmol AITC/headspace volume). The AITC mixture was diluted serially to concentrations of 2.2 and 1.1 µmol·L⁻¹. A 1-µL aliquot of the AITC mixture was injected onto a 7.0-cm filter paper disk (Whatman qualitative no. 2) in a 500-mL jar that was covered immediately with an inverted petri dish containing a 5-mm plug of *R. solani* or *P. ultimum*. The petri dish was sealed to the jar with two layers each of Parafilm and labeling tape. A control was prepared by adding 1 µL of methanol to a filter paper disk in a jar and covering the jar with a petri dish containing *R. solani* or *P. ultimum*. Each fungus was exposed to AITC concentrations of 0, 1.1, 2.2, or 3.3 µmol·L⁻¹ in three replications and radial growth was measured at 24-h intervals. The concentration range was selected by comparing chromatographic peak areas of pure AITC in 500-mL jars with AITC peak areas generated by Indian mustard and 'Florida Broad-

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

Brassica taxa	Fungus radial growth (% of control)	
	<i>P. ultimum</i> ²	<i>R. solani</i> ³
<i>B. campestris</i> (Pekinensis Group) 'Michihili Jade Pagoda' Chinese cabbage	96.3 cd	79.6 c
<i>B. juncea</i> 'Florida Broadleaf' mustard	64.9 b	64.9 bc
<i>B. juncea</i> Indian mustard (unknown cultivar)	0.0 a	27.4 a
<i>B. oleracea</i> (Capitata Group) 'Charmant' cabbage	85.5 c	59.6 b
<i>B. oleracea</i> (Botrytis Group) 'Premium Crop' broccoli	91.4 cd	73.8 bc
<i>B. oleracea</i> (Acephala Group) 'Blue Scotch Curled' kale	95.1 cd	62.3 bc
Control, no plant tissue	100.0 d	100.0 d

²*P. ultimum* was cultured on PDA without an antibiotic supplement.

³*R. solani* was cultured on PDA supplemented with a penicillin-streptomycin-neomycin solution at 100,000 units/L, 100 mg·L⁻¹, and 200 mg·L⁻¹ respectively.

⁴Mean separation within columns by Duncan's multiple range test, *P* ≤ 0.05.

Table 2. Volatile compounds detected 30 min after maceration in a 500-mL jar containing 10 g of *Brassica* leaves.

<i>Brassica</i> taxa	Peak area (10 ⁵ area counts) ^z							
	Glucosinolate decomposition products ^y				Lipoxygenase pathway products			
	Allyl cyanide (1.44)	Allyl ITC (2.89)	Sec-butyl ITC (3.50)	3-Butenyl ITC (4.32)	(Z)-3- hexenal (2.07)	(Z)-3- hexenol (2.80)	(Z)-3-hexenyl acetate (5.39)	Unidentified compound (6.10)
<i>B. campestris</i> (Pekinensis Group)								
‘Michihili Jade Pagoda’ Chinese cabbage		0.1 ± 0.1	2.4 ± 1.6	3.3 ± 0.8				
<i>B. juncea</i> ‘Florida Broadleaf’ mustard	0.8 ± 0.3	105.2 ± 20.7	6.3 ± 1.5	2.0 ± 0.5	0.2 ± 0.0		1.4 ± 0.4	0.5 ± 0.1
<i>B. juncea</i> Indian mustard (unknown cultivar)	1.0 ± 0.1	197.5 ± 21.7	5.3 ± 0.3	0.5 ± 0.0	0.2 ± 0.1		1.6 ± 0.5	0.3 ± 0.1
<i>B. oleracea</i> (Capitata Group)								
‘Charmant’ cabbage	tr ^x	0.5 ± 0.3		0.2 ± 0.0	2.7 ± 0.4	19.2 ± 5.5		
<i>B. oleracea</i> (Botrytis Group)								
‘Premium Crop’ broccoli					1.5 ± 0.4	4.1 ± 0.7		
<i>B. oleracea</i> (Acephala Group)								
‘Blue Scotch Curled’ kale				0.1 ± 0.1	2.9 ± 0.8	18.3 ± 2.9		

^zArea counts of chromatographic peaks are the means ± 1 SE of three replications and were measured by a HP 5890 GC with a HP 5972 MSD. Empty cells indicate that compound was not detected; there were no missing data.

^yRetention times (min) beneath each volatile compound are indicated in parentheses.

^xLess than three times noise level.

leaf’ mustard. A standard curve was calculated and used to estimate the quantity of AITC produced by Indian mustard and ‘Florida Broadleaf’ mustard. AITC was procured from Aldrich Chemical Co., Milwaukee, Wis.

The experiments were randomized complete block designs with three replications of each treatment. Analysis of variance and mean separation procedures were performed using SAS statistical software (SAS Institute, 1996). Means and standard errors are provided for chromatographic data.

Results and Discussion

FUNGAL RESPONSE TO GROUND *Brassica* LEAVES. After 48 h, three plants suppressed *P. ultimum* relative to the control (Table 1). Indian mustard was the most inhibitory and completely inhibited *P. ultimum* radial growth. When *P. ultimum* plugs from the Indian mustard treatment were transferred to fresh PDA media outside the jars, there was no subsequent growth, indicating the effect was fungicidal. ‘Florida Broadleaf’ mustard was the next most suppressive treatment of *P. ultimum*, followed by ‘Charmant’ cabbage.

The suppressive potential of the *Brassic*as differed somewhat for *R. solani* compared to *P. ultimum*. All plants were suppressive relative to the control. Although Indian mustard again was the most inhibitory, no differences in inhibition were detected among ‘Florida

Broadleaf’ mustard, ‘Premium Crop’ broccoli, ‘Charmant’ cabbage, and ‘Blue Scotch Curled’ kale. In another study, when 25 g of macerated leaves of *B. juncea* ‘Cutlass’ were used in a similar experimental procedure (as opposed to the 10 g that we used), suppression of *P. ultimum* and *R. solani* was complete and fungicidal (Mayton et al., 1996). The elevated biocidal activity may be attributed to higher levels of volatiles resulting from more leaf biomass and/or to higher rates of volatile evolution specific to the cultivar used.

VOLATILES DETECTED FROM *Brassica* LEAVES AND IMPORTANCE OF SAMPLING TIME. Most of the compounds detected originate from the lipoxygenase (LOX) pathway or are breakdown products of GLs (Table 2). All of the compounds identified have been reported in *Brassica* species (Cole, 1976; Tollsten and Bergström, 1988; Wallbank and Wheatley, 1976). An unidentified compound [major MS ions and intensities were 41 (100), 72 (58), 114 (37), 129 (32), 55 (28), 57 (28), and 100 (9)] was produced by both mustards. The LOX pathway product (Z)-3-hexenyl acetate was generated by every *Brassica* species and (Z)-3-hexenal was identified in all treatments except with ‘Premium Crop’ broccoli. (Z)-3-hexenol was not detected in the headspace of the two mustards, but was found in the headspace of the other *Brassica* species. Since standards were not available for every compound detected, relative response factors could not be determined for all compounds. However, AITC from

Table 3. Time course of volatile organics production by macerated *Brassica juncea* (unknown cultivar) leaf tissue.

Time after leaf maceration (h)	Peak area (10 ⁵ area counts) ^z							
	Allyl cyanide	Allyl ITC	Sec-butyl ITC	3-Butenyl ITC	(Z)-3- hexenal	(Z)-3- hexenol	(Z)-3- hexenylacetate	(E)-2- hexenal
1/30	3.4 ± 0.4	658.4 ± 49.5	3.1 ± 1.1	4.2 ± 0.5	18.1 ± 1.2	tr ^y		tr
1/4	4.0 ± 0.2	706.1 ± 65.7	4.3 ± 1.1	4.1 ± 0.6	2.7 ± 0.6	tr	1.6 ± 0.2	tr
1/2	4.5 ± 0.4	622.2 ± 68.5	3.9 ± 1.0	3.2 ± 0.5		tr	2.1 ± 0.1	
1	4.6 ± 3.4	502.4 ± 51.3	3.3 ± 0.9	2.4 ± 0.4		tr	2.2 ± 0.1	
24	3.4 ± 0.4	506.4 ± 86.9	1.6 ± 0.4	1.2 ± 0.2		tr		
48	3.1 ± 0.5	382.0 ± 15.8	1.5 ± 0.7	0.6 ± 0.2		tr		

^zArea counts of chromatographic peaks are the means ± 1 SE of three replications and were measured by a HP 5890 GC with a HP 5972 MSD. Empty cells indicate that compound was not detected; there were no missing data.

^yLess than three times noise level.

Indian mustard and 'Florida Broadleaf' mustard was the most abundant compound detected based on peak area. A trace amount of AITC was also detected from 'Charmant' cabbage.

In addition to AITC, the only other ITCs identified were 3-butenyl ITC and sec-butyl ITC. No ITCs were detected from 'Premium Crop' broccoli, 'Michihili Jade Pagoda' Chinese cabbage, or 'Blue Scotch Curled' kale. Since isothiocyanates are byproducts of GL metabolism and GLs are common to *Brassica* species, this result was initially surprising and led to our measurements of leaf GL content discussed below.

Several of the volatiles identified have exhibited fungicidal activity. AITC levels in leaves of *Brassica* species have been correlated with suppression of radial growth of *Fusarium sambucinum* Fuckel and bioassays have demonstrated that AITC is suppressive to many fungi (Isshiki et al., 1992; Mayton et al., 1996). 3-Butenyl ITC, (Z)-3-hexenal and (Z)-3-hexenol also suppress fungal growth (Mari et al., 1993; Urbasch, 1984). We did not find literature describing evaluations of sec-butyl ITC for antifungal properties.

The volatile profile produced by Indian mustard changed

rapidly during the first hour after tissue maceration (Table 3). AITC levels were the highest of any compound at all times, and were highest at 1/4 h. At 1/30 h, there was more (Z)-3-hexenal measured than any other compound at any time (excluding AITC), but its level dropped by 85% by 1/4 h and was not detected thereafter. This punctuated burst of (Z)-3-hexenal emission warrants further investigation since Urbasch (1984) showed it suppressed several pathogens including *R. solani*. Its presence at high levels soon after tissue maceration may be a significant factor in fungal suppression.

(E)-2-hexenal and (Z)-3-hexenol were detected from Indian mustard harvested at 70 DAP but not from plants harvested at 54 DAP. Also, the levels of ITCs measured at 1/2 h differed between the two harvest dates. The stage of maturation of the plants and perhaps climatic factors may have contributed to these differences. An earlier study using SPME reported that at 1/4 h after shredding leaves and stems of 'Florida Broadleaf' mustard, (E)-2-hexenal comprised 8.6% of total volatiles (Vaughn and Boydston, 1997). We did not detect (E)-2-hexenal from leaves of this cultivar 1/2 h after shredding. It is possible we did not find this compound because stems

Table 4. Glucosinolates extracted from lyophilized leaf tissue and isothiocyanates detected by SPME in headspace above shredded fresh leaf tissue.^z

<i>Brassica</i> taxa	Glucosinolate ^y	Isothiocyanate ^x
<i>B. campestris</i> (Pekinensis Group) 'Michihili Jade Pagoda' Chinese cabbage	3-Indolylmethyl (1.43) 2-Hydroxy-3-butenyl (1.42) 5-Methylthiopentyl (1.33) 4-pentenyl (1.26) 4-Methoxyindolyl-3-methyl (1.03) Phenylethyl (0.47) 3-Butenyl (0.40) 2-Hydroxy-2-phenylethyl (0.12) 3-Methylsphenylpentyl (0.08)	4-Pentenyl (1.0)
<i>B. juncea</i> 'Florida Broadleaf' mustard	Allyl (22.26) 3-Butenyl (2.15) 3-Indolylmethyl (0.24) 4-Methoxyindolyl-3-methyl (0.08)	Allyl (875.2) 3-Butenyl (76.3) Sec-butyl (13.9)
<i>B. juncea</i> Indian mustard (unknown cultivar)	Allyl (19.52) Phenylethyl (0.53) 3-Butenyl (0.48) 3-Indolylmethyl (0.34) 3-Methylthiopropyl (0.18) 4-Methoxyindolyl-3-methyl (0.13)	Allyl (779.1) 3-butenyl (4.5) Sec-butyl (3.1)
<i>B. oleracea</i> (Capitata Group) 'Charmant' cabbage	Allyl (5.14) 3-Indolylmethyl (2.79) 4-Methoxyindolyl-3-methyl (0.27) 2-Hydroxy-3-butenyl (0.26)	Allyl (0.9)
<i>B. oleracea</i> (Botrytis Group) 'Premium Crop' broccoli	3-Indolylmethyl (1.39) 3-Butenyl (0.34) 4-Methoxyindolyl-3-methyl (0.29) 4-Methylthiobutyl (0.13) 2-Hydroxy-3-butenyl (0.12) 4-Hydroxyindolyl-3-methyl (0.11)	
<i>B. oleracea</i> (Acephala Group) 'Blue Scotch Curled' kale	3-Indolylmethyl (3.63) Allyl (3.08) 4-Hydroxyindolyl-3-methyl (0.32) 4-Methoxyindolyl-3-methyl (0.11)	

^z*Brassica* leaves were divided along the midrib. Half of each leaf was freeze-dried for glucosinolate analysis and half was used for SPME measurement of isothiocyanates.

^yNumbers in parentheses are quantities expressed in $\mu\text{mol}\cdot\text{g}^{-1}$. Response factors relative to benzyl glucosinolate were determined with available standards of allyl, 3-butenyl, 2-hydroxy-3-butenyl, 2-hydroxy-2-phenylethyl, and 4-methylthiobutyl glucosinolates. Other response factors were arbitrarily set to 1.0.

^xNumbers in parentheses are chromatographic peak areas expressed as 10^5 area counts.

Table 5. Radial growth (% of control) of *Pythium ultimum* and *Rhizoctonia solani* exposed to 1.1, 2.2, or 3.3 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC.

AITC ($\mu\text{mol}\cdot\text{L}^{-1}$)	Fungus radial growth (% of control)	
	<i>P. ultimum</i> ^a	<i>R. solani</i>
0.0	100.0	100.0
1.1	19.7	86.9
2.2	0.0	73.1
3.3	0.0	45.3
Linear	**	**
Quadratic	**	*

^aExperiment was terminated at 48 h when *P. ultimum* in nontreated control reached the edge of the petri dish. Experiment with *R. solani* was terminated at 72 h for the same reason.

**, **Significant at $P \leq 0.05$ or 0.01, respectively.

were not included in our sample. Additionally, the rapid disappearance of the aldehydes (Z)-3-hexenal and (E)-2-hexenal after 1/4 h measured for Indian mustard may also have occurred with 'Florida Broadleaf' mustard.

GL CONTENT IN LEAVES. The GL profile found in the leaves varied both qualitatively and quantitatively (Table 4). Allyl GL in Indian mustard was present at the highest concentration of all GLs found and 'Michihili Jade Pagoda' Chinese cabbage had the most different GLs, more than twice the number found in 'Florida Broadleaf' mustard and 'Blue Scotch Curled' kale. The ratio of AITC level (peak area) measured in the jar headspace to allyl GL content ($\mu\text{mol}\cdot\text{g}^{-1}$) in leaves varied considerably among the four *Brassica* species containing allyl GL. This ratio was 39.3:1 and 39.9:1 for 'Florida Broadleaf' mustard and Indian mustard, respectively, 0.2:1 for 'Charmant' cabbage, and there was no AITC measured for 'Blue Scotch Curled' kale. These results indicate that conversion of allyl GL to AITC differed markedly among species, and suggest that GL content alone is not a sufficient predictor of fungicidal potential. Since ITC production depends on GL hydrolysis by myrosinase, the variation in myrosinase activity measured in different species (Bones, 1990; MacGibbon and Allison, 1970) may account for differences in the conversion of allyl GL to AITC. Differences in cellular organization may also be a factor since tissue maceration may not liberate equivalent amounts of myrosinase for GL degradation in different plant species. The relative surface area of myrosin cells viewed by electron microscopy can vary by species (Bones and Iversen, 1985) and could result in different availabilities of myrosinase following tissue disruption.

Indole GLs were found in all species and were predominant in 'Premium Crop' broccoli, 'Michihili Jade Pagoda' Chinese cabbage, and 'Blue Scotch Curled' kale, but no corresponding decomposition products were detected. The isothiocyanate corresponding to 1-methoxyindolyl-3-methyl GL was isolated in a 'low water' system (mainly hexane) but not identified under aqueous conditions (Hanley et al., 1990). Attempts to isolate 3-indolylmethyl ITC from 3-indolylmethyl GL have been unsuccessful and if 3-indolylmethyl ITC exists, it may immediately degrade to thiocyanate ion and indole-3-carbinol (McDanell et al., 1988). The finding by Mithen et al. (1986) that indole-3-carbinol inhibited growth of the fungus *Leptosphaeria maculans* (Desm.) Ces. & de Not. may be important for cruciferous crops that do not produce substantial quantities of isothiocyanates.

Other ITCs would not have been measured if the GL content was insufficient to result in detectable ITC levels. In this experiment but not in previous ones, 4-pentenyl ITC from 'Michihili Jade Pagoda' Chinese cabbage was detected. Changes in GL content with season

and stage of maturity may account for this observation. Production of sec-butyl ITC from the two mustards is noteworthy since there was no sec-butyl GL found in the leaf tissue, nor to our knowledge are there reports of the existence of such a compound. Perhaps sec-butyl ITC results from rearrangement of a decomposition product of some other GL.

FUNGAL RESPONSE TO AITC. AITC was fungitoxic to both fungi (Table 5). There was no growth of *P. ultimum* exposed to 2.2 or 3.3 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC. The AITC concentration needed for fungicidal activity against *R. solani* exceeded the highest concentration (3.3 $\mu\text{mol}\cdot\text{L}^{-1}$) tested in this experiment. Sarwar et al. (1998) reported that 1.6 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC was fungicidal to *R. solani* cultured in the bottom of 250-mL Erlenmeyer flasks. Our fungal cultures were placed at the top of our test vessels. Any tendency for AITC to settle downward would favor suppression of *R. solani* located beneath the point of origin of the AITC, perhaps explaining why our higher nominal levels of AITC were not fungicidal compared to those of Sarwar et al. (1998).

Based on a standard curve created by measuring known levels of pure AITC in 500-mL jars, approximately 1.1 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC was measured in jars with 'Florida Broadleaf' mustard and 2.2 $\mu\text{mol}\cdot\text{L}^{-1}$ from Indian mustard. *Pythium ultimum* was partially suppressed by 'Florida Broadleaf' mustard and 1.1 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC and completely suppressed by Indian mustard and 2.2 $\mu\text{mol}\cdot\text{L}^{-1}$ (Tables 1 and 5). *Rhizoctonia solani* was partially suppressed by both mustards as well as 1.1 and 2.2 $\mu\text{mol}\cdot\text{L}^{-1}$ AITC (Tables 1 and 5).

Results indicate that AITC is a significant factor in the suppression of *P. ultimum* and *R. solani* by volatiles emitted from macerated mustard leaves. All species inhibited both *P. ultimum* and *R. solani* although ITCs were not detected from 'Premium Crop' broccoli, 'Michihili Jade Pagoda' Chinese cabbage, and 'Blue Scotch Curled' kale leaves collected to test for fungal suppression. LOX pathway products were detected and since other investigators have found several of them to be fungitoxic (Mari et al., 1993; Urbasch, 1984), they may contribute to the inhibition we observed. The high level of (Z)-3-hexenal measured immediately after maceration of Indian mustard particularly merits additional investigation. GL content alone is not sufficient for assessing the fungicidal potential of *Brassica* species since some GL may not convert to ITCs, the extent of allyl GL conversion to AITC differs among species, and non-ITC compounds contribute to fungal suppression.

Control of *P. ultimum* and *R. solani* in the tomato industry may be possible by incorporation of Indian mustard residues into infested soil. Since not all cultivars of Indian mustard produce AITC (Mayton et al., 1996) and since AITC suppresses *P. ultimum* and *R. solani*, it will be important to select cultivars that release high levels of AITC upon maceration. The number of pathogens tested for susceptibility to natural plant volatiles should continue to be expanded. Determination that conversion of allyl GL to AITC varies by species indicates that selection of *Brassica* species for pathogen control should not rely solely on GL measurements. Future research should investigate other factors that influence the ultimate production of ITCs from disrupted *Brassica* tissue.

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