EVALUATION OF CHEMICAL AND BIOLOGICAL ISOTHIOCYANATE GENERATORS ON SOILBORNE PEST AND DISEASE CONTROL IN SQUASH

J. A. J. Desaeger, A. S. Csinos, K.W. Seebold
Plant Pathology
University of Georgia
P O Box 748
Tifton, GA 31793

Introduction

Many plants produce compounds called allelochemicals that directly or indirectly impact their biological environment. Glucosinolates (GSLs) are allelochemicals that occur throughout the agronomically important Brassicaceae (Cruciferae) family. There is sufficient evidence to suggest that glucosinolates contained in Brassicaceae tissues produce a variety of allelochemicals that are effective pesticides. Glucosinolate degradation products such as isothiocyanates (ITC's) have broad-spectrum biocidal activity, including insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects. For example, methylisothiocyanate (MITC) is used as a soil fumigant and is the active pesticidal agent produced from the degradation of synthetic dithiocarbamates (e.g., metam sodium) and diazines (e.g., dazomet). Because isothiocyanates have pesticidal activities and are dominant products formed from glucosinolates in soil, the use of Brassica species and other glucosinolate-producing species to control soilborne plant pests could be a valuable component of a methyl bromide alternatives program.

Two biofumigation options were evaluated in this test: (1) Mustard greens (*Brassica hirta)* were grown as a green manure crop and incorporated and (2) an experimental bio-pesticide (Bio-ITC) based on mustard seed components was incorporated. In addition two chemical isothiocyanate generators, metam sodium and dazomet, were included, either by themselves or in combination with the mustard green manure crop.

Materials and Methods

The study was located at the Blackshank Farm, CPES, Tifton, GA. The area had a history of soybeans, tobacco, and assorted vegetables. The area was prepared using all current University of Georgia Extension Service recommendations. The plot design was a split plot design with fumigants as main treatments and mustard cover crop and fallow plots as sub-treatments. Plots were arranged in randomized complete blocks consisting of single bed plots replicated five times. Each plot was 30 feet long and 30 in wide

Mustard (cv. Florida Broadleaf) was planted on 13 February 2003 with a Stanhay planter. Mustard was grown until 30 April 2003. Fallow plots were not planted and remained practically bare throughout the mustard growing period. On 30 April, 2003, the mustard cover was cut with a Flail mower, fertilizer (10-10-10) applied and plots were rototilled. Dazomet treatments were applied at 300 lbs/A, and biopesticide (Bio-ITC) at 1000 lbs/A by spreading the material out over the entire plot area. Beds were shaped and covered with 1 mil black polyethylene with drip tape in the center of the bed approximately 1in. deep. The following day, metam sodium was drip-applied at 50 gal/A (Table1).

Squash seedlings, cv. Croockneck, were produced in nutrient tray system to the 4-leaf stage. A single plant was transplanted using a mechanical type transplanter, which cuts holes in the plastic just ahead of the planters in the center of the plastic bed adjacent to the drip tape on 20 May. Plant spacing was 12 in.

As per the recommendation of the University Of Georgia Extension service, all plots received 500 lbs of fertilizer prior to mustard planting and 700 lbs. of fertilizer (10-10-10) prior to plastic laying. Additional fertilizer on squash was added in the form of liquid fertilizer (NPK 20-20-20 and 8-0-4 alternated) injected through the irrigation tubing during the growing season. All squash plots were sprayed on a 4 to 7 day interval with Manex with Zinc (2.4 qt/A) plus Kocide LF (0.5 gal/A) and Bravo (2 pts/A) for control of foliar diseases, and Ambush (10 oz./A) alternating with Pounce 3.2 (6 oz./A), Asana XL (6 oz./A) and Avaunt (3 oz./A) for insect control.

Stand counts were made to record live plants on 27 May and 10 June and plant vigor ratings were done on 5 and 10 June. Plant vigor was rated on a 1 to 10 scale, 10 representing live and healthy plants and 1 representing dead plants.

Twelve cores of soil, 2.5-cm-diam × 25-cm-deep, were collected from the center of each plot before planting mustard (12 February), at harvest of mustard (30 April), and at planting (20 May) and harvest (4 July) of squash. Nematodes were extracted from a 150-cm^3 soil sub-sample using a centrifugal sugar flotation technique, except at planting when they were extracted in Baermann pans (to capture only active nematodes). On 17 June (at flowering stage) an early root gall evaluation was done on three plants per plot using a 0 to 10 scale, whereby, 0 = no galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small and some big galls, 5 = 25 % of roots severely galled, 6 = 50 % of roots severely galled, 7 =75 % of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, 10 = plant and roots dead. Again following final harvest on 4 July ten plants per plot were evaluated for root galls using that same scale.

All squash fruits were hand-harvested from the 15-ft center area of each bed (15 plants per plot). Each harvest was separated into marketable and cull fruits, counted, and weighed. There were a total of six harvests, on 16, 19, and 23, 26 and 30 June and 3 July.

All data collected was analyzed with an analysis of variance (P = 0.05) and means were separated using Duncan's Multiple range test.

Summary

Both the mustard cover crop and the fallow decreased root-knot nematode soil populations similarly (Table 1). Stubby root and spiral nematode populations increased slightly, more so in the mustard cover. Free-living nematodes decreased similarly in mustard and fallow plots. Soil populations of Pythium and Fusarium were significantly reduced following mustard as compared to fallow plots (Table 1). After incorporation of mustard and application of chemical and bio-pesticides, *Pythium irregulare* propagules in sachets showed survival only in the non-treated plots and lower survival in mustard-amended than non-amended plots (Fig. 1).*Rhizoctonia solani* propagules in sachets were not controlled in non-treated plots and showed lowest survival following dazomet and metam sodium in mustard-amended soils (Fig. 1).

Root-knot nematode populations at plant of squash, after incorporation of mustard and applying chemical and biological ITC, were still low for both mustard and fallow treatments, although somewhat higher following mustard (Table 2). This was due to the fact that mustard roots showed root-knot galls and allowed reproduction of the nematode. This was not the case in the fallow plots, where roots were largely absent (bare soil). Root gall indices were reduced following metam sodium and dazomet (Table 4). Gall indices were increased following mustard at three weeks after planting, but no longer at final harvest (Table 4). By harvest root-knot nematode soil populations were still less following dazomet as compared to non-treated plots (Table 3). Free-living nematodes were significantly greater following mustard plots (Table 3). Overall the mustard cover crop did not affect efficacy of dazomet, but slightly reduced the efficacy of metam sodium and Bio-UCC to control root-knot nematode,.

Squash yields were similarly good throughout the entire test (Tables 5, 6). Root-knot nematode pressure was limited and so were yield differences among different treatments.

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Table 1. Soil populations of plant-parasitic, free-living nematodes and soil fungi at planting and harvest of mustard cover crop, spring 2003, Black Shank Farm Tifton, GA.

	Plant-parasitic nematodes /150 cc soil				Free-living nematodes / 150 cc soil				Fungi (CFU) / g soil		
Time / Cover crop	Root-knot	Spiral	Stubby	Total	Bacteri-ovores	Fungi-ovores	Omni-ovores	Total	Pythium	Fusarium	Total
At planting of cover (14 February 03)											
Mustard	359	9	2	389	845	64	225	1133	22.4	10836	18608
Fallow	359	24	3	385	845	65	238	1147	23.0	11120	18904
At harvest of cover (30 April 03)											
Mustard	5	23 a	56 a	83 a	459 a	48	178 b	685	14.7 b	3907 b	8502
Fallow	4	6 b	16 b	25 b	257 b	37	254 a	548	29.6 a	5180 a	9256

Root-knot nematode (*Meloidogyne* spp.); Spiral nematode (*Helicotylenchus* spp.); Stubby root nematode (Trichodoridae); Free-living nematodes = non-parasitic nematodes

Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 2. Populations of plant-parasitic and free-living nematodes at planting of squash, following incorporation of mustard cover crop and application of pre-plant fumigants, summer 2003, Black Shank Farm Tifton, GA.

		Plant-parasitic nematode soil populations				Free-living nematode soil populations			
Fumigant	Cover crop	Root-knot	Spiral	Stubby	Total	Bacteri-ovores	Fungi-ovores	Omni-ovores	Total
Metam sodium	Mustard	0 b	0	0	0 c	1116 ab	82 bc	2 cd	1200 b
	Fallow	0 b	0	0	0 c	440 d	4 d	8 cd	452 c
Dazomet	Mustard	0 b	0	0	0 c	1390 ab	18 dc	0 d	1408 ab
	Fallow	0 b	0	0	0 c	598 dc	2 d	0 d	600 c
Bio-ITC	Mustard	2 b	0	0	2 c	1996 a	338 a	12 b	2346 a
	Fallow	0 b	0	0	0 c	1878 a	354 a	6 bc	2238 a
None	Mustard	20 a	6	3	29 a	1990 a	354 a	46 a	2390 a
	Fallow	10 a	0	0	10 b	984 bc	104 ab	126 a	1214 b
F probability fumigation effect		<0.01	NS	NS	<0.01	<0.01	<0.01	<0.01	<0.01
F probability mustard effect		0.11	NS	NS	0.03	<0.01	<0.01	NS	<0.01

Nematode samples were collected on April 30; RKN = Root-knot nematode (*Meloidogyne* spp.); SN = Spiral nematode (*Helicotylenchus* spp.); SRN = Stubby root nematode (Trichodoridae); Free-living nematodes = non-parasitic nematodes Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 3. Populations of plant-parasitic and free-living nematodes at harvest of squash, following incorporation of mustard cover crop and application of pre-plant fumigants, summer 2003, Black Shank Farm Tifton, GA.

		Plant-parasitic nematode soil populations				Free-living nematode soil populations			
Fumigant	Cover crop	Root-knot	Spiral	Stubby	Total	Bacteri-ovores	Fungi-ovores	Omni-ovores	Total
Metam sodium	Mustard	90 ab	16 ab	2	108 ab	1122 ab	26	246 ab	1394 ab
	Fallow	34 ab	0 b	10	44 ab	636 c	20	298 ab	954 b
Dazomet	Mustard	4 b	0 b	6	10 bc	940 abc	22	136 bc	1048 ab
	Fallow	6 b	0 b	0	6 c	770 bc	10	86 c	916 b
Bio-ITC	Mustard	134 a	14 ab	12	160 a	1376 a	78	232 ab	1686 a
	Fallow	80 ab	6 ab	12	98 a	892 abc	76	316 a	1284 ab
None	Mustard	106 a	26 a	14	146 a	1382 a	32	268 ab	1682 a
	Fallow	219 a	12 ab	0	231 a	868 abc	32	234 ab	1134 ab
F probability fumigation effect		<0.01	0.08	NS	<0.01	NS	NS	0.01	0.06
F probability mustard effect		NS	0.07	NS	NS	<0.01	NS	NS	0.01

RKN = Root-knot nematode (*Meloidogyne* spp.); SN = Spiral nematode (*Helicotylenchus* spp.); SRN = Stubby root nematode (Trichodoridae); Free-living nematodes = non-parasitic nematodes.

Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 4. Effects of mustard cover cropping and other ITC-generators on plant vigor and root-gall indices (at 20 days and at final harvest) of subsequent croockneck squash, spring-summer 2003, Black Shank Farm Tifton, GA.

		Plant stand	Plant vigor [a]		Root gall index [b]	
Fumigant	Cover crop	At 2w	At 1 w	At 2 w	At 20 d	At harvest
Metam sodium	Mustard	28	7.8 ab	8.8 ab	0.3 d	1.8 abc
	Fallow	30	9.0 a	9.4 a	0.2 d	0.6 c
Dazomet	Mustard	26	6.6 b	7.3 c	0 d	0.6 c
	Fallow	29	8.0 ab	8.4 abc	0 d	0.5 c
Bio-ITC	Mustard	28	7.8 ab	8.6 abc	1.7 ab	2.2 ab
	Fallow	28	7.4 ab	8.3 abc	0.7 cd	1.4 bc
None	Mustard	28	7.6 ab	8.4 abc	2.4 a	2.9 ab
	Fallow	27	7.4 ab	7.9 bc	1.3 bc	3.2 a
F probability fumigation effect		NS	NS	0.03	<0.01	<0.01
F probability mustard effect		NS	NS	NS	0.01	NS

[a] Vigor was done a 1-10 scale with 10= live and healthy plants and 1=dead plants.
[b] Root Gall Index 0-10 scale whereby, 0 = no galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small and some big galls, 5 = 25 % of roots severely galled, 6 = 50 % of roots severely galled, 7 =75 % of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, 10 = plant and roots dead.
Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 5. Effects of mustard cover cropping and other ITC-generators on fruit yield number of subsequent crookneck squash, spring-summer 2003, Black Shank Farm Tifton, GA.

		Number of marketable fruits*							Number of cull fruits
Fumigant	Cover crop	Yield 1	Yield 2	Yield 3	Yield 4	Yield 5	Yield 6	Total	Total
Metam sodium	Mustard	8.6	12.6 abc	18.8	15.0	8.4	6.8	70.2	7.6
	Fallow	6.4	14.0 ab	14.6	10.4	8.0	6.6	60.0	5.8
Dazomet	Mustard	6.0	8.6 bc	19.2	18.4	12.2	8.4	72.8	7.4
	Fallow	7.0	7.4 c	15.4	14.8	12.0	7.8	64.4	6.6
Bio-ITC	Mustard	8.2	8.4 bc	15.8	16.6	11.8	7.0	67.8	5.0
	Fallow	6.4	14.8 a	21.4	11.6	7.6	11.8	73.6	8.8
None	Mustard	4.8	7.2 c	16.6	14.6	11.2	7.0	61.4	7.0
	Fallow	6.2	7.8 c	18.4	10.4	9.0	8.0	59.8	9.6
F probability fumigation effect		NS	0.01	NS	NS	NS	NS	NS	NS
F probability mustard effect		NS	NS	NS	0.04	NS	NS	NS	NS

* per 15 ft bed length

Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 6. Effects of mustard cover cropping and other ITC-generators on fruit yield weight of subsequent croockneck squash, spring-summer 2003, Black Shank Farm Tifton, GA

		Weight of marketable fruits (lbs)*							Weight of cull fruits
Fumigant	Cover crop	Yield 1	Yield 2	Yield 3	Yield 4	Yield 5	Yield 6	Total	Total
Metam sodium	Mustard	1.6	2.8 c	8.4	7.1 ab	5.3	2.4	27.5	1.9
	Fallow	1.9	2.9 c	9.1	4.9 b	4.7	3.1	26.5	3.5
Dazomet	Mustard	1.9	2.7 c	8.5	8.5 ab	7.0	2.6	31.2	3.1
	Fallow	1.8	2.5 c	8.2	7.8 ab	6.7	2.2	29.3	2.1
Bio-ITC	Mustard	2.9	3.2 bc	9.0	10.6 a	6.8	3.0	35.5	2.2
	Fallow	2.2	5.1 ab	10.5	5.7 ab	3.6	4.3	31.4	3.2
None	Mustard	3.1	4.2 abc	9.2	8.7 ab	4.2	2.6	31.9	3.1
	Fallow	2.3	5.4 a	8.1	6.3 ab	4.0	2.5	28.7	2.8
F probability fumigation effect		NS	0.01	NS	NS	NS	NS	NS	NS
F probability mustard effect		NS	NS	NS	0.04	NS	NS	NS	NS

* per 15 ft bed length

Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Table 7. Effects of mustard cover cropping and other ITC-generators on fungal soil populations (CFU/g soil), spring-summer 2003, Black Shank Farm Tifton, GA

		At plant squash			At harvest squash		
Fumigant	Cover crop	Pythium	Fusarium	Total	Pythium	Fusarium	Total
Metam sodium	Mustard	16	608	13712	22	2128	24320
	Fallow	6	192	5680	1	2544	24832
Dazomet	Mustard	1	12112	8096	26	1392	12400
	Fallow	3	160	8720	12	1200	15488
Bio-ITC	Mustard	10	3376	6848	19	6160	24528
	Fallow	26	2432	7968	30	5248	33936
None	Mustard	12	6912	19664	25	5568	20880
	Fallow	10	3872	19584	35	7360	16896
F probability fumigation effect		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
F probability mustard effect		0.01	<0.01	0.05	NS	NS	NS

Data are means of five replications. Means in the same column followed by the same letter are not different (P = 0.05) according to Duncan's multiple range test. No letters indicate non-significant difference; NS = not significant.

Fig. 1. Effects of mustard cover cropping and other ITC-generators on fungal propagule survival following bio- and chemical fumigation, spring-summer 2003, Black Shank Farm Tifton, GA