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Weed Control in Soybean (*Glycine max*) with Green Manure Crops[1]

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Abstract: Greenhouse and field experiments were initiated to evaluate rapeseed and mustard species as green manure crops for weed suppression. Under greenhouse conditions incorporating 20 g fresh wt leaf and stem tissue of rapeseed, two white mustards, and brown mustard into 450 g Sharpsburg, silty clay loam soil resulted in significant reductions in weed emergence, biomass, and height. Kochia, shepherd's-purse, and green foxtail emergences were reduced by all green manure crops. Redroot pigweed emergence was reduced by all green manure crops except brown mustard, and velvetleaf emergence was reduced by white mustards only. Kochia and shepherd's-purse fresh weights were reduced by all green manure crops, while redroot pigweed and velvetleaf fresh weights were reduced by brown mustard and white mustard var. Salvo. Green foxtail fresh weight was reduced by all green manure crops except rapeseed. With the exception of shepherd's-purse, no relationship between glucosinolate content of the incorporated green manure and suppression of weed growth was found. Under field conditions, early spring-planted green manure crops reduced early season weed biomass in soybean at one of the two locations. Mustard species as green manure crops reduced total weed biomass in soybean by 40% 4 weeks after emergence (WAE) and 49% 6 WAE. Soybean biomass and yield were sometimes reduced by the incorporation of green manure crops in treatments containing weeds; however, hand-weeded plots with green manure treatments yielded similar to hand-weeded plots without green manure.

Nomenclature: Green foxtail, *Setaria viridis* (L.) Beauv. #[3] SETVI; kochia, *Kochia scoparia* (L.) Schrad. # KCHSC; large crabgrass, *Digitaria sanguinalis* (L.) Scop. # DIGSA; redroot pigweed, *Amaranthus retroflexus* (L.) # AMARE; shepherd's-purse, *Capsella bursa-pastoris* (L.) Medikus. # CAPBP; velvetleaf, *Abutilon theophrasti* Medikus # ABUTH; brown mustard, *Brassica juncea* (L.) 'Greenwave'; rapeseed, *Brassica napus* (L.) 'Jupiter'; soybean, *Glycine max* (L.) Merr.; white mustard, *Brassica hirta* Moench. 'Martigena' and 'Salvo.'
Additional index words: Allelopathy, glucosinolate, isothiocyanates, mustard, rapeseed, SETVI, KCHSC, DIGSA, AMARE, CAPBP, ABUTH.
Abbreviations: DAE, days after emergence; DAP, days after planting; ITC, isothiocyanate; WAE, weeks after emergence; WAP, weeks after planting.

INTRODUCTION

Herbicides are and will continue to be a key component in most integrated weed management systems in the foreseeable future (Worsham 1991); however, herbicides are not without negative effects. These include crop injury due to herbicide residues or adverse environmental interactions, potential groundwater contamination, increase in herbicide-resistant weeds, weed species shifts, and increased cost to the producer. It is unlikely that weeds can be managed by any single method because of weed species diversity, variable germination periods, and highly divergent life cycles (Waddington 1978). There is a need to develop ecologically and environmentally sound methods of weed control (McWhorter and Barrentine 1988).

Allelopathy, recognized since the third century BC, is a possible alternate weed management strategy (Putnam and Duke 1978). Phytotoxic green manure crops and cover crops represent readily available weed control strategies that could be used in combination with conservation tillage practices such as no tillage and minimum tillage (Wolf et al. 1984). Members of Brassicaceae have frequently been cited as allelopathic crops (Bell and Muller

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[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

1973; Chew 1988). Some *Brassica* species have harmful effects on crops including reduced seed germination and emergence of subsequent small-grain crops when grown in rotation (Bialy et al. 1990; Erickson and Duke 1978; Muehlchen et al. 1990).

In a natural grassland community, allyl-isothiocyanates (ITC) isolated from black mustard [*Brassica nigra* (L.) W.J.D. Koch] residues inhibited establishment of grass species, and *Bromus rigidus* Roth, sensu Am. anctt. seed germination (Bell and Muller 1973). Benzyl-ITC, a breakdown product of white mustard (Josefsson 1968; Tollsten and Bergstrom 1988), was phytotoxic to velvetleaf, sicklepod [*Senna obtusifolia* L. formerly *Cassia obtusifolia* L.], and sorghum [*Sorghum bicolor* (L.) Moench]. Other breakdown products of glucosinolate like ionic thiocyanate (SCN-) inhibited the root or shoot growth of many crop species (Brown and Morra 1993; Brown et al. 1991). Volatile compounds like isoprenoid and benzenoid released from *Brassica* tissue degradation may suppress weed growth (Tollsten and Bergstrom 1988).

Rapeseed and mustard green manure crops suppress several common potato (*Solanum tuberosum* L.) pests (Boydston et al. 1994; Mojtahedi et al. 1991; Muehlchen et al. 1990). *Brassica* species synthesize large quantities of glucosinolates (Fenwick et al. 1989), which are hydrophilic, nonvolatile thioglucosides (Vaughn et al. 1993). Glucosinolates themselves possess limited biological activity until they are hydrolyzed by the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1) to produce isothiocyanates (Chew 1988).

The glucosinolates are stored in vacuoles, while myrosinase is cell-wall bound (Chew 1988). When plants are injured or mechanically damaged, this enzyme catalyzes the rapid hydrolysis of glucosinolates to D-glucose, HSO^{-4}, and a multitude of physiologically active products including isothiocyanates, thiocyanates, organic nitriles, and oxazolidine-2-triones. The ITCs exhibit a wide range of physiological effects in plants, insects, and microorganisms (Brown et al. 1991; Chew 1988; Fenwick et al. 1982; Tollsten and Bergstrom 1988). Therefore, rapeseed and mustard may have the potential to provide short-term weed suppression.

Field studies indicate that the critical period to control weeds growing with soybean to prevent yield loss is approximately the first 4 to 6 wk after soybean emergence (Worsham 1991; Zimdahl 1980). Experiments have shown that allelopathic suppression of weeds is usually adequate only for the first few weeks after planting, and subsequent weed control strategies are often necessary (Worsham 1989). Due to their allelopathic potential, rapeseed and mustard species may have a role in early season soybean weed management.

The objectives of this research were to determine the effects of rapeseed and mustard green manure crops on the emergence and growth of kochia, shepherd's-purse, green foxtail, redroot pigweed, and velvetleaf in the greenhouse, and to evaluate weed control and potential crop injury using rapeseed and mustard species as green manure crops preceding soybean in the field.

MATERIALS AND METHODS

Greenhouse Experiments. Green manure crops tested were rapeseed, two white mustards, and brown mustard. The five weed species studied were kochia, green foxtail, shepherd's-purse, redroot pigweed, and velvetleaf.

Green manure crops were planted in 23-cm-diam fiber pots filled with sand, soil (Sharpsburg, silty clay loam), and peat in the ratio of 1:1:1 by weight. Plants were randomly thinned to four plants per pot 1 week after emergence (WAE). Pots were watered daily and fertilized weekly with 100 ml of solution containing 0.6 g N/L, 0.2 g P/L, and 0.4 g K/L. The day/night temperature was 27/21 C, respectively. Day length was extended to 16 h with sodium vapor lamps delivering a photosynthetic photon flux density of 700 $\mu E/m^2/s$.

Shoots and leaves of mustard and rapeseed species were harvested 4 and 8 WAE, respectively. Mustard species had begun to flower at harvest, while rapeseed never flowered for the duration of the experiment. Rapeseed averaged 40 cm in height and produced eight leaves; mustard species were 80 cm tall with 12 leaves. Plants from five 23-cm pots provided enough biomass to treat thirty 500-ml pots.

Stems and leaves of green manure crops were cut into 0.5-cm^2 pieces, and 20 g fresh wt was uniformly mixed into 454 g air dry soil. The amended soil was placed into 500-ml polypropylene pots with holes drilled for drainage. Nonamended soil was included for comparison. Twenty-five weed seeds were placed on top of moistened soil and covered to a depth of 5 mm with nonamended soil. Pots were watered to field capacity each day and fertilized once a week with 50 ml of fertilizer solution mentioned previously. Weed seeds were randomly thinned to 10 per pot 1 week after planting (WAP), if emergence was more than 10. Weed emergence was recorded 1 WAE, and plant height, fresh weight, and dry weight 2 WAE.

The experimental design was a randomized complete block with six replications, and the experiments were conducted twice. Data were subjected to analysis of variance

Table 1. Emergence, height, and fresh weight of five weed species after incorporation of 20 g fresh tissue of green manure crops to 454 g dry silty clay soil in the greenhouse.[a]

	Emergence (1 WAE[b])					Height (2 WAE)					Fresh weight (2 WAE)				
Green manure	KCHSC	CAPBP	SETVI	AMARE	ABUTH	KCHSC	CAPBP	SETVI	AMARE	ABUTH	KCHSC	CAPBP	SETVI	AMARE	ABUTH
	No./pot					cm					g/pot				
None	8.9 a	9.2 a	8.8 a	7.8 a	7.4 a	9.3 a	4.3 a	15.3 a	8.5 a	6.2 a	0.34 a	0.14 a	0.49 a	0.54 a	0.55 a
Rapeseed	6.4 b	4.9 b	6.5 b	4.8 bc	6.0 ab	4.5 bc	3.1 bc	13.7 ab	8.0 ab	6.9 ab	0.15 bc	0.06 b	0.38 ab	0.43 a	0.42 ab
Brown mustard	4.5 b	5.7 b	5.8 b	6.6 ab	6.4 ab	6.9 b	4.2 ab	10.6 b	5.4 bc	4.3 bc	0.23 b	0.07 b	0.25 bc	0.31 b	0.18 c
White mustard															
Martigena	5.2 b	4.3 b	5.9 b	4.2 c	5.2 b	5.7 b	2.5 c	11.3 b	5.1 bc	4.8 bc	0.15 bc	0.04 b	0.23 bc	0.42 a	0.46 ab
Salvo	4.6 b	5.6 b	5.5 b	5.5 bc	5.1 b	2.7 c	2.8 bc	5.2 c	3.6 c	3.3 c	0.10 c	0.06 b	0.13 c	0.31 b	0.37 b

[a] Means within a column followed by the same letter are not different at 5% level by LSD test.

[b] WAE, weeks after emergence.

within and across experiments and were averaged across experiments where appropriate. Treatment means were separated using Fisher's Protected LSD at the 5% level.

Field Experiments. Experiments were conducted during 1994 at the University of Nebraska-Lincoln and at the University of Nebraska, Northeast Research and Extension Center, Concord, NE. The soil at Lincoln was a Zook silty clay (fine montmorillonitic, mesic, Cumulic Haplaquolls) with 2.7% organic matter and pH 6.3. The soil at Concord was a Kennebec silt loam (fine-silty mixed mesic Cumulic Hapludolls) with 3.1% organic matter and pH 5.9.

The six treatments included four green manure plots, one no green manure control plot, and one no green manure herbicide-treated plot. The experimental design was a split plot with four replications. The 9- by 6-m main plots were green manure treatments, and the subplots were weedy vs. weed free.

The green manure crops were planted on March 24, 1994, in Lincoln and on April 4, 1994, in Concord at a seeding rate of 6.7 kg/ha and a row spacing of 25 cm. Two samples for biomass from 1 m^2 each and one 0.5-m^2 sample for glucosinolate analysis were taken at 46 and 56 days after emergence (DAE) at Lincoln and Concord, respectively. The samples for glucosinolate analysis were frozen at -20 C for later analysis. The green manure crops were then cut on the same day using a sickle bar mower at Lincoln and a rotary mower at Concord and were immediately incorporated to a depth of 10 cm using a power-driven, tractor-mounted rototiller. Control plots were rototilled in addition to green manure plots.

'Resnik' soybean was planted immediately after green manure incorporation in 76-cm-wide rows. The main plots contained eight rows of soybean. Imazethapyr was applied at 0.07 kg ae/ha, 3 WAE of the soybean, in the herbicide control plots. Half of all plots were kept weed free by cultivation and hand weeding the remainder of the season. Soybean emergence and height were recorded 4 WAP in 1 m of row. Weed and soybean biomass samples were taken from each plot 4 and 6 WAE. Weed species composition, height, and number were also recorded. Major weed species present in both locations were velvetleaf, redroot pigweed, green foxtail, and large crabgrass. Soybean yields were harvested from two 5.5-m rows per plot 179 days after planting (DAP) and four 5.5-m rows per plot 183 DAP in Lincoln and Concord, respectively.

Data were analyzed as a randomized complete block design for weed biomass only since data were collected from only weedy plots. Data were subjected to analysis of variance within and across experiments and were averaged across experiments where appropriate. Treatment means were separated using Fisher's Protected LSD at the 5% level.

Glucosinolate Analysis. Freeze-dried plant material from greenhouse and field experiments was ground and glucosinolates were extracted by the procedure described by Tholen et al. (1989). Two extractions were made with boiling water. The supernatant was removed, and lead and barium acetate were added. The supernatant was applied to a DEAE-Sephadex A-25 (50 mg) column. The column was washed with aqueous formic acid followed by water, and glucosinolates were eluted with potassium sulfate. Thymol and sulfuric acid were added to the eluent and heated in a water bath at 100 C for 45 min. A spectrometer[4] was used to measure solution absorbance at 505 nm. Standard curves were prepared with known concentrations of sinigrin (allyl glucosinolate).

RESULTS AND DISCUSSION

Greenhouse. All green manure crops reduced emergence of kochia, shepherd's-purse, and green foxtail 1 WAE (Table 1). Pigweed emergence was reduced by all green manure crops except brown mustard, and velvetleaf emer-

[4] UV-2101 PC Shimadzu UV-VIS Scanning Spectrometer, Shimadzu Corporation, Kyoto, Japan.

Table 2. Total glucosinolate incorporated in greenhouse and in field experiments.[a]

Green manure	Greenhouse			Lincoln			Concord		
	Glucosinolate content	Dry wt	Glucosinolate incorporated	Glucosinolate content	Dry wt	Glucosinolate incorporated	Glucosinolate content	Dry wt	Glucosinolate incorporated
	μmole/g	g/m²	mmole/m²	μmole/g	g/m²	mmole/m²	μmole/g	g/m²	mmole/m²
Rapeseed	9.9 a	246 a	2.5 a	—[b]	—	—	—	—	—
Brown mustard	9.8 a	246 a	2.4 a	8.5 a	109 a	0.9 a	6.5 a	77 a	0.5 a
White mustard									
Martigena	6.6 b	246 a	1.6 b	6.6 a	50 b	0.3 b	5.7 a	62 a	0.4 a
Salvo	9.3 a	246 a	2.3 a	7.9 a	139 a	1.1 a	8.7 b	134 b	1.2 b

[a] Means within a column followed by the same letter are not different at 5% level by LSD test.

[b] Data from rapeseed are not included due to low germination in the field experiments.

gence was reduced only by the white mustards, Martigena and Salvo.

Height of all weeds 2 WAE was reduced by adding the white mustards, Martigena and Salvo, to the soil (Table 1). Brown mustard added to the soil reduced height of all weed species except shepherd's-purse, while rapeseed reduced height of only kochia.

Mean separation of treatments was similar for fresh weight and dry weight; hence, only fresh weight data are presented. Brown mustard and Salvo white mustard reduced the fresh weight of all weeds compared to nonamended controls (Table 1). Rapeseed reduced fresh weight of kochia and shepherd's-purse. Martigena white mustard reduced fresh weight of kochia, shepherd's-purse, and green foxtail. Results from these data are in agreement with previous research by Boydston and Hang (1995). In their experiments, the addition of rapeseed and mustard tissue reduced emergence and/or biomass of hairy nightshade (*Solanum sarrachoides* Sendtner) and green foxtail.

The amounts of glucosinolate incorporated for rapeseed, brown mustard, and Salvo white mustard were similar (Table 2); however, rapeseed caused height and biomass reduction of only two of five weed species tested. Brown mustard and Salvo white mustard reduced height and fresh weight of all species; with one exception, brown mustard did not affect shepherd's-purse height. Martigena white mustard did not reduce fresh weight of pigweed and velvetleaf, but total glucosinolates were lower. White mustard with a total glucosinolate content of 4 to 5 μmol/g dry wt reduced hairy nightshade and green foxtail biomass by 84 and 70%, respectively (Boydston and Hang 1995). In our studies, total glucosinolate content averaged 6 to 9 μmol/g dry wt. Shepherd's-purse was the only weed species in which glucosinolate content correlated significantly (−0.86) with reductions in growth (data not shown). No other relationship was found between glucosinolate content and observed weed suppression.

Upon plant injury or mechanical damage, glucosinolates undergo enzymatic hydrolysis to produce ITC and other breakdown products, which have been demonstrated as toxic to a number of plant species (Putnam 1983). Differences in weed emergence may be due to variation in both quality and type of ITC produced. Smelt and Leistra (1974) reported that concentration and length of exposure to ITC influences weed emergence. If allelopathy from ITCs was involved in reducing weed growth and development shown in our experiments, a relationship should exist between kind and amount of glucosinolates produced, and inhibition. Rapeseed, brown mustard, and Salvo white mustard produce 3-butenyl-ITC, allyl-ITC, and p-hydroxybenzyl-ITC, respectively (Van Etten and Tookey 1979). Thus, individual ITC may suppress weeds differently (Dale 1986; Teasdale and Taylorson 1986; Wolf et al. 1984), and conversion rates from glucosinolate to ITC may be different between species. However, this hypothesis was not tested in these experiments.

Field. Rapeseed failed to emerge at both locations in 1994; therefore, data were not included. Dry matter yield and glucosinolate content varied among green manure crops, affecting the total glucosinolate incorporated into soil at each location (Table 2). In both locations, Salvo white mustard produced the greatest amount of glucosinolate incorporated into soil, followed by brown mustard and Martigena white mustard. However, these amounts were 63 to 82% lower than the amount incorporated in greenhouse experiments. Glucosinolate contribution from roots was not taken into account. Lower green manure production in the field could be due to the short amount of time in spring to develop an adequate biomass before soybean planting, moisture availability, and/or fluctuating spring temperatures in Nebraska.

Table 3. Effect of green manure crops on weed height and weed biomass in field experiments.[a]

Green manure	Height (4 WAE[b])			Total weed biomass dry wt			
	Lincoln			Lincoln		Concord	
	AMARE	ABUTH	Grasses[c]	4 WAE	6 WAE	4 WAE	6 WAE
	cm			g/m²			
None	71.9 a	65.4 a	61.4 a	33.2 a	132.7 a	15.2 a	104.3 a
Brown mustard	49.3 b	49.6 b	40.6 b	19.8 b	76.0 b	14.6 a	92.6 a
White mustard							
Martigena	47.9 b	45.5 b	33.2 c	20.6 b	78.0 b	14.9 a	96.4 a
Salvo	46.6 b	48.2 b	41.0 b	15.2 b	64.0 b	15.0 a	78.2 a

[a] Means within a column followed by the same letter are not different at 5% level by LSD test.

[b] WAE, weeks after emergence.

[c] Grass species include green foxtail and large crabgrass.

Weed Growth. Herbicide-treated plots remained weed free throughout the growing season. There were no significant differences among weed species and no interaction between green manure crops and weed species, so data were averaged over weed species.

At Lincoln, the three green manure crops reduced height of pigweed, velvetleaf, and annual grasses (mixture of green foxtail and crabgrass) at 4 WAE (Table 3). Mustard species reduced total weed biomass in soybean by 40% 4 WAE and 49% 6 WAE.

At Concord, heights of the same weed species were not reduced by incorporation of green manure crops. At both sampling dates, the green manure crops did not reduce weed biomass compared to the control. Green manure crops were more mature and beginning to set seeds at Concord when incorporated. Clossais-Besnard and Larher (1991) examined glucosinolate profiles of *Brassica* species at different growth stages. The greatest reduction in growth occurred when the *Brassica* species were incorporated into the soil just before and during early flowering. Soil type and moisture conditions were different between the sites. Concord received a higher amount of rainfall after incorporation of green manure crops. Also, there was less uniformity in growth and distribution of weeds in Concord, resulting in greater variability.

Soybean Growth. The influence of green manure on crop growth and yield is as important as reductions in weed growth. Although no difference in soybean emergence occurred, height reduction was observed 4 WAE in green manure-treated plots at Lincoln; however, there was no difference at 6 WAE (data not shown). At Lincoln and Concord, no significant differences in soybean biomass were observed in the weed-free subplots of green manure crops compared to herbicide control plots at 4 and 6 WAE (Table 4). Furthermore, no significant differences in soybean yield were observed. However, in the weedy plots, growth reduction was evident in all green manure treatments in Lincoln 6 WAE and two of the green manure treatments in Concord 4 WAE. Soybean yield in weedy no-green manure treatments was reduced 57 and 30% in Lincoln and Concord, respectively, compared to herbicide-treated plots (Table 4).

Although significant reduction in weed growth occurred in Lincoln early in the season, weed suppression by the green manures was not enough to prevent soybean

Table 4. Soybean biomass and yield from green manure plots in Lincoln and Concord, NE, in 1994.[a]

Green manure	Biomass								Yield			
	Lincoln				Concord							
	4 WAE[b]		6 WAE		4 WAE		6 WAE		Lincoln		Concord	
	WF[b]	W[b]	WF	W	WF	W	WF	W	WF	W	WF	W
	g/m²								kg/ha			
Herbicide	28 a	22 ab	91 a	75 a	30 a	29 a	95 a	92 a	3,374 a	3,341 a	2,953 a	2,860 a
None	31 a	26 a	98 a	68 a	33 a	32 a	93 a	92 a	3,273 a	1,437 b	3,042 a	2,002 b
Brown mustard	26 a	16 c	89 a	53 b	27 a	23 b	92 a	64 b	3,278 a	1,437 b	2,687 a	1,487 bc
White mustard												
Martigena	29 a	17 bc	94 a	43 b	31 a	30 a	85 a	75 a	3,509 a	1,470 b	2,628 a	1,173 c
Salvo	28 a	20 bc	88 a	53 b	29 a	24 b	87 a	79 a	3,273 a	1,069 b	2,805 a	2,116 ab

[a] Means within a column followed by the same letter are not different at 5% level by LSD test.

[b] WAE, weeks after emergence; W, weedy plots; WF, weed-free plots.

yield reductions. Cultivation of weed-free plots could have alleviated potential green manure effects on soybean growth. Weed control provided by the green manure crops might not be expected to prevent yield loss completely, but it should reduce it.

Significant allelopathic responses under greenhouse conditions are difficult to repeat in the field consistently (Boydston and Hang 1995). In the greenhouse, weed seeds were exposed directly to breakdown products of glucosinolates and only for a short time (2 wk), during which ITC and other products were present in high concentration. In the field, the data were recorded over a longer period of time and the ITCs may have volatilized (Tollsten and Bergstrom 1988). Teasdale and Taylorson (1986) have suggested that concentration of ITC and length of exposure is critical for weed suppression. Differences in kind and amount of glucosinolates present in green manure crops from greenhouse and field may also explain variable weed suppression. The reduction in weed biomass is not likely due to high carbon to nitrogen ratio created by incorporation of green manure in the soil because the plants were adequately fertilized in the greenhouse (Boydston and Hang 1995).

These data suggest that spring-planted *Brassica* green manure crops alone did not adequately suppress weed growth to prevent soybean yield loss. However, refinements like increasing green manure biomass by fertilization, using high glucosinolate content varieties, and understanding conversion rates of glucosinolates to ITC could make *Brassica* green manure crops a useful part of integrated weed management systems. In conclusion, these data suggest that the green manure crops reduced the emergence and growth of weeds in the greenhouse, but there was no relationship between total glucosinolate concentration and growth reduction. In the field, green manure crops reduced early season weed biomass, but response was varied between the two locations.

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