Vegetable crop emergence and weed control following amendment with different *Brassicaceae* seed meals

A.R. Rice¹, J.L. Johnson-Maynard^{1,*}, D.C. Thill², and M.J. Morra¹

¹Division of Soil and Land Resources, University of Idaho, Moscow, ID 83844-2339, USA. ²Plant Science Division, University of Idaho, Moscow, ID 83844-2339, USA. *Corresponding author: jmaynard@uidaho.edu

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Abstract

Brassicaceae seed meals produced through the oil extraction process release biologically active glucosinolate secondary products and may be useful as a part of biological weed control systems. Before meal can be used most efficiently, recommendations for suitable planting dates that maximize weed control but reduce crop injury must be determined. Our objectives were to determine the impact of 1 and 3% (w/w) meal applications of Brassica napus L. (canola), Brassica juncea L. (oriental mustard) and Sinapis alba L. (yellow mustard) on crop emergence and weed biomass in a growth chamber and field study. Results from the growth chamber experiment indicated that lettuce emergence was reduced by at least 75% when planted into 3% S. alba-amended soil earlier than 5 weeks after meal application. After 5 weeks, emergence was not different among treatments. Crop emergence was not reduced by any meal treatment as compared to the no-meal treatment in year 1 of the field study. In year 2, crop emergence in each 1.2-m row was inhibited by all meal treatments and ranged from 16 plants in the 3% B. juncea treatment to 81 plants in the no-meal treatment. The difference between emergence results in year 1 and year 2 is likely due to differing climatic conditions early in the season prior to irrigation, and the method of irrigation used. Redroot pigweed (Amaranthus retroflexus L.) biomass was 72–93% lower in 1% B. juncea and 3% treatments relative to the no-meal control in the first weed harvest of year 1. These same treatments had 87–99% less common lambsquarters (Chenopodium album L.) biomass. By the second weed harvest, redroot pigweed biomass in meal treatments $(0.02-1.6 \text{ g m}^{-2})$ was not different from that in the no-meal treatment (0.97 g m^{-2}) . Redroot pigweed biomass in 3% B. juncea plots was reduced by 74% relative to the no-meal treatment in the first harvest of year 2. This treatment also reduced common chickweed [Stellaria media (L.) Vill.] biomass by 99% relative to the 1% meal treatments. While pigweed biomass was reduced by 3% B. juncea in the early part of the season, by the second harvest this same treatment had the greatest pigweed biomass. Despite significant variability between years, 3% B. juncea did provide early season weed control in both years. Repeated meal applications, however, may be necessary to control late season weeds. Inhibition of crop emergence appears to be highly dependent on the amount and distribution of water and needs to be further studied in field settings.

Key words: mustard meal, canola, biological weed control, lettuce, beets, allelopathy

Introduction

Allelopathy is defined as 'any process that involves secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of biological systems'¹. The ability of allelopathic plants to inhibit seed germination, emergence, and growth may be useful as part of biologically based weed control systems^{2,3}. Certain plants in the *Brassicaceae* family are known to possess allelopathic properties, and their use as cover crops

is currently being studied in many different cropping systems⁴.

Biologically active secondary compounds such as isothiocyanates (ITCs), ionic thiocyanates (SCN⁻), nitriles, and oxazolidinethiones (OZT) are produced through the degradation of chemicals called glucosinolates that are found within *Brassicaceae* plant tissues^{2,5–7}. Myrosinase, the enzyme responsible for catalysis of glucosinolate degradation, is physically separated from glucosinolates, thus preventing secondary glucosinolate product formation

in living $plants^{7-9}$. Physical disruption of plant tissue through insect feeding or grinding is necessary for further degradation of the glucosinolates into ITCs and other secondary products^{8–10}.

Suppression of plant emergence by glucosinolate-derived allelochemicals has been documented^{3,11}. Reduced germination of wheat planted into pots amended with either chopped black mustard [Brassica nigra (L.) Koch] or garden cress (Lepidium sativum L.) tissues has been reported⁵. In the same study, highly volatile allyl-ITC released by Brassica juncea tissue was found to be as effective as the commercial soil fumigant methyl ITC at inhibiting germination of several crop species⁵. A greenhouse study by Ju et al.³ showed that SCN⁻, released by Sinapis alba¹², inhibited the growth of tobacco and bean. Spiny sowthistle [Sonchus asper (L.) Hill], scentless mayweed (Matricaria inodora L.), barnyardgrass [Echinochloa crusgalli (L.) Beauv.], and blackgrass (Alopecurus myosuroides Huds.) were susceptible to the allelochemicals produced by B. juncea and Brassica napus mulches in a greenhouse study¹¹. ITCs appear to be capable of inhibiting germination while seeds are still in the dormant stage¹³, suggesting that they not only inhibit growth, but also germination.

The studies discussed above have focused on the allelopathic properties of Brassicaceae tissues either in fields where Brassicaceae crops have been used as green manures or cover crops, or in greenhouse studies where chopped tissues have been added to soil. While results of these studies generally report a significant reduction in weed seed germination, cover cropping may not be applicable in northern latitudes where the growing season is limited. An alternative to the use of the Brassicaceae cover crops is the seed meal, a by-product of the commercial oil pressing process for food and industrial oil and biodiesel production. Glucosinolates are concentrated within the seed material and are retained in the coldcrushing procedure¹² used in this study. An advantage of use of the meal is that myrosinase is preserved during crushing², but the low moisture content of the meal allows glucosinolates to remain stable in storage for extended periods of time. Once in contact with moisture, glucosinolate degradation to secondary allelopathic compounds is initiated¹⁴.

ITCs and other glucosinolate hydrolysis products released by *Brassicaceae* seed meal can inhibit crop seed germination, potentially limiting its use². Published studies of suitable planting dates for vegetable crops known to be sensitive to ITCs, however, are not found in the current literature. In order for *Brassicaceae* meal to be a viable and feasible means of weed control in farming systems, planting dates that optimize weed control, but reduce the phytotoxic potential of the allelopathic compounds to the crop must be determined, especially when crops are seeded directly into meal-amended soils. The main objective of this work was to determine suitable planting dates for lettuce, a crop known to be highly sensitive to ITCs² in a

growth chamber study. The potential of several *Brassica-ceae* meals to inhibit weed and crop seed germination in the field was also investigated.

Materials and Methods

Seed meal characterization

Meal was defatted and the glucosinolate concentrations determined using methods similar to that of the International Organization of Standardization¹⁵ as modified and reported by Borek and Morra¹². The procedure includes glucosinolate extraction with a 70% methanol/water solution and addition of 4-methoxybenzyl glucosinolate as an internal standard. Glucosinolates were then isolated using a DEAE anion exchanger and desulfated with sulfatase (Sigma-Aldrich, St. Louis, MO), after which time the samples were analyzed on a Waters 2695 HPLC separation module coupled with a Waters 996 photodiode array detector (PDA) and Thermabeam Mass Detector (TMD). Glucosinolates were identified with a combination of expected retention behavior (time, sequence) and their mass spectra, and quantified using response factors¹⁶. Total C and N contents of the meals were determined by dry combustion¹⁷ using a C/N/S analyzer (Elementar, Hanau, Germany).

Growth chamber study

A high-glucosinolate meal (S. alba), low-glucosinolate meal (B. napus), and no-meal treatment were used to determine the impact of active secondary products on lettuce germination over time. Pots $(10.2 \text{ cm} \times 10.2 \text{ cm})$ were filled with soil collected from a local organic-certified farm and amended with either 3% B. napus, 3% S. alba, or no seed meal. The soil was gently ground, passed through a 2-mm sieve and completely mixed prior to being placed in pots. The experiment was designed as a complete randomized block with ten replicates and was repeated twice. Growth chambers were maintained at 60% relative humidity, 24/21°C day/night temperature, and a 14-h photoperiod. Pots were surface-amended with meal every week for 6 weeks so a staggered succession of meal application dates occurred. Soil in pots was uniformly watered once a day and was not disturbed except at planting. Sixteen lettuce seeds were planted 1 week after the last meal application. All seeds were from the same seed lot and source. Emergence was determined by counting plants 3, 7, 10 and 14 days after planting.

Field methods

A field study was conducted on a certified organic farm in the Palouse region of northern Idaho. The soil was classified as Driscoll–Larkin silt loam, a Mollisol association¹⁸. For 3 years previous to the study, the field was fallowed and weeds were controlled using mechanical methods. The experimental design was a randomized

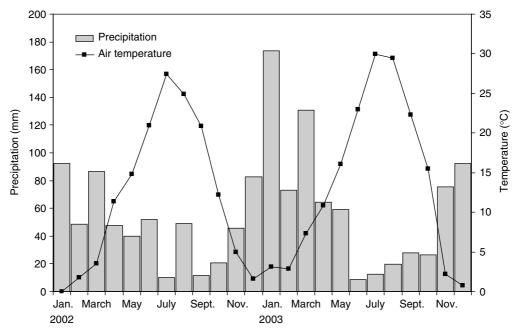


Figure 1. Total monthly precipitation and average monthly air temperature recorded for both years of the study. Data were recorded at a weather station located in Moscow, Idaho, approximately 8 km from the study site.

complete block split plot with six blocks as replicates and two crops as the split plot. Individual plots were 1.2 m^2 with 0.3-m borders between plots and a 1.5-m border around the entire site. Plots were re-located to a new area (approximately 20 m from the first plots) within the same field in the second year to eliminate any residual effects of the meal. Meal was obtained from the varieties Ida Gold (S. alba), Pacific Gold (B. juncea), and Athena and Sunrise (B. napus). Sunrise was used during the second year because Athena seed was not available. Treatments in year 1 included 1% B. napus, 3% B. napus, 1% B. juncea, 3% B. juncea, and no seed meal. Based on results from year 1, a second high-glucosinolate meal (S. alba) was added to the experimental design and treatments included 1% B. napus, 3% B. napus, 1% B. juncea, 3% B. juncea, 1% S. alba, 3% S. alba, and no seed meal in year 2. The seed meal rates of 1% (503 g meal plot⁻¹) and 3% (1509 g meal plot⁻¹) were chosen based on unpublished laboratory data that showed that these were the minimum rates necessary to control weeds, while minimizing phytotoxicity to the seeded crops. The weight of meal added to each plot was calculated on a meal: air-dry soil weight basis and a 3-cm depth of incorporation. The weight of soil in each plot was calculated using bulk density (mean value of $1.13 \,\mathrm{g \, cm^{-3}}$) measured by the core method¹⁹. A single application of meal was incorporated into all treatment plots with a rototiller on the same day. Lettuce and beets were chosen as crops for this study based on differences in seed size. Research has indicated that allelopathic sensitivity is possibly dependent on the size of the seed¹¹.

In the first year, plots were seeded on May 20, 14 days after meal application. Germination was extremely poor and the plots were re-planted 14 days later on June 3. Plots were planted 28 days after meal application during the second year (June 22). The seeding rates were determined based on the supplier's suggested rate that resulted in a 0.64-cm seed spacing for both beets and lettuce. Neither beet nor lettuce stands were thinned. Seeds were all obtained from the same source although lots varied between years. All plots were cleared of weeds at the time of seeding.

Since moisture is required for the production of secondary glucosinolate products, meal-amended plots were irrigated prior to planting in year 1, using a drip system. In year 2, precipitation from March to May equaled 255 mm, 80 mm greater than the amount received during this same time period in year 1 and 60 mm greater than the 30-year average value (Fig. 1). Due to wet field conditions, meal application, seeding, and weed and crop harvests in year 2 were carried out approximately 3 weeks later than in year 1. Irrigation in year 2 was not initiated until a week prior to planting and an overhead sprinkler was used. Although sprinkler and drip irrigations have been shown to produce similar lettuce yields²⁰, the use of two different irrigation systems in this study may complicate comparisons of seedling emergence between years. However, any impact of irrigation type on germination within a year should have been equal across all treatments.

Stands were assessed once each year by counting the number of crop plants that emerged in the entire middle row (1.2 m) within each plot. Weed biomass was determined two times during the growing season, on 25 June and 15 July for year 1 and 14 July and 11 August for year 2. Biomass of each weed species present in each 1.2-m^2 plot was collected by clipping at the soil surface. The biomass was dried in an oven at 60° C for 48 h and weighed.

Table 1. Glucosinolate concentrations in Brassicaceae seed meals.

Glucosinolate R-group (response factor)	Brassica napus 'Athena'	B. napus 'Sunrise'	Sinapis alba 'Ida Gold'	Brassica juncea 'Pacific Gold'
	μ mol g ⁻¹ of sample			
(2 <i>R</i>)-2-hydroxy-3-butenyl (1.09)	1.5 (0.30)	1.3 (0.09)	3.4 (0.25)	0.5 (0.18)
2-propenyl (1.00)				123.8 (15.31)
(2S)-2-hydroxy-3-butenyl (1.09)	0.4 (0.05)			
2-hydroxy-4-butenyl (1.09)	0.2 (0.09)		1.8 (0.66)	
(2R)-2-hydroxy-4-pentenyl (1.09)				0.5 (0.12)
4-hydroxy-benzyl (0.28)			148.1(26.75)	
Unknown (1.00)			9.1 (1.71)	
3-butenyl (1.11)	2.8 (0.60)	2.7 (0.31)		
4-hydroxy-3-indolylmethyl (0.28)	11.3 (3.52)	10.9 (3.25)		0.7 (0.26)
Unknown (1.00)			2.6 (0.75)	
Unknown (1.00)			0.74 (0.15)	
4-pentenyl (1.11)	1.3 (0.29)	1.4 (0.30)		
3-indolylmethyl (0.29)	0.9 (0.19)	0.8 (0.21)		
4-methylthiobutyl (1.00)	1.7 (0.48)			
<i>N</i> -methoxy-3-indolylmethyl (0.29)		0.1 (0.02)	0.01 (0.04)	0.6 (0.08)
Unknown (1.00)				1.3 (0.17)
Total	20.1 (2.71)	17.2 (2.16)	165.8 (20.32)	126.1 (14.38)

Blanks indicate the absence of a particular glucosinolate within a meal type. Standard deviations (based on five subsamples taken from the same lot of bulked meal) are shown in parentheses following means.

Data were statistically analyzed using the general linear model within SAS (Release 8.02) software (SAS Institute Inc., Cary, North Carolina). Emergence data from the growth chamber study were transformed by using an arcsine square root transformation. Due to the uneven distribution of weed species across the plots (certain species were only found in one or two plots) weed biomass data were separated by weed species and only the most common species were evaluated statistically. Means were separated with a pairwise *t*-test based on P < 0.05.

Results and Discussion

Meal characterization

The meals selected for this study provided a range from high (*B. juncea* and *S. alba*) to low (*B. napus*) glucosinolate content. The two *B. napus* meals contained the lowest total glucosinolate concentrations, ranging from 17 µmol g⁻¹ in Sunrise to 20 µmol g⁻¹ in Athena (Table 1). Total glucosinolate concentrations in the mustard meals substantially exceeded those in the *B. napus* control meals with *S. alba* meal containing 166 µmol g⁻¹ and *B. juncea* meal 126 µmol g⁻¹ (Table 1). The inclusion of different meals allowed us to not only compare glucosinolate type but also concentration. The experimental design did not allow us to specifically identify possible impacts of the meal on soil physical properties; however, any such effects should have been consistent among meal treatments.

ITCs produced by the specific glucosinolates contained in each seed meal have unique allelopathic properties. Similar to the findings of Minchinton et al.²¹, S. alba (variety Ida Gold) contained 4-OH-benzyl (Table 1), a glucosinolate that can form SCN⁻²² at pH values expected in soil¹². SCN⁻ is known to inhibit seed germination and seedling emergence^{3,22}. Mechanisms behind the toxicity of SCN⁻ are unclear, but a positive relationship between SCN⁻ tissue concentration and chlorosis has been demonstrated, suggesting that the physiological effect of SCN⁻ is related to its ability to chelate iron, thereby reducing iron availability for chlorophyll synthesis³. Similar to results reported by Quinsac et al.²³, the dominant glucosinolate in both B. napus varieties was 4-OH-indolyl-3-methyl $(10.9-11.3 \,\mu\text{mol g}^{-1})$. Although 4-OH-indolyl-3-methyl is a non-ITC-producing glucosinolate² it can potentially degrade to SCN^{-} in soil²⁴.

B. juncea (variety Pacific Gold) contained 2-propenyl glucosinolate (Table 1), consistent with the findings of Hanley et al.²⁵. 2-Propenyl ITC has been shown to be inhibitory to a number of organisms²⁶. Mechanisms for toxicity of 2-propenyl ITC are unclear, although it has been proposed that seed germination inhibition is a result of the ability of ITCs to bind to proteins, resulting in inhibition of metabolic processes²⁴.

The N contents of the low glucosinolate meals (5.2%) for Athena and 5.3% for Sunrise) were similar, to those found in *S. alba* (5.8%) and *B. juncea* (5.6%) meals. The C:N ratios (8.5:1 for *B. juncea*, 8.1:1 for *S. alba*, and 9.1:1 for *B. napus*) were also similar, suggesting that

differences in C:N ratios among meals should not confound the allelopathic effect on germination or weed growth.

Growth chamber

Analysis of data from the first and second growth chamber experiments showed that the treatment means had the same rank order and thus data were combined over experiments and analyzed using a pooled analysis. Lettuce plant emergence was the same for 7- and 14-day counts, thus only the 14-day count is presented. Seedling emergence in the B. napus treatment was lower than emergence in the nomeal treatment when lettuce was planted 1 or 2 weeks after meal amendment, indicating that this treatment had a shortterm effect on lettuce germination (Fig. 2). This may be due to the ability of 4-OH-indolyl-3-methyl found in *B. napus* meal to produce SCN^{-24} or the formation of nonglucosinolate-derived allelopathic products. Inhibition of lettuce seed germination by non-glucosinolate degradation products from *B. napus* seed meal has been suggested². The authors of this previous study concluded that nonglucosinolate-derived compounds were probably responsible for inhibition of seed germination although such compounds were not identified². In a two-year cover crop study, researchers found the high-glucosinolate mustard (var. Ida Gold) crops reduced weed biomass to the same degree as rapeseed (var. Dwarf Essex) and a lowglucosinolate canola (var. Hyola)⁴. Low-glucosinolate B. napus meal was also shown to suppress apple root infection by Rhizoctonia spp. and Pratylenchus penetrans²⁷. Together, results of past research suggest that B. napus tissues do produce biologically active compounds that are not derived from glucosinolates. Our data, however, suggest that these compounds are active over a relatively short period of time under optimal environmental conditions and that lettuce emergence was no longer delayed when planted 2 weeks after meal amendment.

Emergence was 3-17% of planted seeds in S. albaamended soil from week 1 to week 4 after meal application (Fig. 2), indicating that S. alba meal can suppress lettuce emergence if it is planted less than 5 weeks after meal application under these conditions. This corresponds to other literature suggesting that S. alba can inhibit plant emergence¹³. Although the influence of varying environmental conditions experienced in field situations on seedling emergence was not tested, the data suggest that it may be necessary to wait approximately 5 weeks before planting lettuce into meal-amended soil. Since lettuce grows best under cooler temperatures, meal must be applied early enough in the season to avoid temperatures above the optimum range for lettuce seed germination. Early season application dates may pose a problem in areas where the soil is too wet to work in early spring. Although it was not evaluated as part of this study, transplanting established plants may help reduce toxicity, allowing for earlier planting.

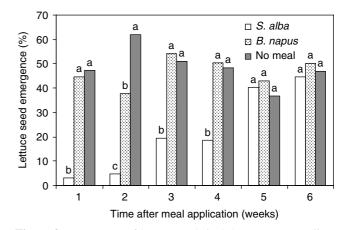


Figure 2. Emergence of lettuce seeds in 3% *B. napus-*, *S. alba-*, and no-meal-amended soils 2 weeks after planting in a growth chamber. Within a week, different letters indicate statistical difference among treatments.

It is important to note that the soils were kept moist throughout the growth chamber study. Pronounced wet and dry periods experienced in the field may alter the planting date to some extent, due to the requirement of moisture for the production of biologically active secondary compounds and its impact on volatilization, degradation reactions and leaching of these same compounds.

Field study

Lettuce and beet emergence. No significant interactions between crop and seed meal treatments for crop stand counts occurred in the field study for either year when crops were planted 28 days after meal amendment, thus the data are presented as averages for the two crops. For the first year, average stand counts, taken 22 days after planting, ranged from 39 plants per 1.2-m row in 1% B. juncea and 3% B. napus treatments to 23.5 plants per 1.2-m row in the no-meal treatment (Fig. 3A). Three percent B. napus and 1% B. juncea (both 39 plants per 1.2-m row) had significantly higher stand counts compared to the no-meal treatment (23.5 plants per 1.2-m row). Unlike data from year 1, emergence in the no-meal treatment was significantly higher (80.5 plants per 1.2-m row) than in all other treatments for the second year stand count (Fig. 3B). Crop emergence in the 3% B. juncea treatment (16.3 plants per 1.2-m row) was lower than in all other meal treatments.

Amendment with *B. napus* and *B. juncea* meals did not inhibit seedling emergence in year 1 compared to that measured in the no-meal plots when plots were seeded 28 days following meal application. This is consistent with results of the growth chamber study for *B. napus* that indicated no significant inhibition of germination after 2 weeks. In year 2, all meals reduced emergence relative to the no-meal treatment and 3% *B. juncea* inhibited crop emergence by at least 58% relative to the other meals when plots were seeded 28 days following meal application. The

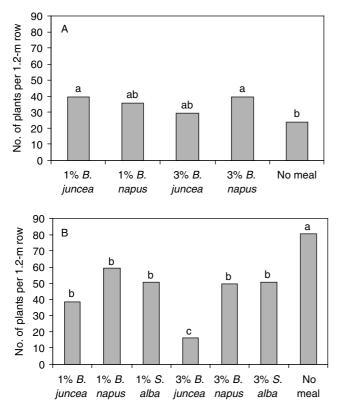


Figure 3. Crop stand counts for (A) year 1 and (B) year 2 of the field study. Data are pooled over beets and lettuce because there was no crop by seed meal interaction for either year. Different letters indicate statistical differences among treatments (P < 0.05).

only major differences between years 1 and 2 were in early season climatic conditions (80 mm more precipitation from March to May in year 2) (Fig. 1), type of irrigation system and the physical location of plots in the same field. Any impact of the change in plot location between years should have been apparent across all treatments and cannot explain inhibition in one year and not in the other, especially considering that the plots in both years were established in the same field. In year 1, irrigation water was added to the meal-amended plots using a drip system, while sprinklers were used in year 2. The lack of crop and weed emergence in the first planting in year 1 (14 days following meal application) indicated that sufficient water was present to initiate the formation of secondary glucosinolate products. Irrigation was continued during the 2-week period between the first and second seedings of year 1 ensuring that glucosinolate products were no longer being produced. Drip irrigation results in wetting of a relatively small area of soil²⁸, while the overhead sprinklers used in year 2 resulted in the wetting of the entire soil surface across each plot. Differences in wetting fronts could have altered the release and distribution of secondary products within the rooting zone between years, with the drip system favoring more rapid formation and disappearance (through volatilization or microbial decomposition) of secondary compounds.

Although we cannot entirely explain the variation between years, our field study results indicate that crop emergence can be reduced by high-glucosinolate seed meals. The data also highlight the importance of understanding the influence of environmental conditions and irrigation on the formation and retention of biologically active secondary compounds in field soils. The type and concentration of glucosinolates present in S. alba and B. napus meals are different (Table 1) yet these treatments inhibited emergence to the same degree in year 2 of the field study. This was not consistent with results of the growth chamber study. The lack of differences between these two treatments in the field after 28 days suggests that environmental conditions in the field allowed secondary compounds produced by *B. napus* to persist longer than was found in the growth chamber study (2 weeks). Distribution and losses of soluble SCN⁻ within the root zone in the field may have also been different than within the pots used in the growth chamber study.

Weed biomass

Due to the rarity of species in replicates (several species only occurred in one plot within a treatment) and the overall low weed biomass present, weed biomass from only the most predominant species present in each collection will be discussed. For the first weed biomass harvest during year 1, redroot pigweed and common lambsquarters were the dominant species present. Redroot pigweed biomass ranged from a mean of 3.5 g m^{-2} in the no meal treatment to $0.26 \,\mathrm{g}\,\mathrm{m}^{-2}$ in the 3% *B. napus* treatment. All meal treatments significantly reduced (P = 0.027 - 0.001) redroot pigweed biomass (59-93%) relative to the no-meal treatment (Fig. 4A). Biomass of common lambsquarters ranged from 0.01 g m^{-2} in the 3% *B. napus* treatment to 1.18 gm^{-2} in the no-meal treatment. All meals except 1% B. napus (P = 0.109), the lowest glucosinolate-containing meal, suppressed lambsquarters biomass relative to the nomeal treatment (P = 0.0048 - 0.0018). In the second weed harvest of year 1, redroot pigweed was the dominant species present and the only weed occurring in all treatments. Average redroot pigweed biomass ranged from 0.02 g m^{-2} in the 1% *B. napus* meal treatment to 1.6 g m^{-2} in the 1% B. juncea treatment (Fig. 4B). There was a marginally significant (P = 0.056) increase of redroot pigweed biomass in the 1% B. juncea treatment relative to that in plots treated with 1% B. napus.

Weed biomass in year 1 was low across all treatments. This is likely a reflection of the aggressive use of physical weed control methods such as tillage in the three-year fallow period previous to this study and the use of an efficient drip system that limits soil moisture outside of the crop rooting zone. Although the low weed biomass during year 1 makes interpretation of results somewhat difficult, both rates of *B. juncea* and the 3% *B. napus* treatment had significantly lower levels of redroot pigweed and common lambsquarters biomass in the first sampling. The lack of

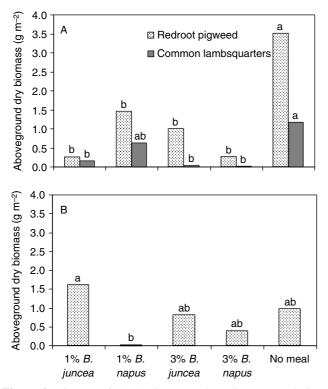


Figure 4. Biomass of the dominant weed species present during the (A) first and (B) second weed harvests of year 1. Differing letters indicate statistical differences among treatments (P < 0.05) within a weed species.

differences in weed biomass between meal treatments and the no-meal control during the second harvest indicates that weed control following meal application is short-term. This is consistent with laboratory results demonstrating the short half-lives (20–60 h depending on soil type) of ITCs in soil¹⁴. Concentrations of SCN⁻ are also expected to decrease relatively rapidly in soils (half-life of 60–120 h), as a result of microbial degradation²⁹.

The only weed species present in the first harvest of year 2 were redroot pigweed and common chickweed (Fig. 5A). Redroot pigweed was the dominant weed, with common chickweed making up only 4–23% of the total weed biomass within plots. Although it is difficult to clearly infer treatment effects, due to the low biomass of common chickweed present, biomass did tend to be higher in the 1% meal treatments (Fig. 5A) relative to the 3% treatments. Biomass of redroot pigweed was suppressed 74% by 3% *B. juncea* relative to the no-meal treatment and 3% *B. juncea* was the only treatment that inhibited redroot pigweed biomass compared to the no-meal treatment (Fig. 5A).

By the second harvest, redroot pigweed biomass within the 3% *B. juncea* treatment was 52% higher than it was in the no-meal treatment and was significantly higher than in all the other treatments (Fig. 5B). This is similar to the increase in redroot pigweed biomass in the 1% *B. juncea* treatment during the second harvest of year 1. Increased redroot pigweed biomass within *B. juncea* plots at the second harvest may be explained by direct and indirect

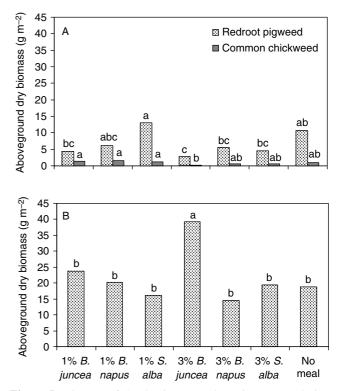


Figure 5. Biomass of the dominant weed species present during the (A) first and (B) second weed harvests of year 2. Differing letters indicate statistical differences among treatments (P < 0.05) within a weed species.

impacts of this meal and the specific growth characteristics and requirements of this weed species. Redroot pigweed is considered a nitrophile³⁰ and therefore its growth may be favored by mineralized N in meal-amended plots following the dissipation of allelochemicals. Although the C: N ratio and total N content of each meal were similar, ITCs produced by B. juncea meal are known to directly impact micro-organisms²⁴, the group of soil organisms that are responsible for N mineralization. Application of B. juncea meal may, therefore, result in patterns of N mineralization different from those expected from non-biologically active materials with similar C:N ratios. The impact of specific glucosinolates on mineralization in meal-amended soils should be further researched. Redroot pigweed is also able to germinate at any time during the season if soil moisture is adequate³¹. The ability to germinate throughout the season combined with reduced competition due to the impact of 2-propenyl ITC produced by B. juncea meal on earlier weeds would give redroot pigweed a competitive advantage. The redroot pigweed data indicate that meal application may result in higher weed biomass later in the season after the allelopathic effect has dissipated. The ability to promote late season weeds may limit the use of meal or require repeated applications during the growing season.

Contrary to results in the growth chamber study, significant weed suppression was not apparent in the *S. alba* treatment, the seed meal expected to produce the

most pronounced herbicidal effects due to the formation of SCN⁻²². The lack of statistical differences in weed biomass between S. alba and the no-meal treatment is likely due to a combination of the relatively high degree of variability in weed biomass and the expected behavior of SCN⁻ in soils. SCN⁻ has a relatively short soil residence time, with 40-95% being lost within a 6-day period in one study²⁹. Although 2-propenyl ITC released by B. juncea meal also has a short half-life of $20-60 h^{14}$, it is likely that the high precipitation plus irrigation water added during the second year resulted in retention of 2-propenyl ITC while the SCN⁻, being more water soluble and less volatile than 2propenyl, was leached through the soil profile. Thus, it is likely that SCN⁻ concentrations in the soil were low enough at the end of the 28-day-period before planting that it had no detectable influence on weed emergence. Because weeds were removed at the time of planting, any impacts of meal application on weed biomass prior to planting were not quantified.

Despite the difference in environmental conditions, irrigation, and planting and harvesting dates between years, 3% B. juncea inhibited early season (first harvest) weed biomass relative to the no-meal treatment in both years. The lack of significant differences between weed biomass in 3% B. juncea (high glucosinolate) and 3% B. napus (low glucosinolate) treatments during the first harvests indicates that glucosinolates may not be the only allelochemicals causing the reduction of weed biomass. The lack of weed control in the second harvests of both years indicates that meal application provides short-term weed control. An additional concern is an increase of redroot pigweed biomass in the second collections. The data suggest that weeds that are able to germinate throughout the year, or later in the growing season when temperatures are high, may benefit from mineralized N and/or lower competition once secondary agents that have relatively short half-lives in soil are no longer present.

Conclusion

Data from the growth chamber study suggest that sensitive crops such as lettuce should not be seeded directly into fields amended with high glucosinolate meals until 5 weeks after application. Meal, therefore, may need to be applied when relatively wet conditions are present in the field. For crops sensitive to ITCs, transplanting established seedlings or increasing the seeding density may help increase plant survival and yields in meal-amended soils. The influence of glucosinolate-derived secondary products on crop emergence appears to be highly dependent on soil moisture conditions. The negative impacts of relatively soluble SCN⁻ produced from *S. alba* meal may not be as drastic as expected from the growth chamber study, due to leaching of this soluble compound in irrigated field soils.

The field study results suggest that *Brassicaceae* meal can be used to control early season weeds. These results

were most consistently seen with the 3% *B. juncea* amendment. Single applications of meal, however, will likely not be adequate to control weeds throughout the growing season and may result in higher late-season weed biomass due to possible increases in plant-available nitrogen and reduced intraspecific competition. For these reasons, meal may not be a suitable method of weed control in all systems. Meal amendments, however, may still be beneficial in organic-certified fields. Without the use of synthetic pesticides organic growers face significant challenges relating to weed control^{32,33} and therefore may especially benefit from any ability to control early season weeds if conditions allow early application of meal.

Due to the dependence of ITC formation and degradation on soil moisture conditions, climatic conditions and irrigation practices must also be accounted for when determining planting dates. Further understanding of the mode of action and persistence of glucosinolate secondary products under field conditions needs to be gained to better predict planting dates that avoid crop inhibition.

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