

ARTICLES

Ionic Thiocyanate (SCN^-) Production, Fate, and Phytotoxicity in Soil Amended with Brassicaceae Seed MealsDANIEL HANSSON,[†] MATTHEW J. MORRA,^{*,†} VLADIMIR BOREK,[†]
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Brassicaceae seed meals produce ionic thiocyanate (SCN^-), a bioherbicide compound. This study determined the fate of SCN^- in a field soil amended with seed meals of *Sinapis alba*, *Brassica juncea*, and *Brassica napus* and quantified crop phytotoxicity by monitoring carrot (*Daucus carota*) emergence. Meals were applied at 1 or 2 t ha⁻¹, and soils were sampled to 35 cm for SCN^- . Maximum SCN^- (211 $\mu\text{mol kg}^{-1}$ of soil) was measured at 5 days in 0–5 cm samples from plots amended with *S. alba* meal at 2 t ha⁻¹. Less than 30 $\mu\text{mol kg}^{-1}$ of soil was measured at soil depths below 15 cm. At 44 days, SCN^- was <15 $\mu\text{mol kg}^{-1}$ of soil in all treatments. Emergence inhibition of carrots seeded 15–36 days after meal amendment was found only in *S. alba* treatments. The rapid decrease of SCN^- concentrations in Brassicaceae meal-amended soil indicates limited potential for off-site environmental impacts.

KEYWORDS: Glucosinolates; Brassicaceae; *Sinapis alba*; allelochemicals; mustard; phytotoxic; sinalbin; thiocyanate; SCN^-

INTRODUCTION

With increasing interest in biodiesel as a fuel, more research is being devoted to Brassicaceae oilseed crops. Meals remaining after oil extraction from seed of *Brassica napus* L. (canola), *Brassica juncea* L. (oriental mustard), and *Sinapis alba* L. (yellow mustard) contain glucosinolates, which in themselves have limited biological activity (1, 2). After hydration of the seed meals, however, glucosinolates are hydrolyzed enzymatically by thioglucoside glucohydrolase (EC 3.2.1.147) (myrosinase) to form a number of allelochemicals including isothiocyanates, ionic thiocyanate (SCN^-), organic cyanides, and oxazolidinethiones (2, 3). The unique glucosinolate profile of each seed meal, in combination with environmental conditions including pH, Fe^{2+} concentration, and the presence of coenzymes, determines which reaction products will result.

Meals of glucosinolate-containing tissues applied as soil amendments have been reported to have herbicidal, insecticidal, nematicidal, and fungicidal effects (1, 4–8). Because of these biocidal properties, Brassicaceae seed meals resulting as a

byproduct from oil extraction could become a valuable biopesticidal material. *S. alba* meal has been shown to inhibit the emergence of various annual weed (9) and crop species (4, 6). The primary glucosinolate found in *S. alba* seed meal is sinalbin (4-hydroxybenzyl glucosinolate), a glucosinolate producing an unstable isothiocyanate that nonenzymatically hydrolyzes to SCN^- (10, 11). Seed meals of other species including *B. napus* contain indole glucosinolates that also degrade to SCN^- via unstable isothiocyanate intermediates (1, 12–15). SCN^- is a known phytotoxin (6, 16–20) and is likely the major allelochemical responsible for weed suppression after soil amendment with *S. alba* meal (10).

The environmental fate of SCN^- released from Brassicaceae meal soil amendments is of interest because of its known toxicity to aquatic organisms and mammals (21, 22). Toxicity to fish has been observed in water containing 90 mg of $\text{SCN}^- \text{L}^{-1}$ (23), with sudden death syndrome in freshwater trout, and fish embryo deformities resulting from SCN^- concentrations at or below 85 mg L^{-1} (24–26). Mammalian feeding studies indicate an LD₅₀ for rats of 750 mg of $\text{SCN}^- \text{kg}^{-1}$ of body weight (21). SCN^- is considered to have a low acute human toxicity, but effects on the central nervous system resulting in irritability, hallucinations, and convulsions have been noted (21). A recent EPA superfund record of decision raised concern of groundwater

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contamination by SCN⁻ from subsurface soil polluted by industrial sources (27).

Although sorption and degradation of SCN⁻ have been studied under laboratory conditions (28), to our knowledge no previous publication has reported field data on the fate of this anion as produced in soils amended with Brassicaceae seed meals. Our objectives were to quantify SCN⁻ in field soil amended with Brassicaceae seed meals and assess any phytotoxic response of the crop to meal amendments. To achieve these objectives, we measured SCN⁻ release from seed meals of *S. alba*, *B. juncea*, and *B. napus* in a soil planted to carrots (*Daucus carota* L. var. *sativus*), determining SCN⁻ leaching potential and residence time. Phytotoxicity of this anion was ascertained by determining inhibitory effects on emergence of carrots seeded at different times following meal application.

MATERIALS AND METHODS

Glucosinolate Analysis. The seed meals used in our study were produced from three locally adapted oilseed varieties: *B. napus* 'Sunrise' (29), *B. juncea* 'Pacific Gold' (30), and *S. alba* 'IdaGold' (31). Mature seed was cold-pressed using a mechanical seed crusher to remove 85–90% of the oil (32). For glucosinolate analysis only, meal samples were further processed by extraction with petroleum ether to remove any residual oil. Glucosinolates were extracted with a 70% methanol/water solution, and 4-methoxybenzyl glucosinolate was added as an internal standard. Glucosinolates were isolated using a DEAE anion exchanger and desulfated with sulfatase (Sigma-Aldrich, St. Louis, MO). Methanol extracts were analyzed for glucosinolate concentrations using a Waters 2695 HPLC separation module coupled with a Waters 996 photodiode array detector and a ThermoFinnigan mass detector. For quantitative purposes all glucosinolates detected by PDA were measured at a wavelength of 229 nm. To correct for different absorbance coefficients at 229 nm, detector absorbance values were multiplied by response factors (33). Separation was performed on a Phenomenex Aqua 5 μ m C18 125Å, 250 \times 2.00 mm column. Complete details of glucosinolate analysis have been described previously (10).

Field Study Design. A field study was initiated in 2005 at the University of Idaho Plant Science Farm in Moscow, ID (46° 43' 25'' N, 116° 57' 30'' W). The soil at the field site is a Latahco silt loam (34) containing approximately 20% sand, 60% silt, 20% clay, and 2.7% organic matter. At a depth of 0–20 cm, a soil pH (1:1, soil:water) of 6.3 and a CEC of 18.7 cmol_c kg⁻¹ of soil were measured. Concentrations of preplant macro- and micronutrients in the soil were tested and found to be adequate to support carrot growth, with no apparent elemental deficiencies (unpublished data). Prior to the 2005 growing season, a crop of spring canola (*B. napus* L.) had been grown at the experimental plots.

The experiment was a randomized complete block design with five meal treatments and four replications (blocks). Within a block there were four beds, each of which was planted with carrots at one of four different dates post meal amendment. The beds were divided into 1 \times 2 m plots to which the seed meal treatments were applied. The same seed meal treatment was applied to the four plots spanning all four beds within a block. Buffers of 0.6 m were placed between both the beds and the meal treatments (Supporting Information, Figure S1).

Meal amendments were prepared from oil-extracted seeds of the three cultivars previously described. The crushing procedure yielded meals with a residual oil content of approximately 13% as expressed on a weight basis. The meal was used in a flake form as produced by the press, with 1 mm thick flakes ranging in diameter from approximately 5 to 15 mm. Amendment and dispersal operations resulted in flakes typically <5 mm in diameter. For each meal type, amendments were applied at a treatment rate of 2 t ha⁻¹. For *S. alba*, an additional treatment of 1 t ha⁻¹ was included. Unamended control plots were also included in the experimental design.

On June 8, 2005, all seed meal amendments were applied manually to the rototilled 1 \times 2 m plots and incorporated 2 cm deep using a landscaping rake. Immediately following treatment application, 27 mm of water was applied to all plots by drip irrigation. An additional 3

mm of water was subsequently delivered by a tractor-mounted sprayer to thoroughly hydrate the surface layer of the seed meal.

Each bed was planted with four carrot rows having a between-row spacing of 25 cm. A pressure-compensated drip irrigation system was designed and constructed to ensure quantitative and reliable irrigation, not possible with overhead irrigation. Two parallel lengths of emitter tubing were installed in each replicate bed, with each length of tubing approximately 25 cm from the center of the bed. Emitters located every 23 cm along the tubing length delivered an average of 1.9 L of water h⁻¹ per emitter from a source well. This placement resulted in drip irrigation tubing located approximately 12 cm from each of the four carrot rows in a single bed (Supporting Information, Figure S1).

Beds were seeded 15 (June 23, 2005), 22 (June 30), 29 (July 7), and 36 days (July 14) post meal treatment. On the planting dates, pelleted seed of the 'Nelson' carrot variety (*D. carota* L. var. *sativus*) (Johnny's Selected Seeds, Winslow, ME) was seeded at 78 seeds m⁻² using an EarthWay precision seeder fitted with a custom seeding plate (EarthWay Products, Bristol, IN). A mixture of peat moss, perlite, gypsum, and limestone (Sun Gro Horticulture, Bellevue, WA) was spread in a uniform 1 cm deep and 3 cm wide layer over the seeded carrot rows to minimize emergence inhibition resulting from soil crusting. Intense spring precipitation and high winds often induce soil crusting in the fine-textured soils of the region. Hand-weeding was performed immediately before each seeding, after emerged carrots had been counted, and as necessary throughout the growing season.

During the 36 days that elapsed between meal application and the final planting, a total of 419 mm of water was applied by pressure-compensated drip irrigation at a rate of 18 mm ha⁻¹. After carrot seeding, an irrigation schedule based on gravimetric and tensiometric measurements was established to retain moisture in the top 20 cm of soil at an amount \geq 50% of the available water capacity. The minimum soil moisture content was 0.15 g of H₂O g⁻¹ of soil with a seasonal mean of 0.22 g of H₂O g⁻¹ of soil. Total precipitation for the 44 day soil sampling period was 36 mm. Carrot emergence was measured for each plot 21 days after seeding by a direct count of seedlings per meter of row. Linear regression analysis of emergence data versus extractable SCN⁻ concentrations (0–5 cm depth increment measured within 2 days of planting) was performed, and Pearson's correlation coefficients were determined.

Analysis of SCN⁻ Concentrations. Plots seeded with carrots 3 weeks post meal amendment were sampled for SCN⁻ at seven different times. We used a random sampling scheme and a 20 mm diameter stainless steel probe to remove three soil cores from a 0.5 \times 0.5 m subplot within each 1 \times 2 m plot (Supporting Information, Figure S1). The three soil cores from each subplot were subdivided into depth increments, and each depth increment was composited. SCN⁻ was measured at soil depths of 0–5, 5–10, 10–15, 15–25, and 25–35 cm at 5 (June 13), 8 (June 16), 13 (June 21), 21 (June 29), 29 (July 7), 36 (July 14), and 44 (July 22, 2005) days after meal amendment. Mean values calculated using the four separate SCN⁻ measurements (one from each block) are shown in all figures.

Soil samples were air-dried until a constant weight was measured. The dried samples (75–130 g of soil) were extracted using 150 mL of 5 mM CaCl₂ in deionized water, with the addition of 1.0 mL of 100 mM KBr as an internal standard. Bottles of sample solution were agitated on a reciprocating shaker for 60 min, after which time they were centrifuged at 2000 rpm using an IEC preparatory centrifuge (Thermo Fisher Scientific, Waltham, MA). Supernatant was drawn off with a syringe and immediately filtered into marked autosampler vials using in-line disposable filters (PVDF filter media, 25 mm diameter, 0.45 μ m pore size; Whatman, Florham Park, NJ). SCN⁻ in soil extracts was quantified by ion chromatography using a Dionex 4000i ion chromatograph (Dionex, Sunnyvale, CA) equipped with a GP40 gradient pump, ED40 electrochemical detector, AS40 automated sampler, and a 250 μ L sampling loop as previously described (35). An IonPacAS16 4 \times 250 mm column was used in combination with gradient elution from 5 to 80 mM KOH during a period of 15 min. A standard solution of 100 mM KSCN in deionized water was diluted to obtain calibration solutions in the range between 1 μ M and 10 mM. Samples of all calibration standards were analyzed in triplicate in the same way as the field soil sample extracts. A calibration curve was

Table 1. Glucosinolate Concentrations and Their Respective Standard Deviations in the Three Brassicaceae Meal Amended Field Plots

glucosinolate R group (common name)	$\mu\text{mol g}^{-1}$ of defatted seed meal		
	<i>B. juncea</i> 'Pacific Gold'	<i>S. alba</i> 'IdaGold'	<i>B. napus</i> 'Sunrise'
2-propenyl (sinigrin)	152.0 \pm 12.3		
4-hydroxybenzyl (glucosinalbin)	156.8 \pm 4.7		
3-butenyl (gluconapin)	2.9 \pm 0.1		
2-hydroxy-3-butenyl (progoitrin)	7.1 \pm 0.8		
4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin)	1.2 \pm 0.3		
4-methoxy-3-indolylmethyl (4-methoxyglucobrassicin)	10.0 \pm 1.0		
	0.5 \pm 0.1		
total glucosinolates	153.2	163.9	14.8

obtained by linear regression of SCN^- peak areas versus SCN^- concentrations. All results are expressed on a soil dry weight basis.

To measure the production of SCN^- from glucosinolates in *S. alba*, *B. juncea*, and *B. napus* seed meals alone, we hydrolyzed the contained glucosinolates by adding deionized water to triplicate samples of each respective meal (1 g of meal/20 mL of water) in polyethylene bottles. After agitation of bottles on a reciprocating shaker for 24 h, suspended particles were allowed to settle. The supernatant was drawn off with a syringe and analyzed using ion chromatography as described above for soil extracts.

RESULTS AND DISCUSSION

Glucosinolate Content in Seed Meals. Distinct profiles of different glucosinolates were observed in the three seed meals (Table 1). *S. alba* seed meal was dominated by 4-hydroxybenzyl glucosinolate (glucosinalbin) (156.8 $\mu\text{mol g}^{-1}$ of defatted seed meal), whereas *B. juncea* seed meal was dominated by 2-propenyl glucosinolate (sinigrin) (152.0 $\mu\text{mol g}^{-1}$). Glucosinolates measured in *B. napus* seed meal included mainly 4-hydroxy-3-indolylmethyl glucosinolate (4-hydroxyglucobrassicin) (10.0 $\mu\text{mol g}^{-1}$), 3-butenyl glucosinolate (gluconapin) (2.9 $\mu\text{mol g}^{-1}$), and 2-hydroxy-3-butenyl glucosinolate (progoitrin) (1.3 $\mu\text{mol g}^{-1}$). The glucosinolates detected and their respective concentrations are consistent with what was expected (4, 5).

Concentrations of SCN^- in Seed Meal Amended Soil.

SCN^- concentrations measured in extracts from soils amended with *S. alba* seed meal at 2 t ha^{-1} are shown in Figure 1. A complete data set with measures of standard errors is provided in Supporting Information, Table S1. At the initial sampling date (5 days after meal amendment), a maximum SCN^- concentration of 211 $\mu\text{mol kg}^{-1}$ of air-dried soil was observed at a depth of 0–5 cm. On the same date, 32 μmol of SCN^- kg^{-1} of soil was measured at a depth of 5–10 cm, 10 $\mu\text{mol kg}^{-1}$ at 10–15 cm, 6 $\mu\text{mol kg}^{-1}$ at 15–25 cm, and 6 $\mu\text{mol kg}^{-1}$ at 25–35 cm. At the second sampling date 8 days after meal amendment, we measured a 58% decrease in SCN^- at a depth of 0–5 cm (123 $\mu\text{mol kg}^{-1}$) and a 3-fold increase in SCN^- at 5–10 cm (108 $\mu\text{mol kg}^{-1}$). During the same interval, SCN^- concentrations at the 10–15 and 15–25 cm depths increased to 17 and 20 $\mu\text{mol kg}^{-1}$, respectively, but decreased to 1 $\mu\text{mol kg}^{-1}$ at 25–35 cm.

Thirteen days after meal amendment, concentrations of SCN^- decreased in the 0–5 cm depth to 78 $\mu\text{mol kg}^{-1}$ and in the 5–10 cm depth to 89 $\mu\text{mol kg}^{-1}$. Increased concentrations relative to the prior sampling were measured at the lower soil depths (Figure 1). In soils sampled 21, 29, 36, and 44 days post meal amendment, SCN^- concentrations at all sampled depths were always lower than those measured for the same

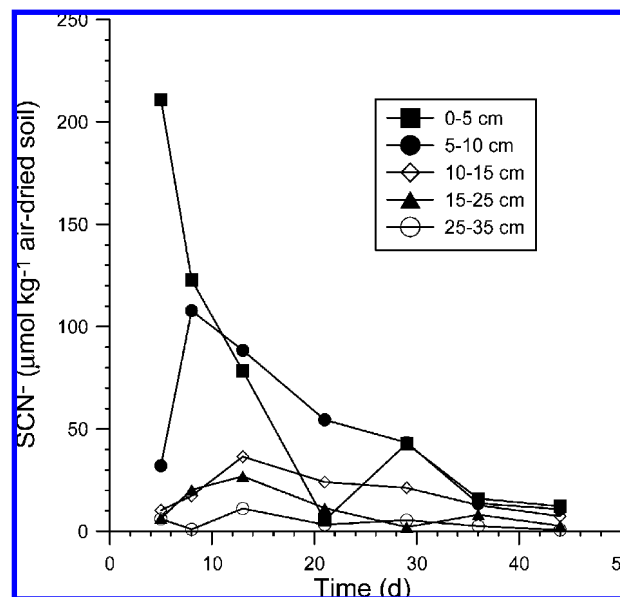


Figure 1. Distribution profile of SCN^- in soil amended with *S. alba* seed meal at 2 t ha^{-1} within 44 days of soil amendment.

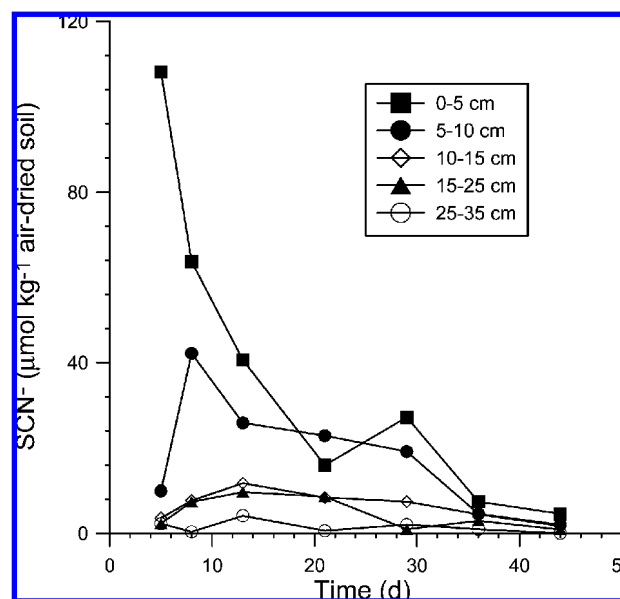


Figure 2. Distribution profile of SCN^- in soil amended with *S. alba* seed meal at 1 t ha^{-1} within 44 days of soil amendment.

depths at 13 days. No SCN^- was measured in the 44 day sample from the 25–35 cm soil depth.

The distribution pattern of SCN^- at different soil depths that we observed for the 1 t ha^{-1} *S. alba* (Figure 2) treatment was similar to that described above for soils amended with 2 t meal ha^{-1} (Figure 1). As expected, we found approximately 50% lower SCN^- concentrations in the 1 t ha^{-1} *S. alba* treatment than in soils amended with 2 t *S. alba* meal ha^{-1} . We conclude that changes in SCN^- concentrations with depth in the soil profile are related to the degradation and mobility of SCN^- , as occurs predominantly at depths at or above 10 cm, and that changes in concentration were moderate between soil depths of 10 and 35 cm.

Soil concentrations of SCN^- for the *B. napus* 2 t ha^{-1} treatment are displayed in Figure 3, and concentrations for the *B. juncea* 2 t ha^{-1} treatment are shown in Figure 4. The SCN^- concentration distributions with time throughout the soil profile in these two treatments follow an overall pattern analogous to

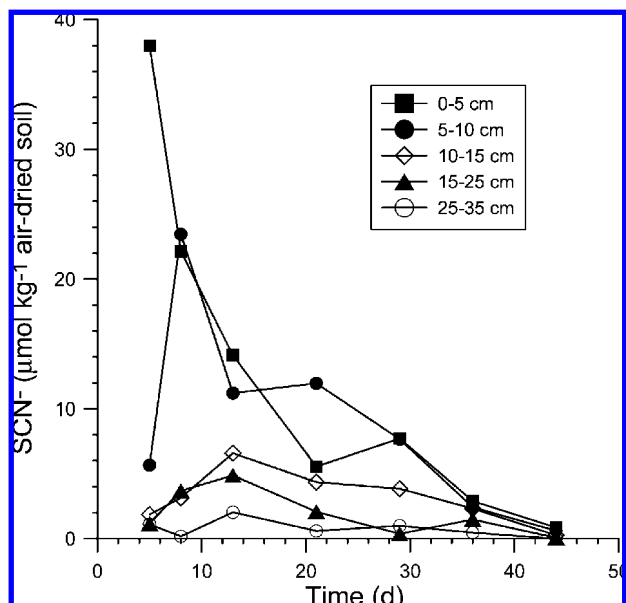


Figure 3. Distribution profile of SCN⁻ in soil amended with *B. napus* seed meal at 2 t ha⁻¹ within 44 days of soil amendment.

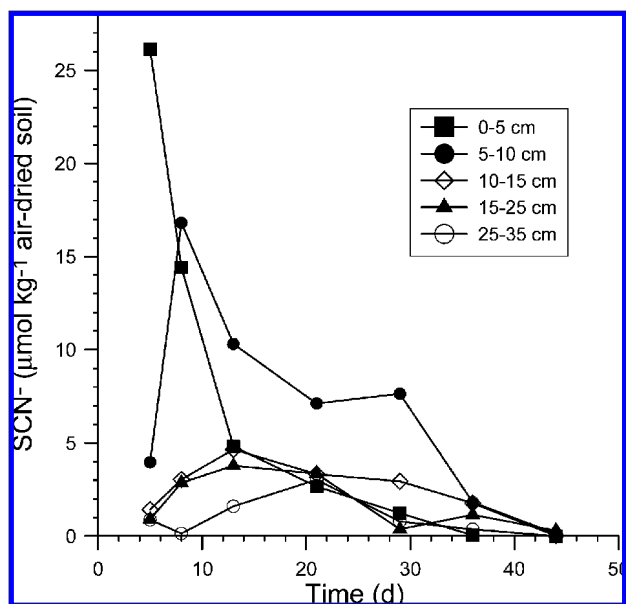


Figure 4. Distribution profile of SCN⁻ in soil amended with *B. juncea* seed meal at 2 t ha⁻¹ within 44 days of soil amendment.

that found for *S. alba* treatments. Throughout all Brassicaceae meal treatments in our study, concentrations of SCN⁻ remained <30 µmol kg⁻¹ of dry soil at depths below 15 cm (Figures 1–4).

The measured SCN⁻ in our samples was produced from the hydrolysis of glucosinolates in the applied seed meals as catalyzed by myrosinase. Some of the SCN⁻ measured in the 0–5 cm samples from all treatments may have resulted from hydrolysis occurring during the extraction procedure, because these samples contained not only soil but seed meal as well. However, we minimized such secondary SCN⁻ production by thoroughly hydrating the meal immediately after field application and by waiting 5 days between initial wetting of the meal and our first sampling. Because meal amendment was confined to the top 2 cm of soil, elevated SCN⁻ concentrations at 5–10 cm and below are explained by SCN⁻ leaching downward with irrigation water or rainfall.

Glucosinolates having *p*-hydroxybenzyl or indole groups attached to the thioglucose moiety are expected to produce unstable isothiocyanate intermediates that spontaneously degrade to SCN⁻ over a pH range from 3 to 7 (10, 28). Borek and Morra (10) observed stoichiometric release of SCN⁻ from *S. alba* meal at 48 h when buffered at pH values down to 4.0. The pH of our sampled soil was 6.3 and, thus, within the range where SCN⁻ is expected to be the major breakdown product of 4-hydroxybenzyl glucosinolate. As confirmed here (Table 1), this glucosinolate has been shown to constitute >90% of the total glucosinolate content in *S. alba* seed meal (10). At pH values near neutral, SCN⁻ is also expected to be the product of 4-hydroxy-3-indolylmethyl glucosinolate (the major indole glucosinolate in *B. juncea* and *B. napus*) and 4-methoxy-3-indolylmethyl glucosinolate (a minor indole glucosinolate in *B. napus*) (1, 2). In contrast, the primary glucosinolate in *B. juncea* meal, 2-propenyl, produces an isothiocyanate that reportedly does not degrade to SCN⁻ (1, 2).

Production of SCN⁻ concentrations in soils amended with *B. napus* and *B. juncea* meals lower than those observed in *S. alba* amended soils is consistent with the fact that *B. napus* and *B. juncea* meals contain lower concentrations of SCN⁻-yielding glucosinolates (Table 1). The concentrations of indole glucosinolates in *B. juncea* and *B. napus* tissues are approximately 1 order of magnitude lower than the concentration of 4-hydroxybenzyl glucosinolate measured in the tissue of *S. alba* meal (Table 1). Under optimal environmental conditions, 4-hydroxybenzyl glucosinolate, as well as indole glucosinolates, yields SCN⁻ on a 1:1 molar ratio via the corresponding isothiocyanate intermediates (1). As predicted, we found initial SCN⁻ concentrations in the 0–5 cm samples that were approximately 1 order of magnitude lower in the *B. juncea* and *B. napus* treatments relative to the 2 t ha⁻¹ *S. alba* treatment (Figures 1–4).

Estimates of maximum SCN⁻ produced in soil having an average bulk density of 1.3 g cm⁻³ can be made by using the measured meal glucosinolate concentrations (Table 1) and a predicted 1:1 stoichiometric conversion of 4-hydroxybenzyl and indole glucosinolates to SCN⁻, or by using the laboratory-measured SCN⁻ yields obtained from wetted meal extracts (Table 2). Calculations for *S. alba* demonstrate that the measured amount of SCN⁻ in field-amended soil is of the same order of magnitude, but lower than predicted either from glucosinolate stoichiometry or from laboratory meal extractions. Losses through SCN⁻ degradation, leaching, incomplete hydrolysis in the field, or incomplete extraction contribute to the observed difference in predicted and actual values. Specific microorganisms degrading SCN⁻ have been identified (36–41), and microbial degradation of SCN⁻ in soil has been observed (28). From a laboratory incubation study it was concluded that microbial activity was the main factor responsible for SCN⁻ disappearance in soil incubated at or below 30 °C (28).

Although measured amounts of SCN⁻ in field soil amended with either *B. napus* or *B. juncea* follow the expected relative ranking, absolute amounts of SCN⁻ are higher than predicted from both meal glucosinolate concentrations and laboratory meal extractions (Table 2). We have not been able to determine whether the differences between predicted and observed soil SCN⁻ concentrations are a result of unmeasured glucosinolates, unanticipated reactions such as isothiocyanate conversion to SCN⁻, or imprecision in the employed methods.

Phytotoxicity of SCN⁻ in Agricultural Soil. To assess possible adverse crop effects of Brassicaceae seed meal amendments, we used emergence inhibition of carrots as an indicator

Table 2. Predicted and Measured Concentrations of SCN⁻ Produced in Soil to Which Brassicaceae Seed Meals Were Added (2 t ha⁻¹), Assuming an Average Soil Bulk Density of 1.3 g cm⁻³ and That All SCN⁻ Was Released at Soil Depths of 0–5 cm

seed meal	SCN ⁻ -yielding glucosinolates ($\mu\text{mol g}^{-1}$ of meal)	SCN ⁻ in H ₂ O extract ($\mu\text{mol g}^{-1}$ of meal)	predicted SCN ⁻ ($\mu\text{mol kg}^{-1}$ of soil)		measured SCN ^{-b} ($\mu\text{mol kg}^{-1}$ of soil)
			glucosinolate ^a	H ₂ O extract	
<i>S. alba</i>	157	106	420	328	278
<i>B. napus</i>	10.5	5.8	28	17.8	50.0
<i>B. juncea</i>	1.2	3.7	3.2	11.4	35.1

^a Calculated amounts include adjustment for the presence of 13% (w/w) residual oil in the seed meal applied to the field plots. ^b Reported concentrations were calculated by summing micromoles of SCN⁻ measured in 0–35 cm of soil as measured at the first sampling date 5 days after meal amendment.

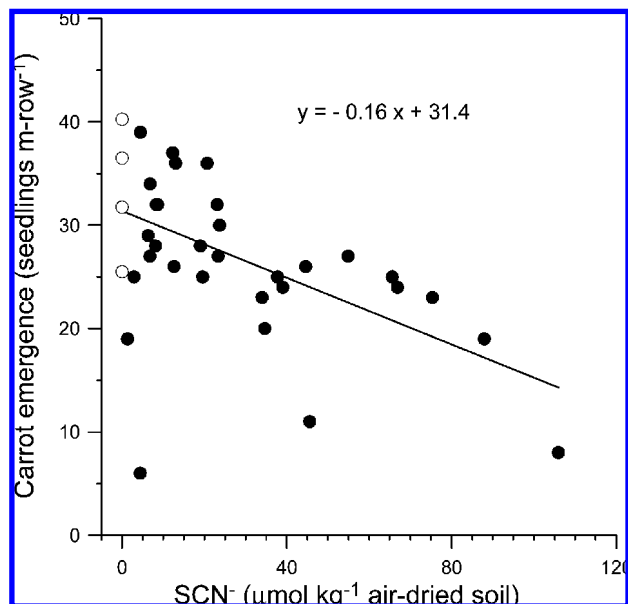


Figure 5. Carrot seedling emergence as a function of SCN⁻ concentrations measured in extracts from soils sampled at depths of 0–5 cm. The linear regression model was obtained using data from plots to which *S. alba* seed meals were applied, excluding the replicate in which only six seedlings emerged. Open circles represent the means of the control plots in each block to which no Brassicaceae meals were applied.

of phytotoxicity. For this purpose, we considered SCN⁻ concentrations in the upper 5 cm of the soil profile where carrot germination took place. The Pearson correlation between SCN⁻ concentration and carrot emergence count was weak and insignificant ($p > 0.05$) for *B. juncea* (-0.199) and *B. napus* (-0.296) treatments (Supporting Information, Figures S1 and S2). A stronger correlation was observed for the two *S. alba* treatments, for which higher SCN⁻ concentrations were measured. Carrot emergence and SCN⁻ concentrations in soil extracts from the two *S. alba* treatments yielded a linear association with a Pearson correlation of -0.635 ($p < 0.01$) after exclusion of the one outlier in which only six carrot seedlings emerged (Figure 5). Control plots without meal amendment yielded carrot emergence rates similar to those observed at lower SCN⁻ concentrations, further supporting the conclusion of a causal relationship. There is thus evidence of crop phytotoxicity, but only in *S. alba* treatments where SCN⁻ concentrations were relatively high compared to the other meal amendments. It is possible that the moisture regimen during our study facilitated leaching of SCN⁻ below the zone of seed germination, which would otherwise have resulted in greater phytotoxic effects on the crop if retained in the 0–5 cm layer of the soil for a longer period of time. It must also be remembered that carrots were seeded 15–36 days after meal

amendment, thus providing time for SCN⁻ dissipation within the zone of seed germination.

Harvey (20) documented complete kill of crops (corn, wheat, and peas) and weeds (*Setaria viridis* and *Chenopodium* sp.) for 4 months at an application rate of 1366 kg of SCN⁻ ha⁻¹. Complete removal of vegetative cover for a period of 4 months has also been reported after application of 270–680 kg of SCN⁻ ha⁻¹ as has cotton defoliation after spray applications of 8.6 kg of SCN⁻ ha⁻¹ (18). Bissey and Butler (19) showed that 342 kg of SCN⁻ ha⁻¹ is needed to inhibit wheat germination, an effect that was no longer observed when wheat was planted 69 days after SCN⁻ application. *S. alba* seed meal used in our study would yield 17.7 and 8.8 kg of SCN⁻ ha⁻¹ for amendment rates of 2 and 1 t of seed meal ha⁻¹, respectively, assuming stoichiometric conversion of the measured glucosinolates (Table 1). Under the same conditions, *B. napus* seed meal would yield 1.2 kg of SCN⁻ ha⁻¹, and *B. juncea* seed meal would produce 0.1 kg of SCN⁻ ha⁻¹ at amendment rates of 2 t seed meal ha⁻¹.

A detailed dose–response relationship is needed to establish guidelines for timing and rates of Brassicaceae meal amendment appropriate to minimize crop emergence inhibition and suppress weeds. Despite a potential phytotoxic effect on the crop, we have shown that concentrations of SCN⁻ remained $<30 \mu\text{mol kg}^{-1}$ of dry soil at soil depths below 15 cm throughout all Brassicaceae meal treatments in our study (Figures 1–4). Diffusion, leaching, and microbial decomposition decreased concentrations of SCN⁻ below concentrations documented as causing negative biological effects within a relatively short time span, even at shallow depths in the soil profile. Different moisture regimens and soil characteristics could have significant effects on the disappearance of SCN⁻ from soil amended with Brassicaceae seed meals, and it is possible that heavy rainfall immediately after amendment could facilitate more extensive leaching of this anion. However, soil concentrations of SCN⁻ that have been measured after amendment with seed meals of *S. alba*, *B. juncea*, and *B. napus* are orders of magnitude lower than concentrations considered to be harmful to humans and wildlife (42, 43). Current results and past evidence of rapid microbial degradation indicate that the use of Brassicaceae meals as agricultural soil amendments does not appear to pose environmental hazards when applied at rates of 2 t ha⁻¹ or less.

Supporting Information Available: Field plot design, soil SCN⁻ concentrations and respective standard errors, carrot emergence with *B. napus* meal, and carrot emergence with *B. juncea* meal. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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