



# The Mechanism for Weed Suppression by a Forage Radish Cover Crop

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## ABSTRACT

Little is known about the mechanism of winter annual weed suppression by forage radish (*Raphanus sativus* L. variety *longipinnatus*) winter cover crops. Previous studies suggest that allelopathy from decomposing residue and competition due to rapid canopy development contribute to weed suppression by other *Brassica* cover crops. Four contrasting experimental approaches were used to identify the mechanism of weed suppression by forage radish cover crops. Results of a field based cover crop residue-transfer experiment supported the hypothesis that fall cover crop weed competition is the dominant mechanism of weed suppression following forage radish cover crops. A high level of early spring weed suppression was observed where forage radish grew in the fall regardless of whether residues were left in place or removed. In contrast, there was limited weed suppression in bare soil treatments that received additions of forage radish tissues. Bioassays using cover crop amended soil or aqueous extracts of cover crop tissues and amended soil did not reveal any allelopathic activity limiting seed germination or seedling establishment. In a field-based weed seed bioassay, forage radish cover crops did not inhibit emergence of winter-planted weed seeds relative to a no cover crop control. Forage radish amended soils stimulated seedling growth of lettuce (*Lactuca sativa* L.) in all types of bioassays. The results of the four experiments in this study point to a common conclusion that fall weed competition is the dominant mechanism for early spring weed suppression following forage radish winter cover crops.

IN THE MID-ATLANTIC region of the United States, forage radish winter cover crops planted before 1 September suppress winter annual weeds from fall through early April (Lawley et al., 2011). This weed suppression may be used by farmers to provide pre-plant weed control for a subsequent crop while taking advantage of the soil quality and nutrient benefits of cover crops (Chen and Weil, 2010; Dean and Weil, 2009; Gruver et al., 2010; Weil and Kremen, 2007; White and Weil, 2011). In contrast to the highly repeatable pre-plant weed suppression observed following forage radish winter cover crops in the coastal plain of Maryland (Lawley et al., 2011), researchers report that weed suppression by other cover crops and their residues is inconsistent (Gallandt et al., 1999; Teasdale et al., 2007). Knowledge of the mechanisms involved could be used to improve cover crop management strategies to suppress weeds and help predict when supplemental weed management strategies will be needed.

Little is known about the mechanism of weed suppression following forage radish winter cover crops. Similar winter-kill-susceptible radishes planted in the late summer or early fall have been observed to suppress weeds in several field studies. For example,

oilseed radish (*R. sativus* L. variety *oleiformis*) winter cover crops suppressed winter annual weeds until March/April in on-farm vegetable studies conducted in western New York (Stivers-Young, 1998). In Michigan, oilseed radish reduced early spring weed density and biomass before vegetable crops and also reduced recoverable weed seeds in the soil seed bank compared to a no cover crop control (Baskin and Baskin, 2001; Wang et al., 2008). In Ontario, Canada, oilseed radish also produced sufficient biomass in 2 of 3 site-years to suppress fall growth of volunteer winter wheat (*Triticum aestivum* L.) by 75% (Swanton et al., 1996).

When planted by early August in the Netherlands, fodder radish (*R. sativus* cultivar Brutus) reduced fall weed biomass by 65 to 95% when grown as a fall cover crop (Kruidhof et al., 2008). However, fodder radish in that study had no effect on lettuce and sugar beet (*Beta vulgaris* L.) test crops or the natural weed population during May. No data were reported on earlier spring weed suppression before May. The authors suggested cover crop competitiveness, allelopathy, and reduced weed seed production as mechanisms for this weed suppression.

Several studies have supported allelopathy as the mechanism of weed suppression for *Brassica* cover crop species (Al-Khatib et al., 1997; Boydston and Hang, 1995; Krishnan et al., 1998; Turk and Tawaha, 2003). Haramoto and Gallandt (2004) and Boydston and Al-Khatib (2006) reviewed *Brassica* cover crops and weed management. They focused on allelopathy as the mechanism responsible for weed suppression and the hydrolysis products of glucosinolates as the source of allelochemicals involved. Glucosinolates are secondary plant metabolites commonly found in *Brassica* species. Glucosinolates are hydrolyzed by the enzyme myrosinase into several products, some of

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**Abbreviations:** BARC-NF, Beltsville Agricultural Research Center North Farm; BARC-SF, Beltsville Agricultural Research Center South Farm; DM, dry matter; EC, electrical conductivity; ITC, isothiocyanate.

which have demonstrated biological activity against weed seeds, pathogens, insects, and nematodes (Brown and Morra, 1995, 1997; Chew, 1988). Isothiocyanates (ITCs) from glucosinolate hydrolysis are volatile and short-lived compounds when released in soil (Al-Turki and Dick, 2003; Borek et al., 1996). These ITCs can inhibit seed germination and seedling growth in a variety of weed and test crop species (Bialy et al., 1990; Brown and Morra, 1995, 1996; Petersen et al., 2001; Turk and Tawaha, 2003).

Forage radish and similar radish cover crops behave differently than many of the other *Brassica* species when planted in the fall. For example, forage radish is sensitive to frost and winter-kills with prolonged exposure to temperatures below  $-4^{\circ}\text{C}$  (Weil et al., 2009). Forage radish cover crop residue decomposes rapidly during the freeze–thaw cycles that characterize winters in the Mid-Atlantic region, leaving little residue on the soil surface the following spring. Because of this rapid decomposition, forage radish cover crops create a unique low residue and weed-free seed bed for planting in the early spring.

These characteristics of forage radish cover crops also create challenges for studying their mechanism of weed suppression. In the Mid-Atlantic, forage radish shoots are first damaged by frost in late November or early December but shoots regrow from the growing point, which is often protected by surrounding foliage, until the growing point is finally killed by colder temperatures in January or February. Thus it is difficult to define a distinct termination date, control the termination event, or create one treatment event with the potential to release a single high dose of allelochemicals.

The objective of this study was to determine the mechanism of weed suppression by forage radish cover crops. Two potential mechanisms of weed suppression were evaluated: (i) allelopathy and (ii) cover crop weed competition in the fall. We hypothesized that allelopathy was the driving mechanism of weed suppression. This study employed four contrasting experimental approaches to test for these two mechanisms.

## MATERIALS AND METHODS

Four experiments were conducted including field and controlled environment experiments. Field experiments involved manipulating cover crop residues to compare the effects of competitive fall growth and allelopathy along with a field bioassay of planted weed seeds. Controlled environment bioassays involving cover crop-amended soil, aqueous plant extracts, and aqueous soil extracts were used to evaluate the allelopathic potential of forage radish. All experiments were conducted between January 2005 and May 2008. Field experiments were conducted at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) ( $39^{\circ}01'51''\text{N}$ ,  $76^{\circ}55'58''\text{W}$ ; 40 m elevation) and South Farm (BARC-SF) ( $39^{\circ}00'56''\text{N}$ ,  $76^{\circ}56'29''\text{W}$ ; 30 m elevation) in the coastal plain of Maryland.

### Field Studies—Site Description and Field Management

All field experiments at BARC-NF and BARC-SF were conducted in fields with a history of conventional tillage and crop rotation that included corn (*Zea mays* L.), soybean [*Glycine max* L. Merr.], vegetable crops, and winter rye. Soils at BARC-NF were classified as Matawan (fine-loamy, siliceous, semiactive, mesic Aquic Hapludults)–Hamonton (coarse-loamy,

siliceous, semiactive, mesic Aquic Hapludults) loamy sands in 2005–2006, 2006–2007 (blocks 1 and 2), and 2007–2008 (blocks 1 and 2). Soils at BARC-NF were classified as Ingleside (coarse-loamy, siliceous, semiactive, mesic Typic Hapludults)–Hamonton loamy sand in 2006–2007 (blocks 3 and 4) and 2007–2008 (blocks 3 and 4). At BARC-SF, all soils were classified as Codorus (fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts) silt loam in 2006–2007 and 2007–2008. Organic matter of all soils ranged from 1.3 to 2.0%.

The experimental design for all field experiments was a randomized complete block design with four replicates. A forage radish cover crop was compared to a no cover crop treatment in all experiments and to a fall-planted oat (*Avena sativa* L. cultivar Ogle) cover crop in 2005 only. Pre-plant soil-incorporated fertilizer applications to ensure adequate cover crop nutrition and growth were applied to both cover crop and no cover crop treatments. Based on soil tests, 50 and 60 kg N ha<sup>-1</sup> were applied in August 2005 to fields at BARC-NF and BARC-SF, respectively, before cover crop planting. In August of 2006, 62 kg ha<sup>-1</sup> N was applied to BARC-NF and BARC-SF. No N fertilizer was applied to BARC-NF and BARC-SF before cover crop planting in August of 2007. Cover crops were seeded using a conventional grain drill with double disk openers and 18 cm row spacing. All cover crops were seeded between 25 and 31 August at rates of 14 kg ha<sup>-1</sup> (forage radish) and 90 kg ha<sup>-1</sup> (oat). Sprinkler irrigation was used to stimulate cover crop germination in August and September of 2005 when conditions were unusually dry. The 2005 oat cover crop had reached panicle emergence (Zadocks stage 59) by the time it was killed by frost in late November. Forage radish cover crops grew vegetatively in the fall until damaged by frost in mid- to late November and eventually winter-killed due to progressively cold temperatures in January and February.

### Experiment 1: Cover Crop Residue Transfer

The residue-transfer experiment was conducted at BARC-NF and BARC-SF in both 2006–2007 and 2007–2008, for a total of 4 site-years. Plot size was 3 by 3 m. Forage radish was planted over the plot area on 31 Aug. 2006 and 28 Aug. 2007 at both locations. Forage radish was removed in the no cover crop treatments by hand hoeing on 14–15 Sept. 2006, and by spraying glyphosate [*N*-(phosphonomethyl) glycine] (1.12 kg ha<sup>-1</sup> a.i.) on 3 Oct. 2007. Residue-transfer treatments (Table 1) were imposed before a killing frost on 13 November, 2006 and 14 November, 2007. Cover crop biomass was measured at the time of residue treatment establishment (Table 2). Tarps and boards were used to minimize soil compaction when removing cover crop residues from the plots. Where forage radish plants were removed, the fleshy taproot was hand pulled carefully to minimize soil disturbance. Two no cover crop treatments were included as control treatments: (i) no cover crop, no weed removal (NC-weedy), and (ii) no cover crop, weeded twice (NC-fall-weeded). The first treatment was not weeded for the duration of the experiment, while the second treatment was hand weeded in October and November. No cover crop plots that received additions of forage radish residues (NC-S<sub>1</sub>R<sub>1</sub> and NC-S<sub>1</sub>R<sub>0</sub>) were weeded before applying the treatments in 2007 but not in 2006.

Visual ratings of percent ground area covered by weeds were made periodically in spring to evaluate weed suppression (Table 3). Data were analyzed by ANOVA using the MIXED procedure of

**Table 1. Residue transfer treatments for Exp. 1.**

Treatment ID†	Fall cover crop	Residue treatment manipulations in late fall
FR-S <sub>0</sub> R <sub>0</sub>	FR	Shoots and fleshy tap roots removed
FR-S <sub>1</sub> R <sub>1</sub>		Shoots and roots remain in place
FR-S <sub>2</sub> R <sub>2</sub>		Add shoots and fleshy tap roots to an existing stand
FR-S <sub>0</sub> R <sub>1</sub>		Remove shoots only, roots remain
NC-S <sub>1</sub> R <sub>1</sub>	NC	Add shoots and fleshy tap roots to plot with no growing forage radish
NC-S <sub>1</sub> R <sub>0</sub>		Add shoots only to plot with no growing forage radish
NC-weedy		No cover crop, weeds never controlled
NC-fall-weeded		No cover crop, weeds periodically removed by hand in fall

† FR, forage radish; NC, no cover crop.

SAS version 9.1 (SAS Institute, Cary, NC). Separate analyses were conducted for each rating date. Data from the two seasons were analyzed separately due to unbalanced treatments between 2007 and 2008. In the model, block within site was considered a random effect. Cover crop treatment and site were considered fixed effects. A natural log transformation was used before analysis of visual rating scores to improve homogeneity of variances. Back-transformed means are reported. When the ANOVA indicated significant differences between cover crop treatments ( $P < 0.05$ ), mean comparisons were made using Fisher's Least Significant Difference test.

### Experiment 2: Planted Weed Seed Bioassay

This experiment was first conducted within an existing experiment at BARC-NF in 2005–2006 (see Field Studies section above for field and management description). After forage radish cover crops were initially damaged by frost and oat cover crops were winter-killed, 2.2 mL of each of the following weed seeds were sown by hand into a 1-cm furrow under cover crop residues in individual 1-m rows between rows of cover crops (19-cm spacing) on 5 Jan. 2006. The weeds planted were common chickweed [*Stellaria media* (L.)], fall panicum (*Panicum dichotomiflorum* Michx.), green foxtail [*Setaria viridis* (L.) Beauv.], henbit (*Lamium amplexicaule* L.), horseweed [*Conyza canadensis* (L.) Cronq.], common lambsquarters (*Chenopodium album* L.), red-root pigweed (*Amaranthus retroflexus* L.) and common ragweed (*Ambrosia artemisiifolia* L.). Lettuce (*L. sativa* L. cultivar Great Lakes) was also planted because lettuce is an established bioassay test species in allelopathy research. In 2008, the experiment was conducted a second time focusing only on common lambsquarters. Seeds were planted on 1 February in a similar manner into forage radish and no cover treatments in existing experiments at BARC-NF and BARC-SF (described in Lawley et al., 2011).

Weed seed emergence counts were taken on a weekly or biweekly basis from January through June and seedlings were hand pulled after counting. Naturally emerging weeds were removed by hand from the area within and surrounding the rows of planted weed seeds. At BARC-NF in fall 2005, mean forage radish cover crop dry matter (DM) before killing frost was 4.5 and 2.3 Mg ha<sup>-1</sup> for shoots and fleshy tap roots, respectively. In fall 2007, forage radish shoot and fleshy tap root DM was 4.1 and 1.5 Mg ha<sup>-1</sup>, respectively, at BARC-NF and was 6.7 and 1.8 Mg ha<sup>-1</sup>, respectively, at BARC-SF. No cover crop treatments were tilled with a field cultivator and cultipacker at the same

**Table 2. Mean fall dry matter of forage radish shoot and fleshy root tissue used to create residue-transfer treatments in November at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF) in 2006 and 2007.**

Year	Location	Forage radish shoot and fleshy root	
		Forage radish shoot	Forage radish root
kg ha <sup>-1</sup>			
2006	BARC-NF	6883	2581
	BARC-SF	5495	1971
2007	BARC-NF	4104	1499
	BARC-SF	4103	2363

time as cover crop treatments before planting but received no subsequent weed control until the time of weed seed introduction. Winter annual weeds present in no cover crop treatments were removed by hand at the time of weed seed planting.

Mean cumulative weed emergence and standard error was calculated using the MEANS procedure of SAS. Data for common lambsquarters emergence over 3 site-years were analyzed by ANOVA in the MIXED procedure of SAS. In the model, cover crop treatment and site were considered fixed factors. Block within site was considered a random factor. Mean emergence was presented by site due to an interaction between cover crop treatment and site.

## Controlled Environment Studies

### Experiment 3: Soil Bioassay

Soils for this bioassay were collected from forage radish and no cover crop treatments in existing experiments conducted in 2006–2007 at BARC-NF and BARC-SF (see Field Studies section above for field and management description). Mean forage radish DM on 6 Nov. 2006 at BARC-NF was 4262 and 1338 kg ha<sup>-1</sup> for shoots and tap roots, respectively. Dry matter at BARC-SF was 5046 and 1138 kg ha<sup>-1</sup> for forage radish shoots and tap roots, respectively, on 6 Nov. 2006. No cover crop treatments were tilled with a field cultivator and cultipacker at the same time as cover crop treatments before planting but received no subsequent weed control before soil sample collection.

Twenty soil cores, 5 cm in diameter and 5 cm deep, were collected from surface soils in each replicate of forage radish and no cover crop treatments at both sites. Soil cores from each plot were composited and stored in a cooler on ice while in the field. Soils were sampled on 18 Jan. 2007, 28 Feb. 2007, and 30 Mar. 2007 representing early, intermediate, and late stages of cover crop residue decomposition.

Fifty lettuce (cultivar Great Lakes) and tomato (*Solanum lycopersicum* L. cultivar Rutgers) seeds were each placed on top of 300 g of field moist soil in a 10-cm long by 8-cm wide by 10-cm deep plastic pot and covered by an additional 100 g of soil. Soil from each field plot was potted and subsamples were reserved to determine gravimetric soil moisture content and soil nitrate content using the salicylic-acid method (Cataldo et al., 1975). Soil samples were air dried for 24 h at room temperature on 28 February because they were too wet for potting.

The potted seeds were incubated for 5 wk in a growth chamber at 23°C, 50% relative humidity, and 17 h d<sup>-1</sup> of light at an average light intensity of 250 μmol m<sup>-2</sup> s<sup>-1</sup>. To prevent leaching of water soluble allelochemicals through the soil, the pots were watered from saucers below each pot. Pots were arranged in the growth chamber in a pattern that reflected the randomized complete block design of the field experiment where the soil samples were collected.

**Table 3. Effect of forage radish cover crop residue transfer treatments on mean percent weed cover in early and mid-spring at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF).**

Year	Location	Date of rating	FR residue treatments†			NC residue treatments†				
			FR-S <sub>0</sub> R <sub>0</sub>	FR-S <sub>1</sub> R <sub>1</sub>	FR-S <sub>2</sub> R <sub>2</sub>	FR-S <sub>0</sub> R <sub>1</sub>	NC-S <sub>1</sub> R <sub>1</sub>	NC-S <sub>1</sub> R <sub>0</sub>	NC-weedy	NC-fall-weeded
Percentage weed cover in early spring										
2006–2007	BARC-NF	20 Mar. 2007	00.1a‡	00.3a	00.0a	00.0a	32b	38b	53c	50.8a
	BARC-SF	28 Mar. 2007	00.0a	00.0a	00.0a	00.3a	20b	20b	71c	16b
2007–2008	BARC-NF	7 Apr. 2008	70.4b	00.7a	–	–	9.0b	–	30c	29c
	BARC-SF	1 Apr. 2008	20.5a	00.0a	–	–	3.8a	–	14b	13b
Percentage weed cover in mid-spring										
2006–2007	BARC-NF	30 Apr. 2007	40.8a	90.0b	50.3ab	50.3ab	96d	98d	85d	47c
	BARC-SF	25 Apr. 2007	30.8a	40.8a	40.8a	40.0a	69b	55b	89b	53b
2007–2008	BARC-NF	3 May 2008	30b	15a	–	–	33b	–	72c	80c
	BARC-SF	3 May 2008	58a	62ab	–	–	78ab	–	81b	62ab

† FR = forage radish, NC = no cover crop, S<sub>0</sub> = shoots removed, S<sub>1</sub> = shoots remain or added to no cover, S<sub>2</sub> = shoots doubled, R<sub>0</sub> = roots removed or absent from no cover, R<sub>1</sub> = roots remain or added to no cover, R<sub>2</sub> = roots doubled, NC-weedy = plots never weeded, NC-fall-weeded = plots hand weeded in October and November.  
‡ Means followed by different letters indicate significant difference (Fisher's Least Significant Difference test  $P < 0.05$ ) within a site-year and rating period.

Lettuce and tomato germination was counted and emerged weed seedlings were pulled weekly. At the end of the first week, seedlings were thinned to eight plants. Those eight plants were thinned to four at the end of 2 wk. Any additional lettuce, tomato, or weed seeds that emerged were counted and pulled. At the end of the 5-wk study, the four seedlings were cut and their aboveground biomass was dried at 65°C and weighed. Data were analyzed by ANOVA using the MIXED model procedure of SAS. Data for lettuce and tomato were analyzed separately. Comparisons were made between pairs of cover crop treatments within sampling date and site. Cover crop treatments were considered fixed effects and block was considered random.

#### Experiment 4: Aqueous Plant and Soil Extracts

Forage radish fleshy root, forage radish shoot, and oat shoot samples were harvested on 7 Nov. 2005 (before frost damage) from cover crops grown at the BARC-NF (see Field Studies section, above, for field and management description). At harvest the forage radish cover crop was in an advanced vegetative stage with large fleshy tap roots. The oat cover crop had reached panicle emergence (Zadocks stage 59). Mean dry weights of sampled cover crops were 4457, 2319, and 7404 kg ha<sup>-1</sup> for forage radish shoots, forage radish roots, and oat shoots, respectively. Winter-killed plant residue samples of forage radish shoots, forage radish roots, and oat shoots were collected on 24 Mar. 2006. Samples were washed to remove soil, dried at 65°C for 2 wk, ground (<2 mm), and stored at 4°C.

Soil samples from 0- to 5-cm depth were collected on 28 Mar. and 30 May 2006 below decomposing forage radish and oat residues as well as from a no cover crop control at BARC-NF. The no cover crop treatments were tilled with a field cultivator and cultipacker at the same time as cover crop treatments before planting but received no subsequent weed control before soil sample collection. At the time of soil sampling, average weed cover in the no cover crop plots were 84 and 95% in March and May, respectively. Dominant weeds in the no cover treatment included henbit, speedwell (*Veronica officinalis* L.), and common chickweed. Soils sampled were a Matawan–Hammonton loamy sand complex with 1.3% organic matter. Samples were homogenized in the field to form one composite sample for each cover crop treatment. Soil samples were collected in the morning and kept on ice until they were extracted in the afternoon. The gravimetric soil water content at sampling was determined with a microwave oven (Weil, 2009).

The extraction and incubation procedure was modified from Rice et al. (2005). Aqueous extractions of plant samples were prepared at 4°C by shaking 15 g of dried ground plant material with 150 mL of distilled water at 100 rpm for 1 h in a glass Erlenmeyer flask covered with parafilm. Soil extracts were prepared in a similar manner using field moist soil equivalent to 15 g of dry soil in 150 mL of distilled water. The slurry was filtered through six layers of cheese cloth and centrifuged (3040 × g) for 10 min at 4°C. The supernatant solution was filtered by Whatman no. 3 filter paper and then a 0.025 μm nylon membrane filter. Extract filtrate was kept on ice during filtration and prepared in dilutions with distilled water to proportions of 0.125, 0.25, 0.5, and 1.0 (full strength extract). Electrical conductivity of the crude extract was determined using a conductivity dip cell on samples that had been stored in the freezer and thawed at 4°C for 24 h.

Fifty lettuce seeds (cultivar Great Lakes) were placed on top of Whatman no. 1 filter paper moistened with 2.5 mL of extract in each of four replicate 100 mm diam. by 15 mm deep Petri dishes. Petri dishes were sealed with parafilm and incubated for 48 h at 25°C on trays set at a 45° angle to allow geotropism to facilitate seedling measurements. After 48 h of incubation, seed germination was assessed. Shoot and root length were measured on 10 randomly chosen seedlings. Percentage relative root length was calculated from the length of the root in the treatment divided by the length of the root for the control treatment for each replicate.

## RESULTS AND DISCUSSION

### Experiment 1: Cover Crop Residue Transfer

To compare the influence of competitive fall growth to that of decomposing forage radish residues on spring weed emergence, forage radish cover crop residues were manipulated in the combinations described in Table 1. Two main hypotheses were tested in this experiment: (i) allelopathy from decomposing forage radish residue is the dominant mechanism of weed suppression if weed suppression is greatest in treatments with residue additions or residues left in place and (ii) fall cover crop weed competition due to rapid canopy development is the dominant mechanism of weed suppression if weed suppression is greatest in treatments that had growing forage radish in the fall, regardless of residue removal or addition.

Weed growth in no cover control plots (NC-weedy) was substantial, ranging from 14 to 71% ground cover in early spring and reaching 72 to 89% ground cover by mid-spring (Table 3). Weed

cover was dominated by the winter annual species chickweed, henbit, and speedwell. As observed in other field experiments (Lawley et al., 2011), almost complete weed suppression was observed in all 4 site-years of this experiment in early spring following forage radish (FR-S<sub>1</sub>R<sub>1</sub>). This suppression began to decline by mid-spring, although weed cover was still lower than that in the no cover control (NC-weedy) in 3 of 4 site-years. Weeds were almost completely suppressed by the standard forage radish treatment (FR-S<sub>1</sub>R<sub>1</sub>), therefore doubling forage radish residue (FR-S<sub>2</sub>R<sub>2</sub>) did not provide any additional weed suppression in either early or mid-spring in 2007, this treatment was eliminated from the experiment in 2008 (Table 3).

Regardless of whether forage radish shoots plus roots (FR-S<sub>0</sub>R<sub>0</sub>) or shoots only (FR-S<sub>0</sub>R<sub>1</sub>) were removed before a killing frost in November or left in place to decompose (FR-S<sub>1</sub>R<sub>1</sub>), there was little difference in weed suppression among these treatments in 3 of 4 site-years (Table 3). Although weed cover was higher in FR-S<sub>0</sub>R<sub>0</sub> than in FR-S<sub>1</sub>R<sub>1</sub> in both early and mid-spring at BARC-NF in 2008, weed cover in FR-S<sub>0</sub>R<sub>0</sub> was still substantially lower than that in the no cover control (NC-weedy) in that site-year. Also, the treatments with residue removal (FR-S<sub>0</sub>R<sub>0</sub> and FR-S<sub>0</sub>R<sub>1</sub>) had lower spring weed cover than those treatments that received residue (NC-S1R1 and NC-S1R0) in 2007.

The fall-weeded control treatment (NC-fall-weeded) also provided insight into the potential mechanism of fall weed suppression by forage radish. Weeds were removed from the NC-fall-weeded treatment until the same time in November that forage radish shoots and roots were removed in FR-S<sub>0</sub>R<sub>0</sub>. If the mechanism by which forage radish suppressed weeds was based on eliminating the establishment of winter weeds during the fall, then it would be expected that manual removal of weeds from the NC-fall-weeded treatment would provide the same level of weed suppression as the FR-S<sub>0</sub>R<sub>0</sub> treatment. However, weed suppression was greater in FR-S<sub>0</sub>R<sub>0</sub> than NC-fall-weeded in all site-years in early spring and in 3 of 4 site-years in mid-spring.

Weed cover for NC-fall-weeded was no different than the NC-weedy control in several site-years across both spring weed assessment dates (Table 3). This indicates that forage radish had an additional mechanism for weed suppression than just preventing weed emergence. Although not quantified in this study, differences between residue removal treatments could be explained by differential interception of red to far red radiation. Light, particularly in the red band of the spectrum, are signals used by weed seeds to identify favorable periods for germination and emergence (Baskin and Baskin, 2001). The growing cover crop canopy intercepts short-wave radiation, reduces the amount of light reaching the soil surface, lowers the ratio of red-to-far red radiation, and reduces the heating radiation absorbed by the soil (Teasdale and Daughtry, 1993; Teasdale et al., 2007). The dense forage radish leaf canopy could have maintained or shifted the phytochrome state of weed seeds at the soil surface to an inactive dormant state, thereby reducing their potential for germination (Smith, 1986). In contrast, surface weed seeds in the treatments with no cover crop would be exposed to full sunlight which could release seed dormancy by shifting phytochrome to an active form and by raising daily temperature amplitude (Baskin and Baskin, 1981).

Adding forage radish shoots and roots (NC-S<sub>1</sub>R<sub>1</sub>) or shoots only (NC-S<sub>1</sub>R<sub>0</sub>) to no cover crop plots resulted in responses that varied considerably across years (Table 3). Weed cover in these

treatments were higher or similar to that in the NC-fall-weeded control in 2007, but similar to that in the FR-S<sub>0</sub>R<sub>0</sub> treatment in 2008. This can be explained by the absence of weed control before placement of residues in 2007, which may have slowed but not completely inhibited weeds already established before residue placement. In contrast, control of preexisting weeds in 2008 before residue placement and the observed suppression of weed cover in spring compared to the NC-fall-seeded treatment demonstrates that shoot and root residues on the soil surface had some capacity to suppress new weed establishment. Given the lack of evidence for allelopathy, this finding suggests that these residues may have had a temporary physical suppressive effect before they decomposed.

In summary, addition of radish residues to plots with no cover crop in the fall did not reduce spring weed growth. Conversely, removing forage radish residues or leaving them on the forage radish cover crop plots generally resulted in similar levels of weed suppression in the spring. The results of this experiment provide evidence that the competitive fall growth of forage radish is the primary mechanism of forage radish weed suppression and suggest that allelopathy from decomposing residues is not involved.

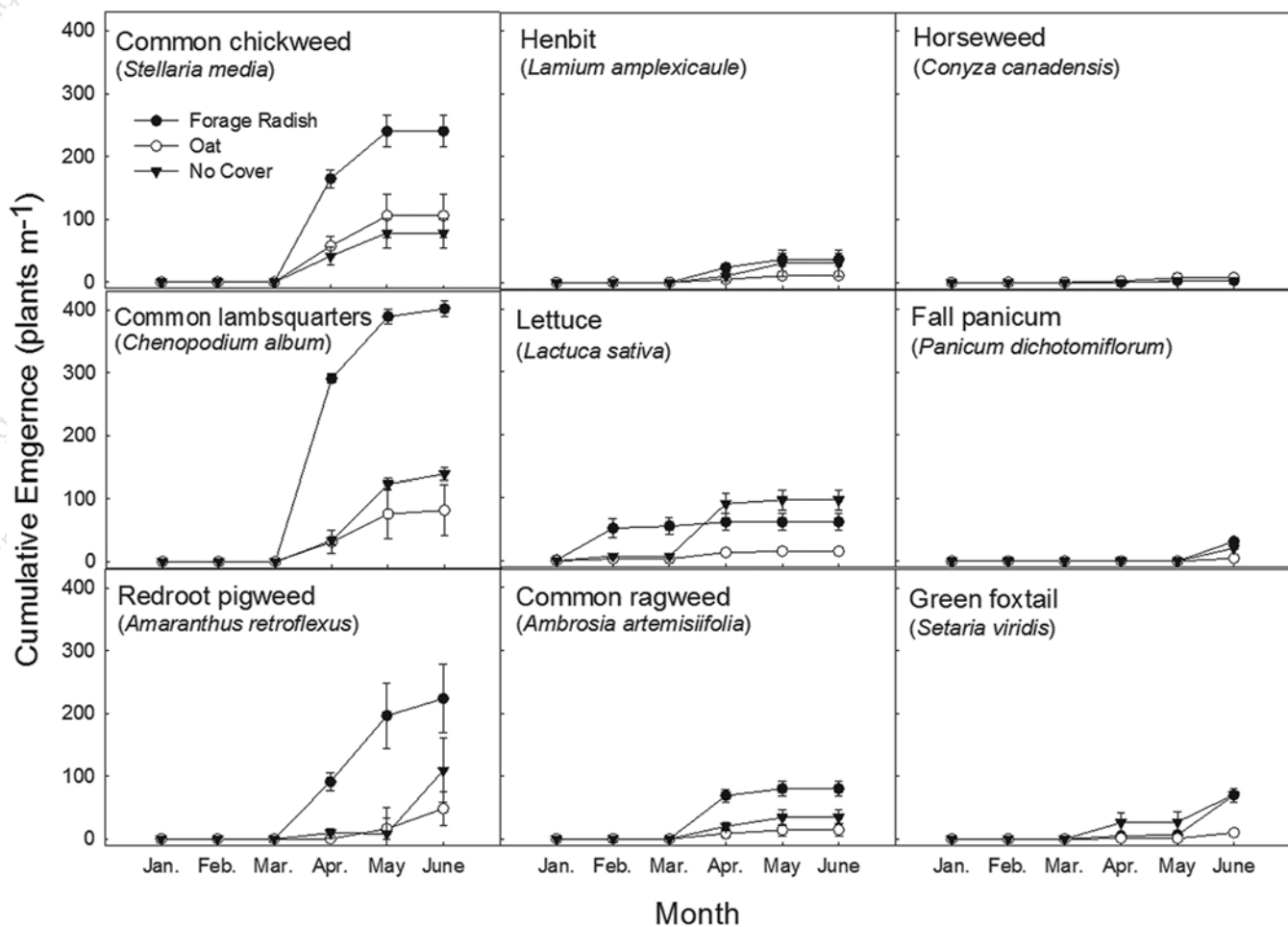
## Experiment 2: Planted Weed Seed Bioassay

This weed seed bioassay was conducted under field conditions because much allelopathy research has overlooked the soil factors influencing the movement and availability of allelochemicals to interact with weed seeds in the soil (Boydston and Al-Khatib, 2006). Both winter annual and summer annual weed species were selected for the bioassay as the type and duration of forage radish weed suppression was unknown at the time. We hypothesized that decomposing forage radish residues would reduce the spring emergence of planted weed seeds relative to a no cover crop treatment if allelopathy was the dominant mechanism of weed suppression.

Weed and lettuce emergence was not suppressed by forage radish relative to the no cover crop control or the oat cover crop treatment regardless of weed type (summer vs. winter annual) or species (Fig. 1). Weed emergence was higher in the forage radish treatment for several of the weeds species planted, including common chickweed, common lambsquarters, redroot pigweed, and common ragweed. Emergence of lettuce occurred much earlier (February) in forage radish treatments than the other two treatments (April) (Fig. 1).

In the Netherlands, field bioassays with fodder radish winter cover crops did not detect any allelopathic effect on emergence of lettuce or sugar beet test crops (Kruidhof et al., 2008). Brown and Morra (1996) observed delayed germination of lettuce seeds when exposed to water-soluble extracts of rapeseed (*Brassica napus* L.) plant tissues. However, field bioassays conducted by Haramoto and Gallandt (2005) did not find consistent reductions or delays in lettuce or tomato seed germination following rapeseed, mustard (*Sinapis alba* L.), or canola (*B. rapa* L.) cover crops in Maine.

Stimulation of lettuce and weed seed emergence may have been due to higher soil nitrate levels in the forage radish treatment (Fig. 2). Some weed species, such as common lambsquarters, use nutrients as a signal to promote germination (Bouwmeester and Karssen, 1993). Following further observations of increased spring emergence of common lambsquarters from the natural weed seed bank following forage radish in other field experiments (Lawley et al., 2011), common lambsquarters was introduced into two subsequent field experiments.



**Fig. 1.** Mean cumulative weed emergence of planted weed seeds and lettuce seeds below decomposing forage radish cover crop, decomposing oat cover crop, and no cover crop control treatments in 2006 at the USDA Beltsville Agricultural Research Center North Farm. Data points are an average of four observations. Error bars represent standard error of the mean.

The results of these field bioassays agree with these earlier observations in 2 out of 3 site-years (Fig. 3).

The stimulatory effect of forage radish cover crops on winter annual weed species observed in this field bioassay contrast with the results of other field experiments (Lawley et al., 2011). In field experiments, Lawley et al. (2011) observed that forage radish cover crops delayed emergence of winter annual weeds relative to no cover crop. One of the differences between these field experiments and the field bioassay was the timing of weed seed introduction and germination. In the field bioassay, winter annual weeds in both forage radish and no cover control plots were forced to germinate in the spring, whereas many would naturally germinate and establish during the fall, as occurred in the no cover crop plots in the field experiments. The winter introduction date in the field bioassay also meant that planted weeds in the forage radish treatment were influenced only by residue decomposition and not by the fall cover crop growth as occurred in the field experiments. This further supports the hypothesis that forage radish weed suppression is the result of fall cover crop weed suppression due to rapid canopy development, rather than allelopathy.

### Experiment 3: Soil Bioassay

We hypothesized that if forage radish was allelopathic, lettuce or tomato germination and seedling growth under controlled environment conditions would be reduced in soils

sampled below decomposing forage radish residues relative to a no cover crop control. We also hypothesized that the allelopathic effects of forage radish cover crops would be greater in soils collected during the time of most active radish decomposition in January than in soils collected during March. However, we reject both hypotheses based on assay results. In all but one case the significant differences between no cover crop and forage radish treatments indicated a stimulatory effect of forage radish, rather than an inhibitory effect, causing improved lettuce seedling biomass or tomato seed germination (Fig. 4).

Tomato seed germination was greater in forage radish treatments relative to the no cover crop control in January and March for soils sampled at BARC-SF. Lettuce seedling DM was greater in forage radish treatments than in the no cover crop control in both January and February. These stimulatory effects of forage radish on lettuce and tomato agree with the findings of Exp. 2 and provide evidence to reject allelopathy as the mechanism of forage radish weed suppression. The stimulation of tomato seed germination and lettuce seedling DM in forage radish treatments could be due to the higher nitrate content of the soil sampled from the forage radish treatment (Fig. 2).

One potential limitation of this experimental approach is higher temperature and moisture in the test chambers than in the field which could have caused loss of volatile allelochemicals, such as many ITCs. Petersen et al. (2001) conducted soil bioassays to

evaluate the allelopathic effect of turnip-rape (*B. rapa* (Rapifera Group)–*B. napus* L.) mulch and identified ITCs present in both the plant tissue and soil. The ITC concentration in their study was 2300 times lower in the soil than in plant tissues and their disappearance from the soil was enhanced by saturated soil conditions and high temperatures. Sampling of soil for the bioassay also resulted in the separation of soil and plant residues, the potential source for a continued supply of newly forming ITCs as these residues decomposed. However, if allelopathy was responsible for the strong weed suppression observed in the field, we would have expected to observe some suppressive activity in these soils, despite the potential attenuating conditions of this assay.

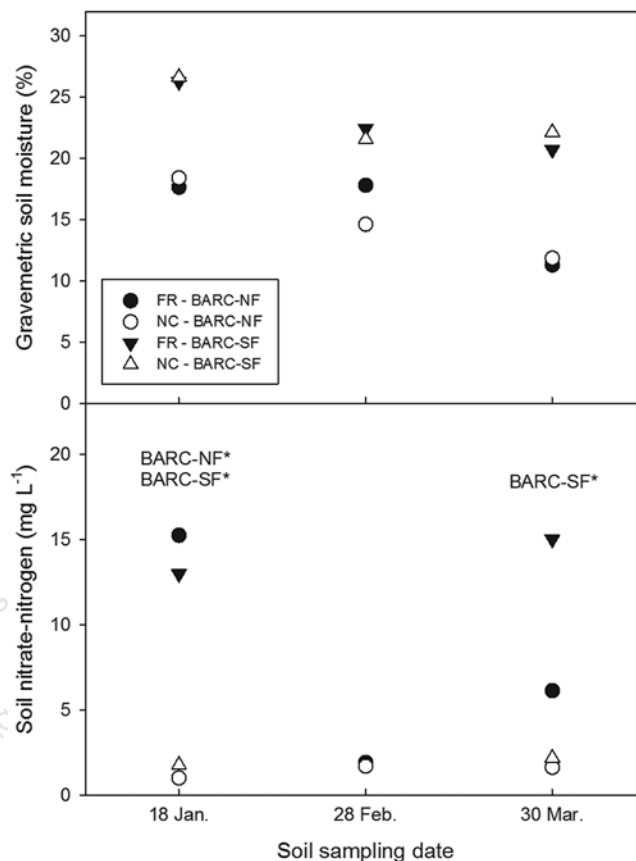
#### Experiment 4: Aqueous Plant and Soil Extracts Plant Tissue Extracts

We hypothesized that if allelopathy was the mechanism of forage radish weed suppression, then aqueous extracts of forage radish tissues would inhibit lettuce seed germination and root growth. Extracts of both forage radish shoot and root tissues were included in the experiment to differentiate the location of potential allelopathic compounds. The allelopathic potential of living forage radish plant tissues was compared to plant residues by preparing aqueous extracts of plant residues harvested in November before frost damage and decomposing plant residues harvested the following March. Oat was included as a treatment because it is another frost sensitive cover crop that is also reported to have allelopathic properties (Inderjit and Keating, 1999).

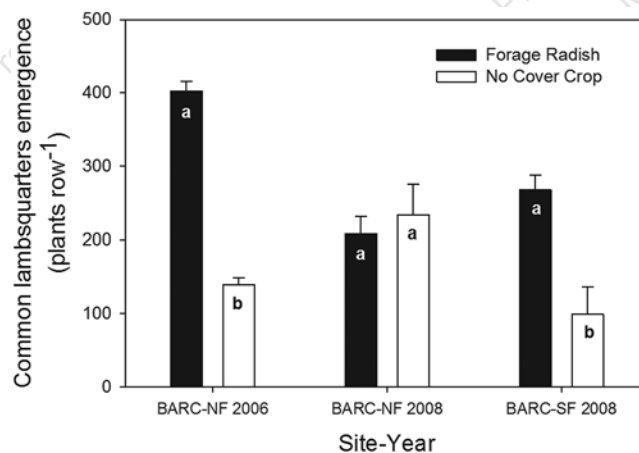
Aqueous extracts of living forage radish tissues harvested before frost in November had an inhibitory effect on lettuce germination and root length relative to a distilled water control treatment (Fig. 5). Aqueous extracts of forage radish residues harvested in March had a stimulatory effect on relative lettuce root length and an inhibitory effect on relative lettuce germination only at the highest extract concentration. Plant tissue extracts had little effect on the relative shoot length of lettuce in both November and March (data not shown).

Despite differences in color and odor of the two extracts (forage radish root extracts had a very pungent odor and dark color), both shoot and root tissues of forage radish had similar effects on relative lettuce germination and relative root growth (Fig. 5). Thus, no differential response was observed between forage radish roots and shoots. Aqueous extracts of living oat tissue harvested in November had similar effects on lettuce germination and root growth to those observed with forage radish shoot extracts (Fig. 5). Extracts of oat residues harvested in March had no effect on relative lettuce germination. Extracts of oat residue harvested in March had the same stimulatory effect on lettuce roots length that was observed with forage radish residues.

Relative lettuce seed germination and relative root length increased with the dilution of the full strength plant tissue extracts for all tissues sampled in November 2005 (Fig. 5). The largest decline in relative germination occurred in forage radish root and shoot tissue extracts in proportions at or above 0.5 of the full strength extract. For extracts prepared from plant residues collected in March, lettuce germination declined only in full strength extracts prepared from forage radish root and shoot tissues. Extracts prepared from plant residue in March had a stimulatory effect on the relative root length of lettuce seedlings at extract proportions of 0.125 and 0.25 (Fig. 5).



**Fig. 2. Initial gravimetric soil moisture and soil nitrate-N content of soils sampled from forage radish (FR) and no cover crop (NC) treatments at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF). Data points are an average of four observations. Significant differences between pairs of FR and NC treatments within a site are indicated by BARC-NF\* or BARC-SF\* ( $P < 0.05$ ). No samples were available from the BARC-SF to measure soil nitrate-N for the 28 February sampling date.**



**Fig. 3. Mean emergence of common lambsquarters below decomposing forage radish residues and a no cover crop control for 3 site-years at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF). Bars represent an average of four observations. Bars topped with different letters indicate significant treatment differences at the  $p = 0.05$  level within a site-year. Error bars represent standard error of the mean.**

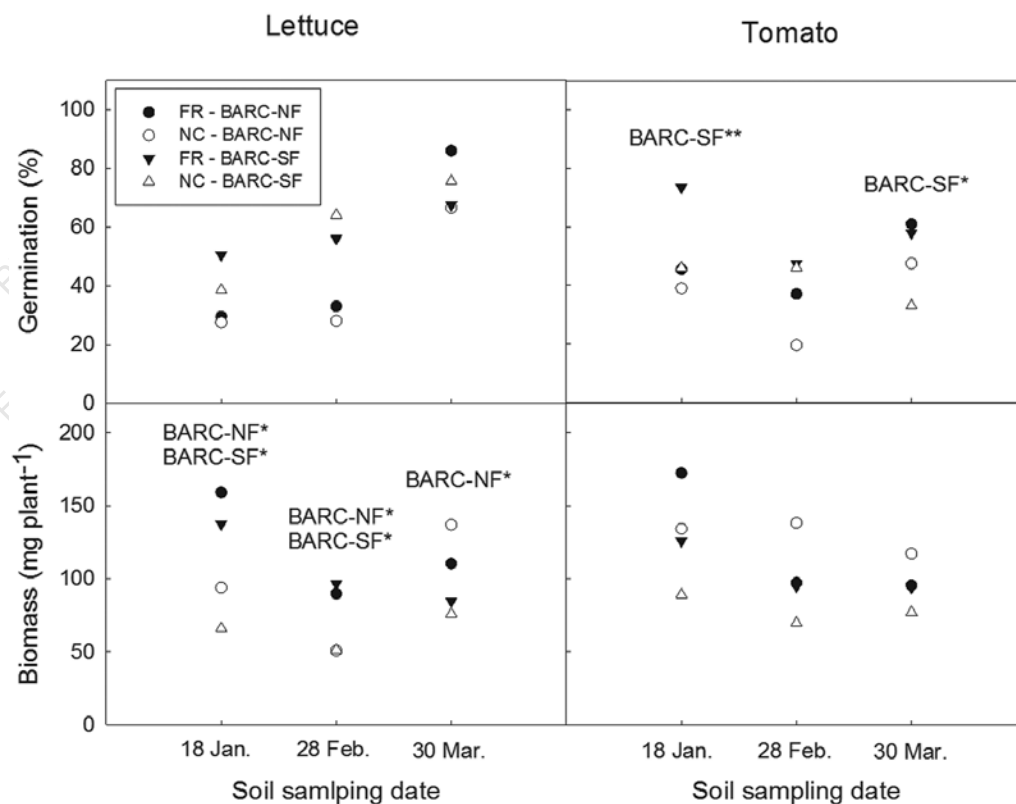


Fig. 4. Effect of soil samples collected below decomposing forage radish residues (FR) or no cover crop (NC) on lettuce and tomato germination and seedling biomass. Soils were sampled from fields at the USDA Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF). Data points are an average of four observations. Significant differences between pairs of FR and NC treatments within a site are indicated by BARC-NF\* or BARC-SF\* ( $p < 0.05$ ).

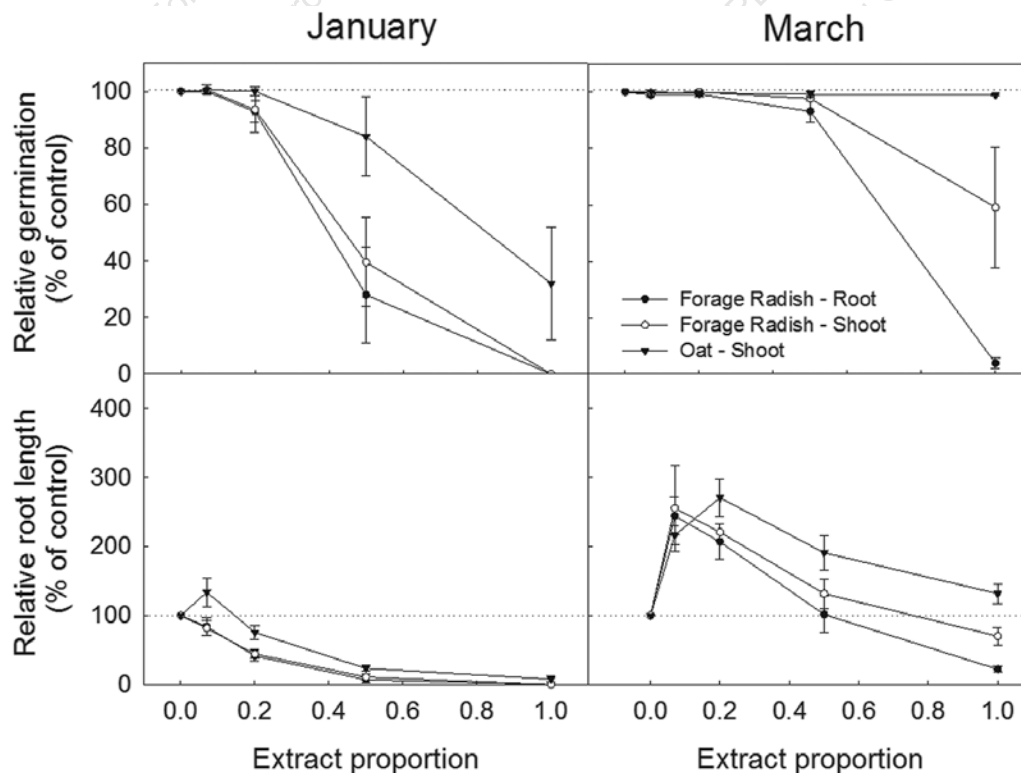


Fig. 5. Relative germination and root length of lettuce seedlings grown in aqueous plant tissue extracts. Germination and root lengths are expressed as a percent of the distilled water control. Extracts were prepared from fresh forage radish shoot, forage radish root, and oat shoot tissues collected on 7 Nov. 2005, and from plant residues collected on 24 Mar. 2006. Data points are an average of four observations. Error bars represent standard error of the mean.

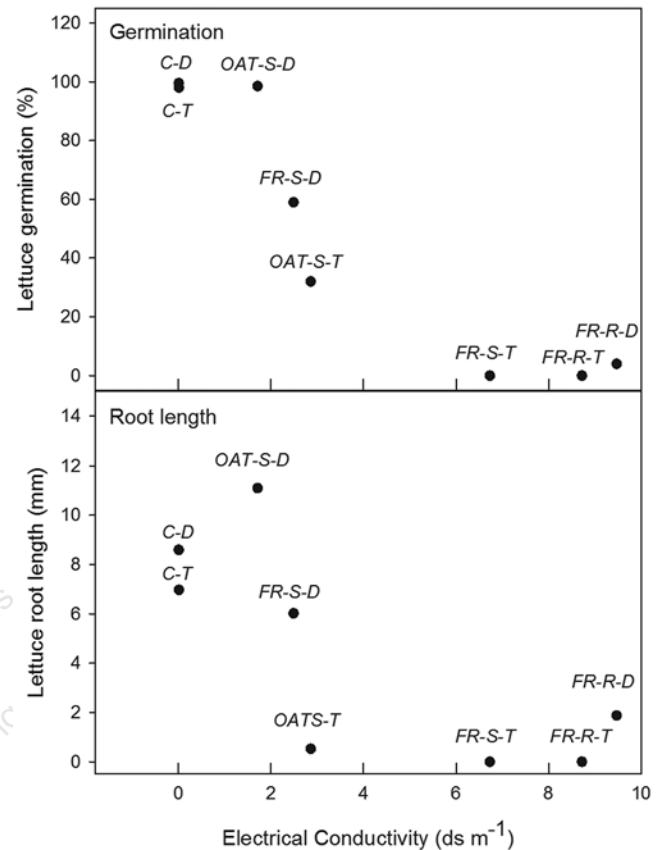


Although these results might suggest allelopathic potential, it is likely that the negative effects of full-strength forage radish and oat extracts on lettuce germination and root growth were due to the osmotic potential of the extract solutions. Regardless of whether the extract was prepared from plant tissue vs. residues or prepared from oat vs. forage radish, there was a general trend of decreasing lettuce seed germination and root length with increasing electrical conductivity, with a threshold between 2 and 4  $\text{dS m}^{-1}$  (Fig. 6). Both types of forage radish tissue extracts also had high electrical conductivity (Fig. 6). The root tissue extract had a higher electrical conductivity and more inhibitory effect on lettuce seedlings than the shoot tissue extract. Previous studies have shown lettuce to be moderately sensitive to salinity with an initial threshold for yield decline at an electrical conductivity of  $1.3 \text{ dS m}^{-1}$  (Shannon and Grieve, 1999).

### Soil Extracts

Soil extracts were included in this experiment to test for potential retention of allelochemicals in the soil that could have a residual effect on weed seed germination and seedling growth. Because weeds naturally encounter allelochemicals within the soil environment, it was thought that soil extracts would provide a more realistic bioassay treatment than those prepared from plant tissues. We hypothesized that soil sampled beneath decomposing forage radish residues would decrease lettuce seed germination as well as root and shoot growth. We also hypothesized that these effects would be greater in March, when weed suppression was previously observed in the field by Lawley et al. (2011), than in May, when no weed suppression was observed.

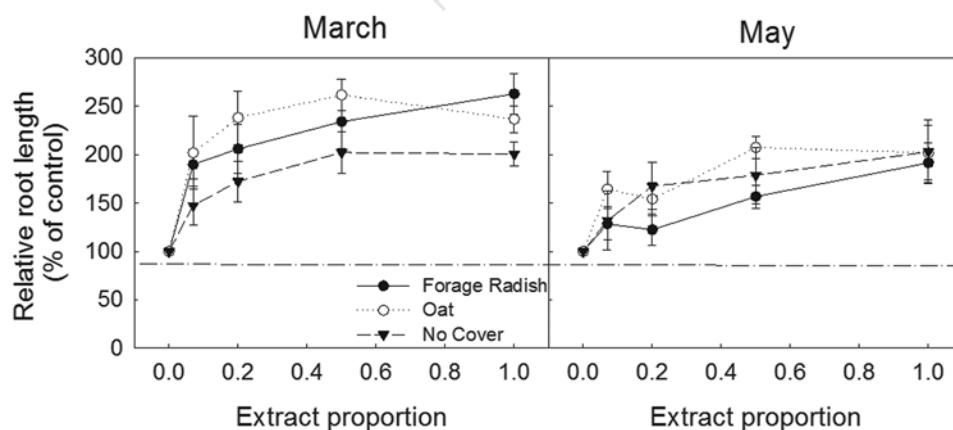
Contrary to our hypotheses, the extracts prepared from cover crop-amended soil did not reduce lettuce seed germination or root growth. However, both cover crop treatment extracts as well as the no cover crop control extract had a stimulatory effect on lettuce root length relative to the distilled water control in March and May (Fig. 7). Unlike extracts prepared from plant tissues, relative root length of lettuce seedlings increased with increasing soil extract proportion. The soil extracts had very low electrical conductivity (EC) ( $<0.1 \text{ dS m}^{-1}$ ). Soil extracts did not have an effect on relative shoot length or lettuce seed germination (data not shown). These results suggest that there were no or very low concentrations of allelochemicals present in the soil extracts and that noncover crop factors were the cause of lettuce



**Fig. 6.** Relationship between lettuce performance and electrical conductivity of aqueous plant tissue extracts and a distilled water control. Extracts were prepared from forage radish root (FR-R), forage radish shoot (FR-S), and oat shoot (OAT-S) and were compared to a distilled water control (C). Plant tissues (T) were harvested November 2005 and residues (D) harvested 24 Mar. 2006. Lettuce root length and germination are an average of four observations. Electrical conductivity readings were measured on one extract.

stimulation, such as nutrients released by organic matter decomposition or from the soil cation exchange.

Results from the bioassay of plant tissue extracts can be explained by high EC levels, and thus only weakly suggest any potential for allelopathy. Certainly the results of the soil extract bioassay suggest that any inhibitory affect, whether due to allelopathy or osmotic potential, were not realized in the soil. Thus,



**Fig. 7.** Relative root length of lettuce seedlings grown in aqueous soil extracts. Root lengths are expressed as a percent of the distilled water control. Soil extracts were prepared from surface soil samples (0–5 cm) collected from forage radish, oat, and no cover crop field treatments on 28 Mar. and 30 May 2006. Data points are an average of four observations. Error bars represent stand error of the mean.

aqueous extract bioassays did not present strong evidence in support of the allelopathy hypothesis for the occurrence of weed suppression following forage radish winter cover crop.

## CONCLUSIONS

By employing multiple experimental approaches, the results of the four experiments in this study point to a common conclusion that early and competitive fall growth of forage radish is the dominant mechanism for weed suppression. Results of the forage radish residue-transfer experiment supported the hypothesis that fall cover crop weed competition due to rapid canopy development is the mechanism of weed suppression following forage radish cover crops. The presence or absence of decomposing residue after winter-kill had relatively little effect on weed suppression. Field and controlled environment bioassays using cover crop-amended soil and aqueous extracts of cover crop tissues and amended soil did not reveal any allelopathic activity limiting seed germination or seedling establishment. In fact, forage radish-amended soils stimulated seedling growth in both types of bioassays.

Cover crop management strategies to maximize weed suppression following forage radish cover crops should ensure that crop rotations allow for early planting of forage radish cover crops. If factors such as late planting, drought, low soil fertility, or early frost limit the rapid canopy development of forage radish in the late summer or early fall, alternative pre-plant weed control is likely to be required the following spring. The results of this study along with the findings presented in Lawley et al. (2011) demonstrate that a competitive fall forage radish cover crop stand can be achieved a relatively weed-free and residue-free seedbed in early spring to facilitate early crop planting operations. The seed bed following forage radish cover crops may be of special interest to organic farmers looking to eliminate or reduce spring tillage for direct seeding of subsequent vegetable or grain crops.

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