Brassica cover cropping: I. Effects on weed and crop establishment

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The Brassicaceae contain glucosinolates, which hydrolyze to form compounds toxic to plants, fungi, nematodes, and certain insects. Lower weed density and biomass in crops grown following incorporation of brassica cover crops suggest that they may contribute to weed management in agricultural systems. Field experiments were conducted to determine whether incorporated brassica cover crops, including canola, rapeseed, and yellow mustard, reduce subsequent weed and crop establishment; a companion paper describes separate but related field experiments that examined the influence of brassica cover crops on plant growth. Emergence rate and total emergence of sixteen weed and crop bioassay species were measured following brassica cover crops, fallow, or incorporated residues of other short-season cover crops including oat, crimson clover, and buckwheat. The bioassay species, representing a range of seed sizes, were chosen to determine whether larger seed size confers protection from residue-mediated effects on emergence. Averaged over bioassay species, brassica cover crops reduced emergence by 23 to 34% compared with fallow; emergence following brassicas was delayed by approximately 2 d. The effects of the incorporated brassica residues were similar to those of the other short-season cover crops, which reduced emergence of the bioassay species by 19 to 39% and delayed emergence by 2 d. Seed size was a poor predictor of a species' establishment. These results suggest that brassica residues are capable of delaying seedling emergence and reducing establishment, although the magnitude of their effects were comparable to other widely available cover crops.

Nomenclature: Canola, 'Hyola', *Brassica napus* L.; rapeseed, 'Dwarf Essex', *Brassica napus* L.; yellow mustard, 'Idagold', *Sinapis alba* L.; crimson clover, *Trifolium incarnatum* L.; oat, *Avena sativa* L.; buckwheat, *Fagopyrum esculentum* Moench.

Key words: Allelopathy, biofumigant, seed size, weed emergence.

Preventative and cultural weed management practices are essential to support and ensure the success of physical weed control on organic farms (Bárberi 2002). Cover cropping is considered pivotal in this regard, contributing to weed management through greater diversity of resource competition and niche disruption (Liebman and Davis 2000) and, ultimately, offering both soil improvement and an ecological approach to weed management (Gallandt 2004; Gallandt et al. 1999; Liebman and Gallandt 1997).

Brassica cover crops have generated considerable interest because of their potential to suppress soil-borne pests. All brassicaceous plants examined to date contain glucosinolates (Brown and Morra 1997; Mithen 2001; Rosa et al. 1997). These compounds are enzymatically hydrolyzed upon tissue disruption to release a variety of biologically active compounds, including isothiocyanates, which are toxic to certain insects (Blau et al. 1978), fungi (Muehlchen et al. 1990), nematodes (Mojtahedi et al. 1993), and plants (Bell and Muller 1973). Isothiocyanates were shown to be potent inhibitors of seed germination (Petersen et al. 2001; Teasdale and Taylorson 1986) and caused stunted seedling growth (Wolf et al. 1984). In greenhouse studies, brassica residues containing these compounds reduced germination rates and the size of emerged weed seedlings (Al-Khatib et al. 1997; Boydston and Hang 1995; Krishnan et al. 1998; Vaughn and Boydston 1997); water-soluble extracts of brassicas also delayed seed germination (Brown and Morra 1996). Evi-

dence from field studies confirm that residues of brassicas, including canola, rapeseed, and mustards, may contribute to weed management (Haramoto and Gallandt 2004). For example, compared with fallow or other nonbrassica cover crops, preceding brassica cover crops reduced weed biomass and early to mid-season weed density in potato (*Solanum tuberosum* L.) (Boydston and Hang 1995), soybean [*Glycine max* (L.) Merr.] (Krishnan et al. 1998), and green pea (*Pisum sativum* L.) (Al-Khatib et al. 1997).

Incorporated cover crop residues may inhibit weed but not crop establishment through seed-size–dependent effects on germination and emergence. The mass of most weed seeds is one to three orders of magnitude smaller than the seed mass of crops they infest (Mohler 1996). Westoby et al. (1996) suggested that seed size plays an important role in determining a seed's response to various stresses, including physical and chemical stresses imposed by cover crop residues.

Although there is considerable evidence that brassica residues are allelopathic and may contribute to weed management, there is a noted lack of field studies examining whether brassica residues act primarily at the level of the population by reducing weed density, at the level of the individual by reducing growth, or both. Red clover (*Trifolium pratense* L.) residues, for example, may both reduce weed density (Gallandt, unpublished data) *and* growth of established plants (Liebman and Gallandt 2002). Two separate but re-

lated sets of field experiments, carried out over 2 yr, were conducted to examine the effect of incorporated brassica cover crop residues on weed and crop emergence and subsequent growth. In this article, we compare the emergence rate and total emergence of weed and crop species following fallow, brassicas, and other short-season cover crops. In a companion article (Haramoto and Gallandt 2005), we examine the impacts of incorporated brassica residues on the growth of established green bean (*Phaseolus vulgaris* L.) and redroot pigweed (*Amaranthus retroflexus* L.).

The objectives of this research were (1) to evaluate the effects of incorporated brassica residues on the establishment of selected weed and crop species; (2) to compare putative weed-suppressive effects of incorporated brassica residues with other short-season cover crops more commonly grown in the northeastern United States, including crimson clover residue, which is known to inhibit seedling emergence (Dyck and Liebman 1994; Dyck et al. 1995; White et al. 1989); (3) to compare the effects of brassicas with a range of glucosinolate content on weed establishment; and (4) to determine whether seed mass would confer selectivity in residue-mediated effects on emergence rate and total emergence.

Materials and Methods

Field Procedures

Field studies were conducted at the University of Maine's Rogers Farm in Stillwater, ME. The 2002 field site was a Lamoine silt loam (fine, illitic, nonacid, frigid, Aeric Epiaquepts), pH 6.1 with 3.9% organic matter; the 2003 field site was a Nicholville very fine sandy loam (coarse-silty, mixed, frigid, Aquic Haplorthods), pH 6.4, and 3.8% organic matter. Both fields were previously cropped to potato. A split-plot design was used in both years, with cover crop treatments as the main plots and the bioassay species as subplots. Main plots were 6 m by 3 m and each cover crop treatment was replicated four times. Because of the highly uniform appearance of the site, main plots were arranged in a completely randomized design in 2002; an apparent gradient in soil texture prompted the use of a randomized complete block design in 2003 to assign main plots. Within each main plot, bioassay species' locations were assigned completely randomly in both years. The cover crop treatments consisted of a fallow control and six early season cover crops that were grown and incorporated before conducting the in-field bioassays. The three brassica cover crops included a high-glucosinolate yellow mustard (var. 'Idagold'), a low-glucosinolate spring canola (var. 'Hyola'), and winter rapeseed (var. 'Dwarf Essex') with an intermediate glucosinolate content; brassicas were sown at 11 kg ha^{-1}. Nonbrassica cover crops included buckwheat, oat, and crimson clover, sown at 150, 123, and 45 kg ha^{-1}, respectively. Buckwheat and oat were chosen because they are commonly grown as cover crops throughout the northeastern United States, and crimson clover was chosen because its residue has demonstrated allelopathic properties (Dyck and Liebman 1994; Dyck et al. 1995; White et al. 1989). Crimson clover seed was inoculated with rhizobium (*Rhizobium leguminosarum* var *trifolii*) before planting. Field preparation consisted of disking and packing in 2002 and harrowing and packing in 2003. All cover crop seed was hand-scattered, and soil was packed with a roller on May 11, 2002, and May 19, 2003. To maintain a similar soil disturbance regime to the cover cropped plots, which were not disturbed after planting, weeds were controlled in fallow plots by periodic cutting of small seedlings with a powered string trimmer.

Cover Crop Sampling

On July 15, 2002, and July 14, 2003, before mowing and incorporation, cover crops were sampled to estimate aboveground biomass; two 0.125-m^2 quadrats were sampled per plot. Samples were dried for 7 d at 65 C and weighed. On July 17, 2002, and July 15, 2003, plots were mowed with a flail-mower, and residues were then incorporated to a depth of 15 cm by two passes of a rotary tiller.

In-Field Bioassay of Incorporated Residues

Immediately following residue incorporation, seeds of the selected weed and crop bioassay species (Table 1) were planted by hand in all plots. These bioassay species were chosen to address applied questions for growers and to provide a wide range of seed sizes to test the hypothesis related to seed size. The density of germinable seeds sown was based on Petri dish assays conducted over a 2-wk period at 20 C in May of each year. Germination ranged from 10 to 70% for weed species, and 80 to 100% for crop species (data not shown). A 1.0-m-long by 0.6-cm-deep trench was made by pressing the edge of a hardwood stake into the soil; each end of the trench was marked with a plastic pot stake. Seeds were placed into the trench, covered with soil, and firmly packed. Field pea and green bean were planted in a similar manner but to a depth of 3 cm. Ten bioassay species were examined in both years of the experiment; additional species representing a greater range in seed size were included in 2003. Fewer seeds of the larger-seeded species were planted to facilitate counting; sowing density was increased for weed species with lower germination rates to ensure sufficient potential emergence for analysis (Table 1). To quantify both the rate of emergence and total emergence, seedlings were counted and pulled daily during the peak of emergence and less frequently as emergence slowed. To minimize possibly confounding effects of the intrinsic soil seed bank, only seedlings emerging directly in the originally seeded 1 m row were counted. The predominant resident weed species in each field was common lambsquarters (*Chenopodium album* L.), with comparatively lower densities of redroot pigweed in both sites, wild mustard [*Brassica kaber* (DC.) L.C. Wheeler] in the 2002 site, and hairy galinsoga (*Galinsoga ciliata* L.) in the 2003 site. All other bioassay species (Table 1) were unique to the experimental sites.

Curve Fitting and Data Analysis

A logistic function was fit to the emergence data:

$$Y = a/(1 + b \times e^{(-c \times DAP)}) \qquad [1]$$

where Y was the cumulative number of emerged seedlings at that date, DAP was days after planting, a was the asymptote or total emergence, and b and c were fit parameters (Dyck and Liebman 1994). The emergence function was fit to the data on a plot-by-plot basis using GraphPad Prism (version 3) software.[1] Because different numbers of seeds

TABLE 1. Species, seed mass, and number of viable seeds sown for in-field bioassays in 2002 and 2003.

Common name (Bayer Code)	Binomial	Cultivar	Average mass per 100 seeds	Viable seeds sown[a]	
				2002	2003
			mg	no.	
Hairy galinsoga (GALCI)	*Galinsoga ciliata*	—	14	160	659
Redroot pigweed (AMARE)	*Amaranthus retroflexus*	—	35	265	343
Common lambsquarters (CHEAL)	*Chenopodium album*	—	71	232	323
Carrot	*Daucus carota*	Danvers	127	268	311
Lettuce	*Lactuca sativa*	Simpson's	131	287	340
Yellow foxtail (SETLU)	*Setaria glauca*	—	165	231	356
Wild mustard (BRAKA)	*Brassica kaber*	—	176	232	309
Tomato	*Lycopersicon esculentum*	Scotia	279	—	312
Broccoli	*Brassica oleracea*	Parkman	422	—	301
Rapeseed	*Brassica napus*	Dwarf Essex	472	—	321
Velvetleaf (ABUTH)	*Abutilon theophrasti*	—	739	108	158
Spinach	*Spinacia oleracea*	Bloomsdale	1035	95	—
Rye	*Secale cereale*	Aroostook	2681	—	197
Cucumber	*Cucumis sativus*	Straight 8	2743	115	106
Field pea	*Pisum sativum*	Trapper	14,769	50	49
Green bean	*Phaseolus vulgaris*	Provider	30,767	—	51

[a] Viability of the bioassay species was determined by placing > 20 seeds of each species into three replicate Petri dishes containing moistened filter paper; these were incubated at 20 C under alternating light/dark conditions for 14 d. Eleven species were included in 2002, 15 in 2003.

were planted depending on the species, the number of emerged seedlings was standardized as the percentage of total viable seeds sown (Table 1). Parameters b and c were used to calculate the number of days elapsed until 50% of the seedlings had emerged (T_{50}),

$$T_{50} = \ln(b)/c \qquad [2]$$

Total emergence (a), as a percentage of the viable seeds sown, and T_{50} were subjected to analysis of variance (ANOVA; SYSTAT 2003). The main plot factor was cover crop, and the subplot factor was bioassay species. Total emergence data were square root transformed in 2002 to meet normality assumptions but did not require transformation in 2003. To satisfy assumptions of normality and equality of variances, emergence rate data were log-transformed in 2002 and log-log transformed in 2003. Our initial hypotheses established a series of preplanned contrasts used to test for effects of residue type on emergence rate and total emergence. These single degree-of-freedom contrasts, because they compared cover crop treatments, were performed using the main plot error term. Average 100-seed mass was used in analysis of covariance (ANCOVA) to assess the role of seed mass in determining emergence, both alone and as an interaction with cover crop residue type.

TABLE 2. Comparison of cover crop, aboveground, dry biomass (± standard error) measured before incorporation in 2002 and 2003.

Cover crop	Above-ground dry biomass	
	2002	2003
	g m^{-2}	
Buckwheat	391 ± 29	339 ± 40
Crimson clover	337 ± 55	333 ± 50
Rapeseed	303 ± 106	218 ± 34
Canola	380 ± 51	342 ± 51
Mustard	439 ± 55	372 ± 62
Oat	405 ± 79	409 ± 60

Results and Discussion

Cover Crop Growth

At the time of mowing and incorporation, crimson clover, canola, yellow mustard, oats, and buckwheat had flowered and set seed, but this seed was not mature (late flowering, or stage 4.4 for brassicas; Harper and Berkenkamp 1975). The winter rapeseed had not flowered at this time. Cover crop biomass was similar in both years of the experiment (Table 2). The amount of yellow mustard biomass incorporated in our studies was similar to the 420 to 450 g m^{-2} of dry white mustard (*Sinapis alba* L.) biomass that reduced weed density in the field studies of Al-Khatib et al. (1997).

Total Seedling Emergence

As blocking failed to explain a significant portion of variability in the 2003 experiment, the block term was removed in an attempt to explicitly test year and year by treatment effects. The 10 species common to both years were included in this analysis. However, because of unequal variances, we could not combine the data sets to assess the effect of year (Gomez and Gomez 1984).

Cover Crop Main Effect

Preplanned, single degree-of-freedom contrasts were used to examine the main effect of cover crop treatments (Table 3). Averaged over bioassay species, brassica cover crops reduced total emergence compared with fallow by 34 and 23% in 2002 and 2003, respectively. Similarly, the nonbrassica cover crops reduced total emergence 39 and 19% compared with fallow in 2002 and 2003, respectively. Total emergence in both years was similar between brassica and nonbrassica cover crops and similar between the higher-glucosinolate yellow mustard and the other brassica cover crops. Crimson clover, compared with the other nonleguminous

TABLE 3. Preplanned contrasts testing the effects of incorporated cover crop residues on total emergence averaged over 11 bioassay species in 2002 and 15 bioassay species in 2003.

Contrast	Mean emergence		P	
	2002	2003	2002	2003
	— % of viable seeds sown —			
Fallow vs.	62	77	< 0.001	< 0.001
Brassica cover crops	41	59		
Fallow vs.	62	77	< 0.001	0.002
Nonbrassica cover crops	38	62		
Brassica cover crops vs.	41	59	0.463	0.296
Nonbrassica cover crops	38	62		
Idagold mustard vs.	42	61	0.626	0.581
Other brassica cover crops	40	58		
Crimson clover vs.	29	48	0.001	0.001
All other cover crops	42	63		

cover crops, reduced total emergence 31% and 24% in 2002 and 2003, respectively.

In Washington state, 420 to 450 g m^{-2} of dry white mustard biomass, grown over winter, reduced weed density by 17% at 30 DAP of green pea; more biomass, 630 to 820 g m^{-2} of lower-glucosinolate rapeseed reduced weed density 34% (Al-Khatib et al. 1997). Also in Washington state, 410 to 580 g m^{-2} of dry overwintered rapeseed biomass was incorporated before planting potato and reduced midseason weed density by 73 to 85% compared with fallow; early season effects on emergence were not measured (Boydston and Hang 1995).

Crimson clover caused the greatest reduction in total emergence in both years (Table 3). Crimson clover suppression of seedling emergence has been noted previously and was attributed to either nitrogen immobilization or phytotoxin release (Dyck and Liebman 1994; Dyck et al. 1995; White et al. 1989). Dyck et al. (1995) found soil nitrate levels were often depressed following crimson clover incorporation compared with plots amended with synthetic nitrogen (N) fertilizer, but lower nitrate levels and reduced seedling emergence did not consistently co-occur, suggesting that low-nitrate concentration is not the sole explanatory factor. Decomposition of incorporated legume plant material may release a large flush of ammonia that can be toxic to newly germinated seedlings (Megie et al. 1967). Further supporting the role of allelopathy in these residue-mediated effects, aqueous extracts of crimson clover residue reduced germination of cotton (*Gossypium hirsutum*), pitted morningglory (*Ipomoea lacunosa* L.), wild mustard, and Italian ryegrass (*Lolium multiflorum* Lam.) seeds in Petri dish assays (White et al. 1989). Bradow and Connick (1990) characterized a number of volatile compounds released from legume cover crops that inhibited germination of onion (*Allium cepa* L.), carrot (*Daucus carota* L.), and tomato (*Lycopersicon esculentum* Mill.). These in vitro studies suggest that crimson clover effects on emergence are a consequence of released phytotoxins in addition to possible nitrogen immobilization or ammonia phytotoxicity.

Seed Size Effect

ANCOVA demonstrated that seed mass failed to explain a significant portion of the variability in total emergence in either year of this experiment (P = 0.179 and P = 0.831

in 2002 and 2003, respectively). Likewise, the interaction between cover crop treatment and seed mass failed to account for a significant portion of variability (P = 0.982 and P = 0.797 in 2002 and 2003, respectively), suggesting that the incorporated cover crops were not affecting smaller seeds more than larger ones. These results conflict with several other studies that have demonstrated the importance of seed mass in determining selectivity of allelopathic effects. After exposing a wide range of weed and crop species to aqueous red clover extracts, Liebman and Davis (2000) reported a strong inverse relationship between seed mass and inhibition of radicle elongation. Petersen et al. (2001) found that smaller seeds, such as spiny sowthistle [*Sonchus asper* (L.) Hill], had lower germination rates after exposure to brassica tissues than larger ones, such as wheat (*Triticum aestivum* L.). Although crimson clover consistently suppressed emergence of common lambsquarters, the residue effects on corn (*Zea mays* L.) emergence were less consistent (Dyck et al. 1995). Factors other than seed size, including seed morphology (Vera et al. 1987; Waddington 1978) and biochemistry (Liebman and Davis 2000), may also be important in regulating a species' tolerance to allelopathic residues.

Species-Specific Responses

In 2002, the eleven weed and crop species included in bioassays (Table 1) responded similarly to cover crop treatments (Bioassay Species by Cover Crop, P = 0.355, Figure 1). However, in 2003 total emergence of 15 species exhibited a range of species-specific responses to the cover crop and fallow treatments (Bioassay Species by Cover Crop, P < 0.001). Notable were the responses of yellow foxtail (*Setaria glauca* L.), which was minimally affected by any treatment (Figure 1H), and carrot (Figure 1D) and cucumber (*Cucumis sativus* L.) (Figure 1N), which had the lowest emergence following crimson clover in both years but was not affected by the remaining cover crops compared with fallow. Similarly, green bean (Figure 1P) and field pea (Figure 1O) emergence was lower following crimson clover than other cover crops in both years.

Unexpectedly, velvetleaf (*Abutilon theophrasti* Medicus) establishment in the field was consistently greater than 100% (Figure 1L). Greater germination of velvetleaf in the field may reflect the warmer soil temperature (i.e., > 20 C) during the mid-July planting (Horowitz and Taylorson 1984)
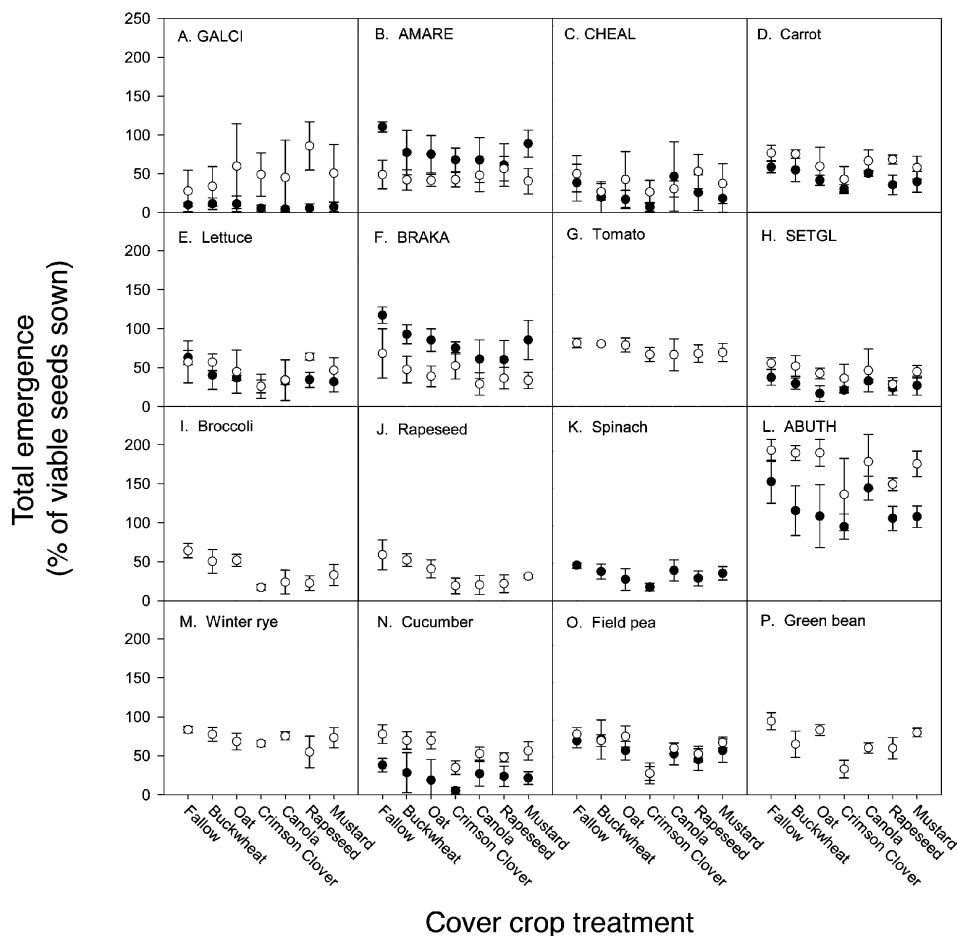
FIGURE 1. Mean total emergence (percent of viable seeds sown) and standard deviation (SD) following cover crop and fallow treatments of all bioassay species, presented in order of relative seed mass in 2002 (●) and 2003 (○). Note spinach was included only in 2002; tomato, broccoli, rapeseed, winter rye, and green bean in 2003.

and, possibly, microbial degradation of the seed's hard coat, which left the seed itself viable (Chee-Sanford and Sims 2004). Because a uniform seed lot was sown across cover crop treatments, increased germination in the field does not compromise our ability to make treatment comparisons.

Seedling Emergence Rate

Cover Crop Main Effect

As with total emergence, unequal variances in emergence rate between years prevented explicit testing of year effects. Analyzed separately, there was a significant interaction between cover crop treatment and bioassay species on T_{50} in both years (P = 0.001 and P < 0.001, in 2002 and 2003, respectively). Averaged over all bioassay species, T_{50} following brassicas was 1.9 and 1.6 d slower than following fallow in 2002 and 2003, respectively (Table 4). Similarly, T_{50} was 2.0 and 1.8 d slower following the nonbrassica cover crops compared with fallow in 2003 and 2002, respectively. In both years, there were no differences in T_{50} between brassica cover crops and nonbrassica cover crops. In 2003, T_{50} following yellow mustard was 1.2 d slower than following canola and rapeseed cover crops; there were no differences in T_{50} between the yellow mustard and other brassica cover

crops in 2002. Crimson clover did not delay T_{50} in either year.

Germination of lettuce (*Lactuca sativa* L.) seed exposed to different volatile and water-soluble extracts from rapeseed leaves, stems, and roots was delayed compared with those exposed to water (Brown and Morra 1996). Although T_{50} was not quantified in those experiments, control lettuce seeds reached their peak emergence at 4 d, whereas seeds exposed to volatiles from rapeseed leaves and stems reached peak emergence at 13 d. Common lambsquarters seeded into incorporated crimson clover residues emerged 5.2 d later than those seeded into bare soil; adding inorganic N lessened the delay to 3.7 d (Dyck and Liebman 1994).

Species-Specific Responses

Like total emergence, T_{50} varied by cover crop and bioassay species. Yellow foxtail, rapeseed, and winter rye (*Secale cereale* L.) exhibited almost no change in T_{50}, regardless of the cover crop treatment (Figures 2H, 2J, and 2M). In contrast, in 2003, T_{50} for hairy galinsoga, common lambsquarters, and wild mustard, was 8 d later following the yellow mustard cover crop than following fallow (Figures 2A, 2C, and 2F). The T_{50} for spinach (*Spinacia oleracea* L.), included

TABLE 4. Preplanned contrasts testing the effects of incorporated cover crop residues on the number of days until 50% emergence (T_{50}) averaged over 11 bioassay species in 2002 and 15 bioassay species in 2003.

Contrast	T_{50}		P	
	2002	2003	2002	2003
Fallow vs.	9.1	7.1	0.006	< 0.001
Brassica cover crops	11.0	8.7		
Fallow vs.	9.1	7.1	0.005	< 0.001
Nonbrassica cover crops	11.1	8.9		
Brassica cover crops vs.	11.0	8.7	0.834	0.331
Nonbrassica cover crops	11.1	8.9		
Idagold mustard vs.	11.7	9.5	0.155	0.001
Other brassica cover crops	10.6	8.3		
Crimson clover vs.	11.5	9.1	0.433	0.174
All other cover crops	10.9	8.7		

only in 2002, was also increased by all cover crop treatments compared with fallow (Figure 2K).

Overall, T_{50} was less variable than total emergence, but there were few consistencies between the two dependent variables. Some species had similar T_{50} regardless of treatment, e.g., redroot pigweed and yellow foxtail (Figures 2B and 2H) in 2003, and total emergence was also similar across treatments (Figures 1B and 1H). For other species, such as rapeseed, T_{50} was similar across treatments (Figure 2J), but total emergence was more variable (Figure 1J).

Conclusion

Averaged over bioassay species, cover crops consistently reduced total emergence, compared with fallow (Table 3), and increased T_{50}, compared with fallow (Table 4). However, brassica and nonbrassica cover crops had generally similar effects on both total emergence and T_{50} (Tables 3 and 4). Thus, brassica cover crops may contribute to weed management by reducing weed density and slowing weed emergence, but the brassicas were generally equivalent to other
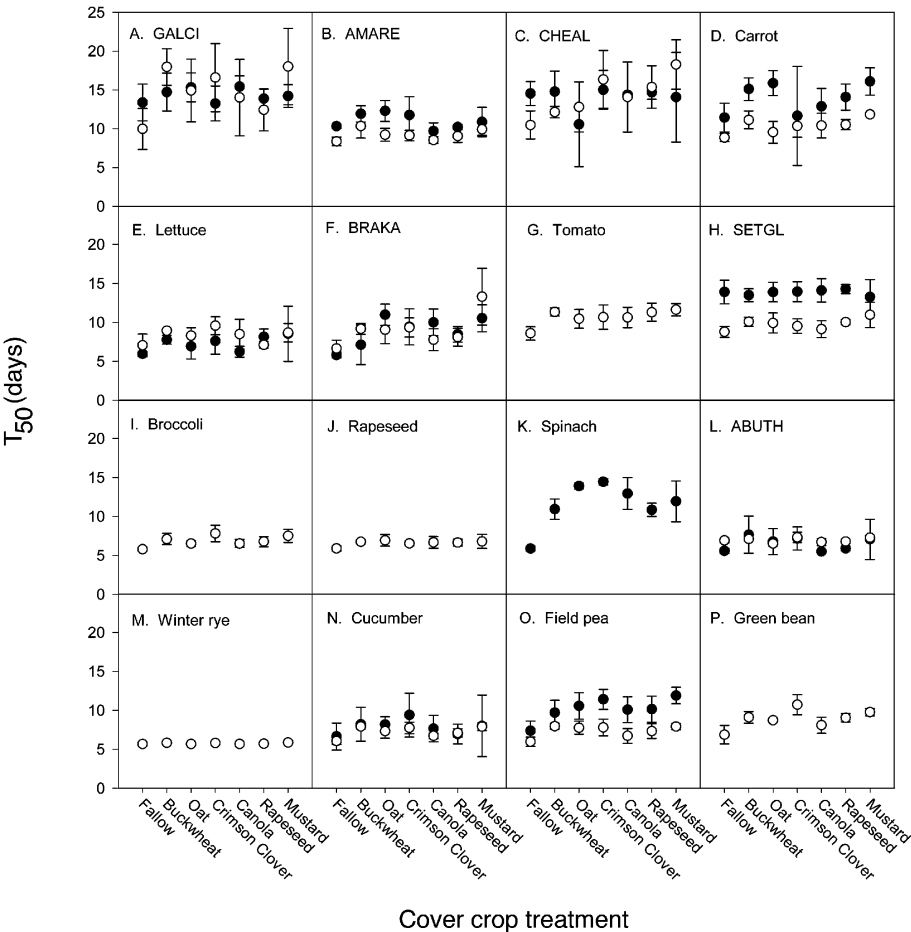

FIGURE 2. Mean time until 50% emergence (T_{50}) and standard deviation (SD) following cover crop and fallow treatments of all bioassay species, presented in order of relative seed mass, in 2002 (●) and 2003 (○). Note spinach was included only in 2002; tomato, broccoli, rapeseed, winter rye, and green bean only in 2003.

short-season cover crops in this regard. Because the magnitude of cover crop effect varied among particular residue and species combinations between years (Figures 1 and 2) and because seed size failed to explain a species' ability to withstand residue-mediated stresses, crop–weed selectivity is not ensured. Increased crop seeding rates would be prudent when planting into freshly incorporated residues. These results suggest that brassica cover crops contribute to weed management by reducing emergence but without apparent advantages over other commonly used cover crops.

Sources of Materials

Acknowledgments

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