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REGULAR ARTICLE

Effect of cover crop extracts on cotton and radish radicle elongation

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ABSTRACT

Research has shown that some cover crops are allelopathic and can inhibit weed germination and growth. Additionally, negative allelopathic effects have been documented in cash crops planted into cover crop residue. However, little literature exists comparing relative the allelopathic potential of cover crops producers utilize in conservation-agriculture systems. This study assessed the effects of twelve cover crop extracts on radish (*Raphanus sativus* L.) and cotton (*Gossypium hirsutum* L.) radicle elongation, in three trials, using an extract-agar bioassay. In Trial 1 the cover crops were black oat (*Avena strigosa* Schreb) cv. SoilSaver, crimson clover (*Trifolium incarnatum* L.) cv. AU Robin, white lupin (*Lupinus albus* L.) cvs. AU Homer and AU Alpha, rye (*Secale cereale* L.) cv. Elbon, wheat (*Triticum aestivum* L.) cv. Vigoro Grazer, and triticale (*X Triticosecale* Wittmack) cv. Trical 2700. In Trial 2 the cover crops were forage rape (*Brassica napus* L. var. *napus*) cv. Licapo, sunn hemp (*Crotalaria juncea* L.), Austrian winter field pea (*Pisum sativum* spp. *arvense* L. Poir), black medic (*Medicago lupilina* L.), hairy vetch (*Vicia villosa* Roth), black oat cv. SoilSaver, and crimson clover cv. AU Robin. Cotton was evaluated using the same bioassay and all of the cover crops mentioned above in a single trial (Trial 3). All cover crop extracts inhibited radicle elongation compared to water. Allelopathic potential was highly variable among cultivars within a cover crop species, and within a cultivar. Allelopathic differences among cover crops give an additional weed control tool in conservation systems. However, winter cover selection may impact on cash crop performance if producers plant their crop into green residue.

Key Words: *allelopathy; conservation agriculture; cover crop residue; winter cover crop.*

INTRODUCTION

Adoption of conservation agriculture systems continues to increase primarily due to concerns about soil quality degradation and the high input costs of conventional tillage. Technological advances in cover crop residue management are allowing producers to maximize cover crop biomass in conservation agriculture systems located in relatively wetter climates where water utilization by a winter cover crop is not usually a concern (Kornecki et al., 2005; Raper et al., 2004). Conservation agriculture systems that utilize high residue cover crops offer many benefits, including enhanced water infiltration, lower soil water evaporation, increased soil organic matter, and increased soil biodiversity. Additionally, high biomass yielding cover crops can suppress early-season weeds; Yenish et al. (1996) reported increased short-term weed control utilizing a rye (*Secale cereale* L.) cover crop in no-till corn (*Zea mays* L.), but not season-long control.

Previous research has shown that some cover crops are allelopathic and inhibit weed germination and growth. However, conclusive field research is difficult to obtain due to the inability to distinguish between chemical allelopathic effects and physical mulch effects (Putnam et al., 1983; Hoffman et al., 1996; Sustainable Agriculture Network, 1998; Inderjit et al., 2001). In some cases, cover crops can negatively affect growth of subsequent crops (Barnes and Putnam, 1986; Hicks et al., 1989; Bauer and Reeves, 1999). Bauer and Reeves (1999) reported that black oat (*Avena strigosa* Schreb), rye and crimson clover (*Trifolium incarnatum* L.) residues inhibited tap root elongation in greenhouse grown radish (*Raphanus sativus* L.) and cotton (*Gossypium hirsutum* L.) plants. However, White et al. (1989) found that corn and cotton emergence and growth were not adversely affected by the root biomass or root and shoot exudates of hairy vetch (*Vicia villosa* Roth) and crimson clover.

Cover crop species differ in their allelopathic potential (Bauer and Reeves, 1999; Price et al., 2006; Reeves et al., 2005). Cereal rye and soft red winter wheat (*Triticum aestivum* L.) are the two most commonly recommended winter cover crops for row crop production in the southeastern U.S. (Monks and Patterson, 1996; McCarty et al., 2003). Both of these cover crops contain allelopathic compounds that inhibit weed growth (Chase et al., 1991; Perez and Ormeno-Nunez, 1991; Yenish et al., 1996; Akemo et al., 2000). Cooperative Extension Service recommendations generally recommend waiting approximately 2 to 4 weeks after desiccating winter cover crops before sowing cotton, citing concerns of excessive residues interfering with sowing, excess moisture depletion, or to the avoidance of allelopathic effects on the following crop (Reeves, 1994; McCarty et al., 2003).

A winter cover crop's allelopathic potential is an important attribute, but is typically overlooked. Selection of cover crops for their allelopathic effects can give an additional weed control strategy in cash crops. However, the effect of the allelochemicals on the subsequent cash crop must also be considered, especially if the time between cover termination and cash crop sowing is minimal. There is little information that ranks the relative allelopathic potential of these cover crops or of other traditional and non-traditional cover crops available to producers.

Field and greenhouse allelopathic screening tests are challenging due to time, labor, and space constraints. In these evaluations allelopathic effects may be difficult to separate from competition or mulch effects. Laboratory screening allows for the selection of promising species or genotypes for field evaluation (Wu et al., 2001). Allelopathic bioassays often use standard indicator species, such as radish, for preliminary allelopathic testing (Wu et al., 2001). Extract-agar bioassays, using indicator species, have been developed to separate allelopathic potential of crop residues from plant competition effects (Pederson, 1986; Ben-Hammouda et al., 1995).

The current study was designed to fill the gaps in the allelopathic potential ranking of available cover crops. This work assessed the relative effects of various cover crop extracts on radish and cotton seedling radicle elongation using an extract-agar bioassay. This information will aid producers in evaluating the advantages and disadvantages of different cover crops in conservation-tillage cotton production.

MATERIALS AND METHODS

Three separate greenhouse trials were conducted to evaluate the allelopathic potential of twelve cover crops on radish and cotton seedling radicle elongation. Trials were arranged as a randomized complete block design with a factorial arrangement of different cover crop options as treatments. Each trial was conducted three separate times. To evaluate radish radicle elongation, twelve cover crops were evaluated in two separate trials, due to time and greenhouse space limitations. In Trial 1, the cover crops were black oat cv. SoilSaver, crimson clover cv. AU Robin, two white lupin (*Lupinus albus* L.) cultivars AU Homer and AU Alpha, rye cv. Elbon, wheat cv. Vigoro Grazer, and triticale (*X Triticosecale* Wittmack) cv. Trical 2700. In Trial 2 cover crops were winter forage rape (*Brassica napus* L. var. *napus*) cv. Licapo, sunn hemp (*Crotalaria juncea* L.), Austrian winter field pea (*Pisum sativum* spp. *arvense* L. Poir), black medic (*Medicago lupilina* L.), hairy vetch, black oat, and crimson clover. Black oat and crimson clover were evaluated in both trials to facilitate relative rankings between trials. To evaluate cotton seedling radicle elongation, all twelve cover crops were evaluated in a single trial (Trial 3). Trials 1 and 2 each had eight treatment replicates and trial 3 had six treatment replicates.

A modified Pederson (1986) procedure was used in all trials. Cover crops were sown in 7.6 L pots containing a peat-based general purpose growing medium in the greenhouse for each treatment replication. At five weeks after sowing, plants were clipped at soil level and cut into 15 mm pieces. Tissues were soaked for 24 h at a ratio of 10 g (fresh weight) to 50 ml distilled water in lidded plastic bottles in the dark at room temperature. A control, of 50 ml of distilled water, was included in each replicate. After 24 h, the extracts were filtered through coffee filters to remove leaf and stem tissue. Granulated agar (12 g L^{-1}) was autoclaved for 15 min at 121°C. Agar was cooled to 50°C before mixing with 20 ml of filtered cover crop extract at a 1:1 ratio. The agar extract solutions were poured into disposable petri dishes (15 x 100 mm) and allowed to solidify.

Radish or cotton seed were surface sterilized in a 0.6% sodium hypochlorite solution for 10 min and rinsed thoroughly with distilled water. Ben-Hammouda et al. (1995) showed that pregermination of indicator species reduced experimental error. Therefore, radish and cotton seeds were pregerminated on moistened paper towels for 24 h and 48 h, respectively. Five germinated seeds with radicles of less than 2 mm in length were placed onto each agar plate. Plates were sealed with parafilm and kept in the dark, at room temperature (approximately 22°C), for 48 h.

Radicle length (mm) of each radish and cotton seedling was measured. The five lengths from each plate were averaged to give a plate value. Plate values from all three runs were combined into a data set for each trial. Plate values were compared for both the radish and cotton radical length using a mixed ANOVA model. Cover crop treatments were specified as fixed effects. To capture potential heterogeneity and dependence across runs the error covariance matrix was modified following procedures stated in Little et al. (2006). Observations from the same run were treated as repeated measures. Thus, covariance parameters between runs were estimated using an unstructured covariance model. Furthermore, replication within each run was treated as a random effect following a compound symmetry covariance structure for the error covariance sub-matrix for each run. All models were estimated using the PROC MIXED procedure in SAS (Littell et al., 2006).

Overall significance of fixed effects were compared for each model using an *F*-test in SAS ($P \leq 0.05$). In addition, least square means were estimated to examine differences between treatment effects. Differences of least square means were calculated using asymptotic *t*-tests. To control Type 1 error rates due to multiple comparisons of treatment means, a simulation approach in SAS following Edwards and Berry (1987) was used to calculate adjusted *P*-values for each t-test to maintain an overall significance level of 0.05. Each test was conducted at a 0.05 level of significance using the adjusted *P*-value. To assess the fit of each model, pseudo R^2 statistics were estimated following Magee (1990) using the likelihood ratio.

RESULTS AND DISCUSSION

RADISH

There were significant differences among cover crop allelopathic effects in both the radish trials ($P \leq 0.05$) (Table 1). The extent of these differences and the relative ranking of each cover crop differed among runs. This suggests that the allelopathic potential of the cover crops is highly variable. However, some relative cover crop differences were consistent in all three runs.

In both radish trials, radicle elongation was significantly greater in plates which containing distilled water than in plates containing any cover crop extract. This supports previous research, which noted allelopathic effects of various cover crops. Radish radicle length was ≥ 60% and 37% longer in plates with distilled water than in plates with any cover crop extract in Trials 1 and 2, respectively.

In Trial 1, radicle inhibition was less with white lupin (cv. AU Homer) and triticale extracts, (≤ 49% and ≤ 67% respectively) than with black oat extracts (≤ 78%) (Table 1). In Trial 2, in all three runs, inhibition was less for forage rape (≤ 35%) than for sunn hemp (≤ 56%). Hairy vetch (≤ 60%) and black medic (≤ 71%) inhibited radicle elongation more than forage rape (≤ 40%) or crimson clover (≤ 52%). White et al. (1989) found that hairy vetch extracts inhibited morningglory (*Ipomoea* spp.) radicle growth more than crimson clover.

In Trial 1, radish radicle length in response to the two lupin cultivar extracts differed significantly in two out of three runs (data not shown). Peters and Mohammed Zam (1981) and Olofsdotter (2001) also showed allelopathic differences among genotypes of other species. Thus, allelopathic potential may vary among genotypes of the same cover crop species. This may be important to a producer in choosing a cover crop cultivar. Further research is needed in this area.

COTTON

As with the radish trials, there were significant differences among cover crop allelopathic potential were observed in the cotton trial ($P \leq 0.05$) (Table 1). Again, the extent of these differences and the relative ranking of each cover crop differed among runs, again implying that allelopathic potential within a cover crop cultivar may be highly variable.

Generally, cotton radicle elongation inhibition was less than in radish. Cover crop extracts decreased elongation ≤ 49% depending on species. Winter pea did not inhibit cotton radicle elongation. Cotton radicle elongation was inhibited by forage rape (≤ 19%), wheat (≤ 23%), rye (≤ 26%), triticale (≤ 28%), black oat (≤ 34%), crimson clover (≤ 30%), white lupin (≤ 40%), sunn hemp (≤ 35%), hairy vetch (≤ 45%), and black medic (≤ 33%) in two out of three runs. In the third run inhibition was dramatically lower (data not shown); crimson clover, white lupin AU Alpha, hairy vetch, and sunn hemp inhibited cotton were the only species to reduce radicle elongation.

Significant allelopathic differences were found among rye, black oat, crimson clover, and wheat that by Bauer and Reeves (1999), Reeves et al. (2005) and Price et al. (2006) as were evident in this study. Allelopathic potential is highly variable among cultivars within a cover crop species, and within a cultivar. The variation among species complicates producer cover crop selection for both weed suppression and crop safety unless the producer follows extension guidelines and waits weeks after cover termination before planting a cash crop. However, early cover crops termination does not allow maximum biomass accumulation, which is a major goal for some conservation agriculture producers.

Table 1. Mixed ANOVA results for all trials examining radish and cotton radical length 48 h after placement on plates containing agar-extract solution and least square mean comparisons across cover crop treatments.

	Trial 1 Radish radical length		Trial 2 Radish radical length		Trial 3 Cotton radical length	
Cover Crop Treatments[a]	77.51 (0.0001)		28.39 (0.001)		14.39 (0.0001)	
Pseudo R^2 [b]	0.74		0.42		0.60	
Least Square Means of Cover Crop Treatments[c]						
Distilled water (control)	34.7	a	33.6	a	33.1	a
Black medic	–	–	13.8	d	24.8	cde
Black oat	11.8	d	16.5	cd	25.9	cd
Crimson clover	16.3	bc	18.2	cd	23.8	e
Forage rape	–	–	26.8	b	28.1	b
Hairy vetch	–	---	12.0	d	20.7	e
Lupin (AU Alpha)	12.1	d	–	–	24.0	de
Lupin (AU Homer)	19.1	b	–	–	25.0	cde
Rye	14.4	cd	–	–	26.7	bcd
Sunn hemp	–	–	17.3	cd	22.8	de
Triticale	16.7	bc	–	–	27.6	bcd
Wheat	13.8	cd	–	–	27.8	bc
Winter pea	–	–	22.0	bc	31.4	ab

[a] Overall significance of cover crop fixed effects was tested using a *F*-test in SAS at a 0.05 level of significance. Test statistic is provided with p-value in parentheses.
[b] Pseudo R^2 were estimated following Magee (1990) using the likelihood ratio.
[c] Least square means followed by the same letter are not significantly different at a 0.05 level of significance using asymptotic *t*-tests adjusted for multiple comparisons to control Type 1 error using a simulation approach in SAS following Edwards and Berry (1987).

All cover crop extracts significantly inhibited radish radicle elongation compared to distilled water. The results suggest that any of these cover crops could inhibit small-seeded weed growth more than the absence of a cover crop due to the allelopathic effects of leaf tissue. While most cover crops reduced cotton radicle elongation, the most commonly used cover crops only moderately decreased radicle elongation compared to radish. This is possibly due to the larger seed size of the cotton. This study shows that the cover crop species evaluated do have relative allelopathic potentials and this potential should be a considered by producers when choosing a cover crop species, cultivar, and cover crop management. More research is needed to evaluate alternative cover crop species and cultivars, cover crop effects on a large-seed indicator weed species as well as alternative large and small-seeded crops, and relative root allelopathic potential.

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