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Field Pea Seed Residue: a Potential Alternative Weed Control Agent

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Field pea seed from bin cleaning operations stored overwinter on nearby cropland was observed to correlate with weed and crop growth suppression for up to three subsequent years. To explore the phenomenon more explicitly, plant growth suppression trials were undertaken with soil sampled 18 mo apart from two locations that had contained field pea seed residues. Test plant species grown in the residue-affected and nearby residue-free soils were compared in greenhouse experiments. Germination was either fully inhibited or emergence was delayed by more than one week. Dry matter accumulation of test species grown in residue-affected soil was significantly reduced compared to dry matter of these test species grown in residue-free soil ($P < 0.0001$). Canola and field pea were inhibited more than wheat and green foxtail over both years. Greenhouse trials also revealed that germination of wild oat was inhibited in the residue-affected soils, although wheat and grassy weeds were less suppressed than dicots overall. Significant reductions of weed species diversity and abundance were correlated to residue-affected soils ($P < 0.0001$) when compared to residue-free soils using multi-response permutations procedures. Germination of wheat and canola seed was inhibited, using aqueous extracts of weathered pea seeds or extracts of the residue-affected soil in bioassays in sterile media. An allelopathic response was proposed to explain the above results, indicating a need for further research on this system. Weed management strategies could be developed with field pea seed residues to provide innovative weed control techniques.

Nomenclature: Green foxtail, *Setaria viridis* L. Beauv. SETVI; wild oats, *Avena fatua* L. AVEFA; canola, *Brassica napus* L.; field pea, *Pisum sativum* L.; wheat, *Triticum aestivum* L.

Key words: Allelopathy, integrated pest management, plant suppression, principal components analysis, sustainable agroecosytems, weed control.

Field pea is the most widely grown pulse crop in Canada, with a 5-yr, mean annual production exceeding 2.5 million tonnes (Anonymous 2008). Occasionally, unsold surpluses are stored outside in exposed piles or under tarpaulin covers. For many years, producers have observed that when field pea seeds were stockpiled outside over winter, soil where the seed was stored did not sustain plant growth in the subsequent three to four growing seasons. Furthermore, a dose-dependent effect was evident when weathered field pea seeds, from cleaning out grain bins, were harrowed into the nearby crop land. The application of a smaller amount of discarded seed demonstrated less effect on plant growth than where the peas had been stockpiled. Crop growth on soil where pea seed was not incorporated (soil lacking field pea seed residues), immediately adjacent to the stockpile area, was not inhibited (D. Wall, personal communication). This phenomenon was suggestive of consequences other than mulching or nutrient overloading effects from storing the crop on open ground, since regularly administered soil tests returned normal values for organic matter and nutrient content. These observations of the farmland led us to examine whether an allelopathic effect might be controlling the plant growth in the residue-affected soils.

Allelopathy is not an uncommon occurrence in crops and represents an underutilized resource for managing agroecosystems (Blum et al. 1999; Weston 1996). Allelopathic crops or crop byproducts offer the potential for integrated weed management, which is particularly attractive as an environmentally responsible opportunity in pest management (Belz 2007). To date, research groups worldwide have identified several crop species such as wheat, annual ryegrass (*Lolium* spp.), *Oxalis* spp., cowpea [*Vigna unguiculata* (L.) Walp.], and sunflower (*Helianthus annus* L.) as possessing potent allelopathic interference mediated by root exudation of allelochemicals (Collins et al. 2008; Hill et al. 2007; Macías et al. 2007; Shiraishi et al. 2005; Singh et al. 2003). Many of the common cereal crops have been found to exude both phytotoxic and plant-growth supporting compounds from their roots (Belz 2007; Eom et al. 2006).

Some plant species comprise aggressive growth habits (e.g., hairy vetch, *Vicia villosa* Roth) or achieve substantial levels of plant suppression (e.g., spotted knapweed, *Centaurea maculosa* Lam.) (Hill et al. 2006; Perry et al. 2005). However, field pea residues have not been examined for phytotoxic effects, although toxicity of field pea seed components related to insect control during storage were attributed to peptides belonging to the albumin family of proteins (Taylor et al. 2004). Similarly, lectins in Indian wild bean [*Lablab purpureus* (L.) Sweet] retarded the development of the larval stage of grain storage pests *Rhyzopertha dominica* Fab. and *Oryzaephilus surinamensis* L. (Janarthanan et al. 2008).

The primary objective of these experiments with field pea seed residues was to evaluate the plant growth suppression phenomena in greenhouse-grown test species, sown into soil collected from residue-affected field sites in two different seasons. Secondly, bioassays were used to test the effects of discarded field pea seed leachates and soil extracts in sterile media. The data were used to predict possible weed control potential and evaluate selectivity.

Materials and Methods

Source of Soil Samples Used in Greenhouse Germination Trials. Soil samples were collected on two occasions (June 2008 and October 2009) directly into standard greenhouse trays (size 1020, 6 cm deep) at two field sites in the Dark Brown soil zone near the town of Borden, SK, Canada in the vicinity of 107°13′48″W, 52°23′52″N. Location 1 was ca. 100 m northeast of Location 2 (Figure 1). The residue-affected zone at these locations had been covered intermit-

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Figure 1. View of cropland where field pea seed residue effects occurred in 2006 to 2008 (photographed in June 2007).

tently with discarded field pea seed from grain-bin cleaning just prior to filling the bins with newly harvested crops. Historically, Location 1 was used for outdoor storage of field pea seed and as a disposal site for discarded field pea seed from a grain bin during the fall of 2000 to 2008, and cleared each spring. Location 2 was used for discarded field pea seed for only three winter seasons (2006 to 2008). The discarded seed was estimated to cover the soil between 8 and 15 cm deep over a 20-m diameter.

Soil was sampled from nine randomly placed quadrats located in each residue-affected location, for a total of 18 samples in June 2008 and 18 mo later (October 2009) from similarly randomized sampling. The soil was removed to a depth of 6 cm ($\pm$ 2) and half of each tray filled. A waterproof divider was placed across the soil edge and the second half of the tray filled with randomly sampled residue-free soil from the adjoining, unaffected cropland, which was used for comparison. Soil was not amended or sieved, and the original soil profiles (top to bottom) were maintained.

Three composite soil samples were taken at each location to be tested at a commercial soil assay laboratory for N, P, K, organic matter, and micronutrients. These samples were made from equivalent volumes taken from the center and, separately, from the edge of the residue-affected site at each location. Residue-free soil at each location, within 5 m adjacent to the residue-affected soil zone, was taken as the third sample. A detailed cropping history was obtained from the grower to account for the effect of weed control measures and crop rotation effects on weed abundance (Table 1).

Planting Design and Test Species. Prior to greenhouse trials, the seed to be used as test species was evaluated for germination according to established procedures and achieved $\geq$ 95% within 72 h (three 50-seed replicated samples) (Anonymous 2007). Four plant species were sown in rows the length of the tray (nine replicates by two locations) ('AC Barrie' wheat, Proven Seeds 9525® canola,[1] green foxtail, and 'CDC Striker' field pea). These test species were chosen for their prevalence as crop and (in the case of green foxtail) a weed species in the Dark Brown soil zone of the region and to represent both dicot and monocot plants. The weed species, green foxtail, is prevalent as a grassy weed and germinated reliably under greenhouse conditions. Other choices for weed species (redroot pigweed [*Amaranthus retroflexus* L.], lamb's quarters [*Chenopodium album* L.] and chickweed [*Stellaria media* (L.) Vill.]) germinated too erratically to be reliable measures.

Ten seeds each of wheat and field pea were planted in the rows, in each half, for a total of 20 seeds per replicate. Canola and green foxtail seeds (225 mg and 150 mg per replicate tray, respectively) were thinly scattered in their designated rows along the length of each tray. Plant species were randomized in row order arrangement in order to eliminate any effect from neighboring plant growth. Plant row markers were used to label each row with the seed species name to facilitate emergence counts.

Greenhouse Conditions. Temperature (day/night), 22/18 C; supplemented by lights[2] 6:00 A.M./11:00 P.M. (on/off) when natural light was $<$ 1,000 mmol m^{-2} s^{-1}; relative humidity, 50%/40% (day/night). Overhead water was supplied as required using local tap water (City of Saskatoon, SK).

Greenhouse Data Collection. Observations and photographs of soil residue effects on plant growth were taken over 14 d (germination and emergence to a minimum of the two-leaf stage). Emergence of test species was recorded every day to reflect differences across locations and treatments. Observations included diversity and total abundance counts of volunteer weed species that emerged in each tray as an estimate of weed seeds that could be suppressed by the field pea seed residues. Weed species diversity and abundance is often measured by removing soil from the field and determining weed emergence in a greenhouse or controlled environment growth chamber (Smith and Gross 2006). Such controlled environment cultivation of field soil as an estimate of the seed bank was established as an acceptable measure of weed diversity because optimum conditions can be applied to facilitate emergence (Smith and Gross 2006).

At the end of the experiment, all the test-seeded plants were washed to remove soil. Plants of each test species from each tray (by location) and soil type (residue-affected or residue-free soils) were bulked separately and dried in a forced air oven (48 h, 80 $\pm$ 5 C) for dry weight biomass (dry matter, DM). DM data for individual test plant species were averaged from the nine replicates in each treatment and from each location, for a total of 36 measurements.

Weed Abundance and Diversity Estimates in the Field. Weed species counts in the field locations were conducted 6 wk after the pre-emergence weed control treatment and immediately prior to the spring post-emergence weed control (treatment history, Table 1). Ten quadrats (0.25 m^2 each) were counted for both residue-affected and residue-free sites at each location, for a total of 40 quadrats. These data were used as an estimate of the weed population in the seed banks in the residue-affected and residue-free soils in the field and to provide a contrast to the greenhouse-germinated weeds, for which the diversity and abundance might be expected to be greater due to optimal water and temperature control.

Statistical Analysis. Significant differences were determined using the Mixed Data Procedure of SAS.[3] The response variable (DM data) was thus tested by using the model "DM

Table 1. Cropping and herbicide history of two locations exhibiting field pea seed residue effects on weed and crop establishment.

Year	Crop	Herbicide	Rate[a]	Group[b]	Timing[c]
2004	Canola[d]	Glyphosate	450	9	Pre and post
2005	HRS wheat	2,4-D	350[e]	4	Post
2006	Field pea	Glyphosate	450	9	Pre
		Imazamox + imazethapyr	34.6	2	Post[e]
2007	HRS wheat	Glyphosate	450	9	Pre[e]
		Tribenuron methyl	15.3	2	Pre
		Bromoxynil + MCPA	560	4, 6	Post[e]
2008	Canola[d]	Glyphosate	450	9	Post
2009	Field pea	Glyphosate	450	9	Pre
		Imazamox + imazethapyr	43.3	2	Post[e]
		tepraloxydim	33.5	1	Post[e]

[a] g ai ha^{-1} except where indicated.
[b] Mechanism of action (http://www.wssa.net/Weeds/Resistance/HerbicideMOAClassification.pdf).
[c] Abbreviations: Pre, pre-emergence; Post, post-emergence.
[d] Roundup Ready®. Monsanto Canada Inc., Winnipeg, MB.
[e] Commercially premixed.

= test species | location | affected or residue-free soil category | replicate | tray'' with the option ''denominator degrees of freedom approximation of Satterthwaite'' to test for any interaction effects and are reported as probability (P) values at the 95% level. Effects of location and of soil categories (residue-affected or residue-free) on test species DM was determined by contrast effect tests with an unstructured covariance matrix chosen to measure the response variable (DM) to all interactions with the fixed effects (test plant species, soil type and location).

Weed abundance and distribution in relation to fixed effects was determined by principal components analysis (PCA; covariance-correlation model). Multi-response permutations procedures (MRPP) (Euclidean distance measures) were used to associate distribution of weed species and abundance with respect to the field pea seed residues in the soil (McCune and Mefford 2006). According to distribution analyses of weeds in similar Saskatchewan farmland (Leeson et al. 2000), these analyses result in a scatter plot (ordination) that reflects the influence of location or ''treatment'' (residue-affected or residue-free soil categories, in our report) on species abundance and distribution in the plant community.

Bioassay Experiments. All water-based reagents and extracts were prepared with HPLC-grade water.[4] Germination and root elongation on sterile media[5] were evaluated in 150-mm plastic petri plates placed in a temperature-controlled incubator with no illumination.[6] Test extracts were assayed with wheat (AC Barrie) and canola (Proven Seeds 9525) (10 seeds each) over a 7-d period incubated for 2 d at 20 C followed by 5 d at 12 C. Field pea seed extracts were applied to sterile filter paper disks[7] in the center of the bioassay plates so as to allow the preparation to diffuse into the media over the bioassay time. Soil extracts were spread directly over the entire surface of the sterile agar medium and left to dry for 24 h in a sterile flow bench. Bioassays were performed twice with two replicates in each test. The results were scored according to whether germination only or germination and root elongation occurred.

Test Extract Preparation. Two types of extracts were tested at full strength and at a 10 × dilution (using sterile water): (1) A water-soluble extract of overwintered field pea seed 'Delta' (discarded from the grain bin) was prepared from 711 g whole seed L^{-1} water, stirred for 48 h at room temperature, refrigerated (10 C) for 3 d and centrifuged (1,364 × *g*). Sequentially filtered[8] supernatant was freeze-dried to produce a dried preparation. The resulting dried extract was reconstituted with sterile water to represent 52.4 g pea seed ml^{-1} (the amount applied to test full strength extracts); diluted extracts were equivalent to 5.24 g pea seed ml^{-1}. Preliminary experiments determined that a leached equivalent of at least 5.0 g seed ml^{-1} was needed to inhibit seed germination. (2) A water-soluble soil extract was prepared from a composite soil sample of the residue-affected soil at Location 1, by using the same method as for the pea leachate but omitting the freeze-drying and reconstitution steps. Several extract rates were tested in preliminary bioassays to establish an effective concentration. As the rate decreased to 1.0 g soil ml^{-1} leachate, the growth of the bioassay test seed surpassed the control assays in germination and elongation. The experimental soil bioassay solution represented 1.78 g soil ml^{-1} residue-affected soil for full-strength tests. In this way, the point at which soil extracts from field pea seed residues in the field could be seen to suppress plant growth were tested in comparison to a dilution that did not inhibit the germinating seed. Control bioassays (sterile water only) were used to compare germination and root elongation/shoot development on sterile media without test solutions.

Results and Discussion

Emergence and Species-Specific Differences in Test Species. Residue-affected soils caused a significant impact on DM accumulation of sown test species in the greenhouse (Figure 2, shown as percent DM relative to residue-free soils). Plant growth was significantly inhibited in both years ($P < 0.0001$), although the significant difference in percent inhibition was less in the 2009 trial (*F*-value = 67.08 vs. 237.57 in 2008). Location 1, where discarded pea seed had been over-wintered longer compared to three seasons in Location 2, reflected the longer time of pea seed residue by a significant crop (i.e., test species) × location interaction in both years at this location (2008, $F = 5.31$ [$P < 0.0018$]; 2009, $F = 16.27$ [$P < 0.0001$]).

Final DM biomass of each test species was similar when grown on soil sampled from residue-affected areas in 2008, (field pea, wheat, and canola, $P < 0.0001$; green foxtail,

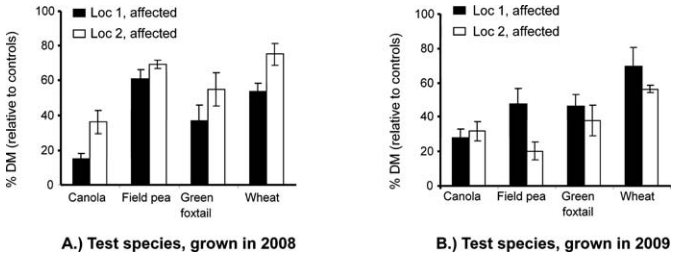

Figure 2. Dry matter accumulation of crop and weed test species in soils sampled in consecutive years at two locations. Results are shown as percent dry matter (DM) with respect to residue-free, control-grown test species for each year: (A) 2008 and (B) 2009. "Loc 1" represents a longer period of field pea seed residue exposure than "Loc 2." Error bars (SE) are the means of nine replicates. The $F_{0.05}$ values by year (2008, 2009) for each crop: canola, 87.6*, 101.1*; field pea, 88.0*, 67.8*; green foxtail, 11.9*, 3.11^{NS}; wheat, 45.3*, 29.2* (values with an asterisk were significantly different from residue-free controls ($P < 0.0001$); NS, not significant).

$P = 0.0008$). Dry matter accumulation from test species grown on residue-affected soil samples collected 18 mo later (October 2009) displayed the same trend, although there was now no significant location effect ($F = 2.06$, $P = 0.1539$). This was likely reflective of a disappearance of the inhibitory factors that had caused a difference in 2008.

The commercial soil tests returned normal values for all nutrient parameters (data not shown) and showed no differences between soil from either location or any difference in residue-free vs. residue-affected soils. The clay-loam soils had an organic matter content of 5.8 to 8.2%, comparable to soil routinely tested from the same general location. The lack of differences in nutrient profiles between the residue-free and residue-affected soil samples supports the hypothesis that field pea seed contributed an agent that suppressed plant growth to the soil.

Canola. In both 2008 and 2009, canola plants grew poorly compared to the control-grown plants in residue-free soil, which reached the three-leaf stage by the 14-d harvest. Very few test-sown canola seedlings emerged in residue-affected soil samples from Location 1. At both locations, canola emerged as sickly, yellowed seedlings in 2008 soil samples, although this effect was less evident in the second year. Canola grown in soil collected in 2009 did not show increased emergence compared to results from soil collected in 2008, particularly relative to the other test species at Location 1 in 2009. This observation was confirmed by DM data where there was a highly significant test plant species × treatment effect (i.e., soil affected or unaffected categories) ($P < 0.0001$, $F_{2008} = 87.64$, $F_{2009} = 101.14$) in both years (Figure 2).

Field Pea. Emergence was delayed by 6 to 7 d in all the residue-affected soil replicates, compared to the seed in residue-free soils. Soils from Location 2 sampled in 2009 exerted a greater effect on DM than the percent growth inhibition in 2008 (Figure 2). Examination of the seedling roots revealed severe root stunting, particularly at Location 2. Lack of root development relative to residue-free soil may be an effect most noticeable in field pea because of the larger fleshy root whereas wheat and canola had fine root systems that might escape detection of such changes.

Wheat and Green Foxtail. Emergence was delayed by more than 4 d in both these species compared to emergence of their counterparts in the residue-free soil. Wheat percent DM relative to plants grown in residue-free soil was not reduced as much as canola and green foxtail in 2008, although the roots were badly stunted. In addition, green foxtail was less inhibited than the other species that were tested (canola, field pea, and wheat, $F > 29.22$, $P < 0.0001$; green foxtail, $F = 3.11$, $P = 0.0804$) when DM data were analyzed statistically by contrast tests. The contrast parameter in the covariance-correlation analysis was reflective of this interaction, showing that DM accumulation of all the test plant species responded negatively to residue-affected soil.

Volunteer Crop and Weed Emergence in the Greenhouse Trials. Weed species flourished in residue-free soil in the greenhouse tests (Table 2). In contrast, weeds were repressed or nearly absent in the residue-affected soils. Green foxtail was very abundant as a volunteer weed in the residue-free soil but virtually absent in the residue-affected soil in 2008 ($P < 0.0001$). Although green foxtail in residue-affected soil sampled in 2008 was evident by 14 d, the comparative abundance of this species was less heavily reduced in soil sampled in 2009 ($P = 0.0804$), with respect to plants growing in the residue-free soil samples (Figure 2). Overall, weed species abundance and diversity were lower in 2009 greenhouse trials (Table 2). A post-emergence herbicide had been applied on the field following weed abundance field counts in 2008 and again in early summer in 2009, after the weed counts were taken (Table 3). This herbicide application would have affected weed seed accumulation in the soil seed bank.

There was no volunteer canola in the residue-affected soil in the greenhouse trials, but other crop volunteers included wheat and barley (*Hordeum vulgare* L.). These volunteer cereals were stunted and did not grow past the two-leaf stage in residue-affected soil in 2008. By comparison, volunteer wheat and barley reached the four- to six-leaf stage in residue-free soil by the end of the experiment. Additional ungerminated volunteer wheat caryopses and field pea seeds were discovered in soil from the residue-affected soils, while washing plant samples for drying and biomass measurements. Volunteer wheat and canola were largely absent in the 2009 greenhouse trials.

When the abundance data from the greenhouse-grown weeds were grouped by the soil categories (residue-affected and -unaffected) and subjected to MRPP analysis, weed abundance and diversity in the residue-affected soil were significantly reduced compared to residue-free soil ($P < 0.0001$). A significance of this degree using an MRPP calculation indicated that the composition and abundance of this plant species community was highly influenced when contrasted by the soil category (residue-affected and residue-free). Within the context of each soil category, however, weed species abundance data were not significantly different when compared by location. Thus, the variation in counts over 2 yr did not change with respect to the inhibition of field pea seed residues in the soil on plant growth.

In the greenhouse, the soils were unsprayed so plant emergence in the "weed category" are numbers that depend upon germination in the soil seed bank. The reduction of total counts for a specific plant species (e.g., green foxtail) is a reflection of the occurrence of the number of seeds in the seed bank (in soil unaffected by pea seed residues). Overall

Table 2. Weed species abundance and diversity in replicated greenhouse trials with soils affected by field pea seed residues.

Latin binomial	Bayer abbrev.	Common name	Residue-free soil (total counts)		Residue-affected soil (total counts)	
			2008	2009	2008	2009
Amaranthus retroflexus L.	AMARE	Redroot pigweed	70	11	9	2
Avena fatua L.	AVEFA	Wild oats	8	52	0	0
Capsella bursa-pastoris (L.) Medik.	CAPBP	Shepherd's purse	24	2	6	1
Chenopodium album L.	CHEAL	Lamb's quarters	75	30	9	2
Crepis tectorum L.	CVPTE	Narrow leaf hawksbeard	0	122	0	1
Erucastrum gallicum (Willd.) O. E. Schulz.	ERGA	Dog mustard	12	0	3	0
Kochia scoparia (L.) Schrad.	KCHSC	Kochia	5	3	1	0
Lolium persicum Boiss. and Hohen.	LOLPE	Persian darnel	0	5	0	0
Malva rotundifolia L.	MALPU	Round-leaved mallow	45	13	9	46
Matricaria perforata Mérat.	MATIN	Scentless chamomile	0	10	0	0
Polygonum aviculare L.	POLAV	Prostrate knotweed	39	15	10	1
Polygonum convolvulus L.	POLCO	Wild buckwheat	59	11	8	3
Setaria viridis L. Beauv.	SETVI	Green foxtail	143	31	21	5
Sinapis arvensis L.	SINAR	Wild mustard	45	9	8	0
Sonchus spp.	SON	Sow-thistle	44	1	13	0
Thlaspi arvense L.	THLAR	Stinkweed	65	131	13	0
Cumulative totals by year and soil type			634	446	110	61

abundance and diversity in the field counts can be related to the subsequent effect on seed set and dispersal due to the previous year's herbicide application. However, since herbicides were applied over the whole area (both residue-affected and residue-free soil), the application does not qualify as a treatment. Certainly, the herbicide application contributed to lack of seed set but despite the chemical control, considerable numbers of green foxtail and other weeds appeared the following spring when the field counts were taken. The salient point is that significant differences between the total number of weeds emerging in residue-affected and residue-free soils occurred under greenhouse conditions.

Volunteer Crop and Weed Emergence in the Field. Farm land where the putative allelopathic effects occurred was observed the following year (June 2009) and showed a continued impact of the original discarded field pea seed. Multivariate analyses to determine the weed species distribution and abundance in the field returned significantly different associations in unaffected residue-free soil compared to residue-affected soil ($P < 0.0001$). When the weed abundance data from the field survey were grouped by the soil categories (residue-affected and residue-free) and subjected to MRPP analysis, the residue-affected soil significantly reduced the weed abundance and diversity ($P = 0.0000$). Similar to the greenhouse abundance results, weed species grouping in the field survey by location was not significant ($P = 0.8820$), within the context of each soil category.

The plant suppression effects were evident throughout the growing season in the cropland. Location 1 continued to display greater plant growth inhibition compared to Location 2 (total counts: 977 vs. 305 [residue-free and residue-affected soils, respectively], Table 3). Soil was sampled before the spring herbicide treatment. The entire cropland including the field pea seed residue locations was treated afterward. The following year, samples were taken in the early fall. Since there was no evidence of herbicide injury to emerging test species in the controls in either timeframe, herbicide effects alone did not account for the depression in DM of plants grown in residue-affected soil. According to the field survey counts of weed and volunteer crop plants, Location 1 was a weedier field with a more diverse species abundance than Location 2 (Table 3). However, it was only by the greenhouse-germinated survey during 2 yr that the species difference became more apparent (Table 2).

Modeling the Interaction of Pea Seed Residues on Weed Abundance and Distribution. These community-level

Table 3. Species abundance (counts) and diversity as influenced by field pea seed residues in the field at two locations in Saskatchewan (June 2009).

Weed species	Abbreviation[a]	Location 1		Location 2	
		Residue-free	Affected	Residue-free	Affected
Green foxtail	GF	419	109	242	90
Hairy vetch	HV	0	0	5	0
Kochia	K	4	0	2	0
Lamb's quarters	LQ	39	46	101	28
Persian darnel	PD	10	1	0	0
Prostrate knotweed	PK	13	1	6	0
Redroot pigweed	RRP	4	1	7	0
Round-leaved mallow	RLM	11	39	0	3
Perennial sow-thistle	PS	0	13	0	0
Stinkweed	SW	31	0	6	0
Volunteer canola	VC	100	95	338	117
Volunteer wheat	V-W	325	0	243	4
Wild oats	WO	4	0	12	0
Wild buckwheat	WB	17	0	10	0
Total counts		977	305	972	242

[a] Designations used in Figure 3.

changes in weed species diversity in response to field pea seed residues, described in the foregoing section, can be visualized by using ecological models that reflect the species-soil residue interrelationship. A multidimensional plot (using PCA, with respect to soil category and the weed species population), provided a perspective of the relative influence of field pea seed residues on the weed species abundance and diversity. The data for residue-free soil vs. residue-affected soil influenced the clustering most strongly and the ordination separated the residue-free soil (Δ) and residue-affected soil (▲) along two axes, accounting for 80.2% of the cumulative variance (Figure 3). The scatter of individual data points (quadrats) within each soil category on the ordination indicated statistically significant distribution differences in weed abundance between the two soil categories as well as the diversity amongst the weed species in a two-dimensional space (Figure 3). Diversity and abundance demonstrated by the germination of weed seed in residue-free vs. residue-affected soil in the greenhouse generated a similar ordination to the farm land distribution (ordination not shown; data, Table 2).

The field pea residue was positively associated with separation along axis 1 (Figure 3). The lower percent variance contributed by a third axis (16.0%, data not shown on ordination) indicated that some weed species (lamb's quarters, kochia [*Kochia scoparia* (L.) Schrad.]), round-leaved mallow (*Malva rotundifolia* L.) occurred at both locations and on both soil categories, even though the counts were significantly different (Table 3). There were two major axes of variation in the PCA ordination. Weed species that fell closer to the center of the ordination along axis 1 (volunteer wheat, round-leaved mallow), were less affected by association with the axis, while others (red-root pigweed, prostrate knotweed [*Polygonum aviculare* L.], and wild buckwheat [*Polygonum convolvulus* L.], for example) grouped together and were strongly influenced by the factors dictating separation on axis 1 (Figure 3). The weed species composition separation in this dimension was influenced by separation along axis 2, which was reflective of the weed community composition. Given that these are community level interpretations, the ordination shows that the weed community is driven by both abundance and distribution. Although this is a qualitative description of the weed species data, the PCA showed that there was an underlying homogeneity to the residue-affected soils. The weed community in the fields with field pea seed residues was altered by the selection pressure of these soil residues, albeit limited in area. For instance, the apparent insensitivity of round-leaved mallow in residue-affected soil shows that this weed could thrive and in time become a predominant weed in the field.

Inhibition of seedling emergence was evident on the farm land that was otherwise very weedy outside the residue-affected zones, thereby indicating that significant horizontal movement of the controlling agent does not occur even in the tilled field. These results prevailed regardless of whether there was a longer (Location 1) or shorter (Location 2) duration of exposure to field pea seed residues. Even though the weed populations in the fields were subject to conventional herbicide control, the weed species diversity was restricted in residue-affected soils and the abundance significantly reduced, compared to the adjacent cropland. Apparently, exposure to field pea seed residue has an effect on soil, although perhaps not noticeable unless discarded or stockpiled seed has remained in place for several months.

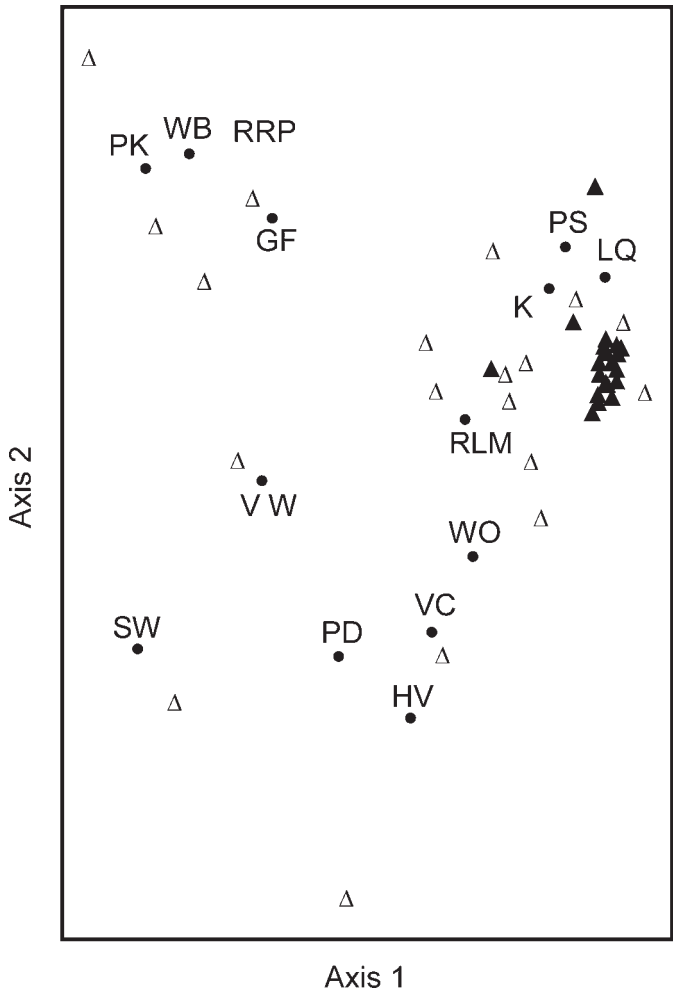

Figure 3. Principal components analysis of the two soil categories that influenced the clustering of the weed species distribution (nonstandard abbreviations [Table 3] are used for legibility). A two-dimensional model of the significant axes where the distance among objects approximate their Euclidean distances (comparative distance, as if measured with a ruler). Axis 1 represents the separation of weed species and their occurrence with respect to field pea residues. Axis 2 reflects the separation of weed communities based on the different weed species, relative to axis 1. Species on the ordination close to the centre of the ordination do not strongly associate with either axis. Legend: control (residue-free) soil (Δ); residue-affected soil (▲); individual weed species on the ordination (●).

Weed Control Selectivity of Field Pea Seed Residue. In the controlled environment of the greenhouse, the cruciferous weed family (the Brassicaceae) was inhibited up to 100% over both years in residue-affected soils (shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.], 73%; stinkweed [*Thlaspi arvense* L.], 93%; wild mustard [*Sinapis arvensis* L.], 85%; dog mustard [*Erucastrum gallicum* (Willd.) O. E. Schulz.], 75%; and volunteer canola, 100%) compared to other plant families (e.g., Amaranthaceae, 89%; Asteraceae, 92%; Poaceae, 80%). Wheat (volunteer or sown as a test) recovered from the residue-affected soils better than any of the dicot crops. Testing the adaptation of plant family tolerance to field pea residues should be the subject of future research so as to establish whether an advantage for organic wheat growers may be realized by incorporating a field pea seed mulch just prior to the year wheat will be planted.

The selectivity of the field pea residue predicted in the greenhouse trials was evident in the field surveys. Volunteer

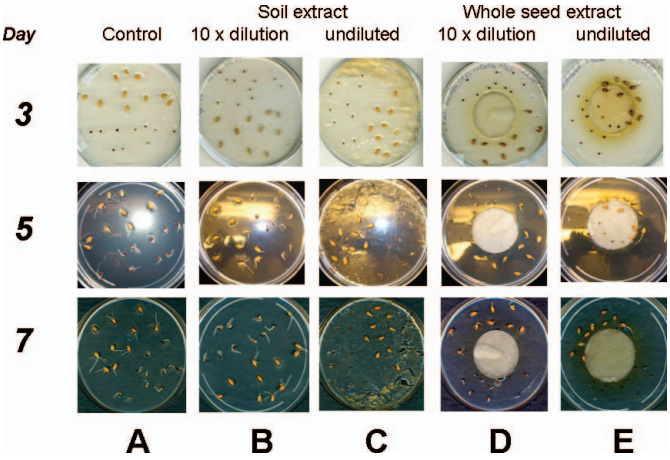

Figure 4. Seven-day bioassay with extracts of soil and of whole field pea seed. Test seed, wheat (AC Barrie) and canola (Proven Seeds 9525), 10 seeds per test species per assay plate. Rows, top to bottom: day 3, day 5, day 7. Columns (left to right): A, residue-free control (water); B, soil extract (10 × dilution); C, soil extract (undiluted); D, field pea seed extract (10 × dilution); E, field pea seed extract (undiluted).

cereals (wheat, oat) and grassy weeds (green foxtail, wild oats, Persian darnel [*Lolium persicum* Boiss. and Hohen.]) emerged but few, if any of the Cruciferous weeds (dog mustard, shepherd's purse, stinkweed, and wild mustard) that appeared in the greenhouse trials were observed at the field sites (Tables 2 and 3). The most abundant Crucifers (stinkweed and volunteer canola) that were present in the farm land residue-free areas were significantly reduced in the residue-affected zones. However, round-leaved mallow, which germinated first in the residue-affected soils in the greenhouse trials, was also evident in the residue-affected soils in the field. Although its occurrence was very patchy (not all sampling quadrats in the field contained round-leaved mallow), the counts increased over time in residue-affected soils (Table 2). These observations were indicative of selectivity that could be exploited for potential weed management, once the effect of field pea seed has been investigated more extensively in field trials.

Bioassay Experiments. The twice-repeated bioassay test produced the same results each time. No germination beyond the initial breaking of the seed coat occurred when the test species were exposed to the full strength extract (representing 1.78 g soil ml^{-1} residue-affected soil or 52.4 g pea seed ml^{-1}, respectively) (columns C, E, Figure 4). In the full strength field pea seed extract, there was 100% inhibition of both wheat and canola closest to or on top of the central disk and up to 80% inhibition of wheat beyond this region (day 5 to 7, column E, Figure 4). Canola was 100% inhibited in the presence of pea seed extract (day 5 to 7, column E, Figure 4). In the undiluted soil extract, canola germination was completely inhibited and wheat was 80% inhibited (column C, Figure 4).

At diluted concentrations, growth was retarded relative to the concentration of the pea seed extract. Wheat appeared to be less suppressed than canola, with the radicle elongating in up to 80% of the caryopses by day 5 (column D, Figure 4). Diluted soil extracts did not inhibit germination and root elongation was not different from controls in the test species (column A, B, Figure 4). Since there was a significant crop × location effect on the greenhouse-grown plants, reflective of Location 1 having a longer history of discarded field pea seed than Location 2, the bioassay evidence for a dilution effect confirms the results in the greenhouse experiments by location.

Detection Systems for Allelopathic Effects of Field Pea Seed Extracts. The bioassay experiments with extracts of field pea seed represent an inhibitory response typical of an allelochemical (Belz 2007; Singh et al. 2003). Allelopathy is the inhibition (or promotion) of plant growth due to compounds released by other plants or plant residues and generally influences the development of neighboring plants (Willis 1985). Allelopathic mechanisms have also been attributed to root exudates rather than compounds leached from surface litter (Bertin et al. 2007; Perry et al. 2005). In some cases, microbial transformation may be needed to convert these root exudates or plant leachates in order to initiate the allelopathic phenomenon (Macías et al. 2007).

The potential allelochemical may be released as an exudate or during plant decomposition. Dry leaf litter (*Oxalis* spp.) was shown to be an effective weed control agent in a ground cover (Shiraishi et al. 2002). Bioassays of the noxious effects of houndstongue (*Cynoglossum officinale* L.) showed that leaf extracts delayed or reduced emergence of desirable forage grasses (Furness et al. 2008). Agronomic practices such as minimum tillage and stubble retention were suggested to increase crop allelopathic interactions according to a field study of crop residue effects on wheat (Lovett and Jessop 1982). Field pea residue effects show a similarity to these foregoing investigations. The inhibitory response presented a classical response in its repression of growth of planted test species in soil and by extract bioassays. Moreover, the methods reported here developed some practical approaches for a more effective bioassay using these test plant species.

It turned out that filter-sterilizing the concentrated pea seed extract was difficult, probably due to albumins (Taylor et al. 2004), which are notorious for clogging membrane filters. Unless the freeze-dried preparation was diluted more than 10-fold (at which point the inhibitory effect was not detectable), the 0.8-μm filter was the smallest pore-size possible to use.[7] When the extract was diluted into an overlay, bacterial contamination developed within 3 d, emphasizing that this classical approach of an overlay of agar with the test extract did not work well with field pea extracts. Experiments with various antibiotics did not resolve contamination and inhibition of the bioassay test plant species (with water blanks) with antibiotic was evident when more potent antimicrobial agents were used (data not shown). Hence, field pea extract assays were most effective with a central paper filter disk saturated with 1 ml of extract, which also conserved the preparation for replicated experiments.

Scoring the allelopathy test in sterile media should be an evaluation of the growth or inhibition beyond a plus or minus. Since the amount of field pea seed extract was applied only to a central disk, there was a gradient effect in the agar, with seed closest to the disk being most inhibited. Although overall inhibition scores per plate were therefore impractical due to the diffusion gradient, the agar plate bioassays confirmed the plant growth suppression phenomenon of field pea seed extracts in the absence of soil, and that suppression did not arise from unintentional greenhouse conditions.

Despite the restrictions on quantifying the sterile-plate data, a dilution effect in the bioassays was evident by using a centrally applied extract. Generally 7-d assays were needed to be certain there was actual inhibition and not just a delayed germination. All the canola and wheat residue-free control test seeds germinated within 24 h and then exhibited slow elongation of the radicle over the next 3 days. Consequently, the growth or inhibition responses to the extracts were most apparent in the 4 to 7 d interval.

The most difficult seed to use in the bioassay was field pea because of the long lag time between initiation of the bioassay, germination, and radicle elongation. Growth suppression of field pea was not observed, possibly because the large cotyledons were a reservoir of nutrition and the effect of extracts was not evident in the 7 to 10 d bioassay periods. Wheat and canola were the best species for bioassay. Their smaller seed size and prompt germination together with rapid radicle elongation in the residue-free control treatments permitted a rapid evaluation of allelopathic response to the extracts. Weed seeds were tested (chickweed and green foxtail) but the germination on sterile media was too erratic to allow a reliable evaluation of plant growth inhibition effects.

Important long-term goals for future work are to determine if weeds can be selectively controlled in major field crops with field pea mulches or seed extracts, to identify the specific allelochemical(s) in field pea seed that exert growth suppression, to examine the role of soil microflora and environmental effects on the phenomenon, and to discover whether the weathering effect of field pea seed is an essential component of the allelopathic effect. In addition, a crucial question to answer is whether the active molecules derive from the hulls or the cotyledons alone. If the allelopathic effect is due to active compound(s) found to be part of the hulls, a valuable end use for a waste product could represent a value-added trait for this crop. Allelopathic activity may prove difficult to establish in terms of tissue sequestration. In a parallel investigation, hull tissue extract analyses have shown that mature yellow cotyledon field pea cultivars lack the phenolic and flavonoid compounds that often exert allelopathic responses (S. M. Marles and K. E. Bett, unpublished data).

Determination of tissue-related allelopathic activity requires extensive tests of a wide variety of compound classes, not just those from water-based extracts. An unidentified agent that suppressed chickweed and pigweed has yet to be determined, although both water-based and organic solvent extracts (methanol and ethyl acetate) of hairy vetch and of cowpea were examined (Hill et al. 2006, 2007). Furthermore, compound identification to establish bioactivity can be difficult to achieve, the procedures being prone to artifacts (Blair et al. 2009). This complexity was demonstrated extensively by reports regarding the allelopathic agent responsible for growth suppression by species of knapweed (*Centaurea* spp.) (Bais et al. 2002; Blair et al. 2006, 2009; Perry et al. 2005).

Despite these unknowns, allelopathic use of field pea seed represents an opportunity to develop an alternative use for this crop as a natural weed control agent. In western Canada, feed-grade field pea carry-over stocks are currently much higher than normal (Anonymous 2008) and new markets for the crop would help to alleviate this situation (Anonymous 2009). An opportunity to develop recommendations for the use of feed-grade field peas as an allelopathic control for Cruciferous weeds would arise if these effects can be substantiated in field trials.

Sources of Materials

[1] Proven Seeds 9525®, Viterra Inc., Regina, SK.

[2] Sylvania high pressure sodium, Manchester, NH; average PAR, 400 to 700 nm.

[3] SAS 9.1 for Windows vers. 5.1.2600, SAS Institute, Cary, NC.

[4] Easypure LF, Barnstead-Thermo Scientific, Waltham, MA.

[5] MS basal salts (supplemented with 0.3% sucrose in 2% agar), Sigma, St. Louis, MO.

[6] General Electric Diurnal Illumination Low Temperature incubator, model 2015, with GE Ecolux F32T8SP41 lighting, VWR International, Edmonton, AB, Canada.

[7] Whatman #1, 2.5 cm diameter, VWR International, Edmonton, AB, Canada.

[8] Whatman (GF/A glass fiber), Whatman # 50 and Millipore 0.8 μm, VWR International, Edmonton, AB, Canada.

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