Allelopathic potential of legume cover crops on selected weed species

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Summary

Laboratory, greenhouse and field studies were conducted to determine the allelopathic potential of legume cover crops on germination and growth of two weed species, viz. *Asystasia intrusa* and *Paspalum conjugatum*. Both the weed species exhibited greater phytotoxic responses from *Calopogonium caeruleum* and *Mucuna cochinchinensis* as compared to other legume cover crops. The germination of *A. intrusa* and *P. conjugatum* decreased by 19 and 34% respectively compared to the control when grown in full-strength aqueous extract (66.6 g L^{-1}) of *C. caeruleum*. When grown in full-strength aqueous extract of *M. cochinchinensis*, the germination of *A. intrusa* and *P. conjugatum* were reduced by 23 and 27% respectively compared to the control. The use of full-strength aqueous extract of *C. caeruleum* and *M. cochinchinensis* significantly reduced the radicle length and dry weight of both weed species. The emergence and dry weight of *A. intrusa* and *P. conjugatum* were affected when these plants were grown under greenhouse conditions in the presence of increasing amounts of *M. cochinchinensis* and *C. caeruleum* debris incorporated into the soil medium. Conversely, the emergence and dry weight of *A. intrusa* and *P. conjugatum* were not affected by the presence of *Centrosema pubescens* debris.

Introduction

It may be economical to prevent infestation with undesired plants in a cultivated area by cultural practices. This has been exemplified particularly in plantation crops where legumes are established in the inter-rows. The legumes provide considerable amounts of nitrogen, increasing the organic matter and thus improving soil physical properties, as well as preventing the encroachment of weeds. The two most common weeds in young rubber and oil palm plantations in Malaysia were *Asystasia intrusa* Bl. and *Paspalum conjugatum* Berg. (Pamplona 1975, Chee 1989). In the field, their populations were reduced with the existence of legume cover crops (Scholaen and Koch 1988).

Legume cover crops are widely used to control weeds in young rubber and oil palm plantations in Malaysia (Abu and Samsudin 1985, Chee 1989). These crops cover the soil and reduce the light reaching soil surface. Thus, reduction of weed growth could be due to competition (Pushparajah 1977, Wilson *et al.* 1982). However, other factors may also play a role in reducing weed growth. It has been reported that many species of leguminous cover crops contain secondary plant products with allelopathic potentials, but only a limited number have been investigated so far (White *et al.* 1989). For example, *Trifolium* sp. and *Vicia* sp. reduced germination and growth of certain weeds and other forage crops (White *et al.* 1989). The objective of the present study was to investigate the allelopathic potential of legume cover crops on the growth of *A. intrusa* and *P. conjugatum*, the two major weed species in rubber and oil palm plantations.

Materials and methods

The allelopathic potential of five species of legume cover crops i.e., *C. caeruleum*, *C. mucunoides*, *C. pubescens*, *P. javanica* and *M. cochinchinensis*, were studied, while two species of weed i.e., *A. intrusa* (broadleaf) and *P. conjugatum* (grass), were used as bioassay species. Above ground plant materials including stems and leaves were collected for the allelopathic study from the Rubber Research Experimental Station at Sungai Buloh Selangor, Malaysia. The collected plant materials were oven-dried at 50°C for three days, then frozen until used.

Soil-root core bioassay

Soil-root cores were collected at the same location and time by using a hand-held soil sampler. The cores (15 cm depth) were obtained from areas where the above ground legume biomass had been harvested for the legume debris and aqueous extract studies. For control treatments the soil cores were collected from adjacent areas of the same field which was free from legume and other plant material. There were 35 samples taken from each area, with or without legume cover crops. The soil samples from each of the areas were mixed thoroughly and filled into 8 × 12 cm polythene bags.

Five seeds each of the bioassay species were sown into polythene bags 1 cm below the soil surface and placed in the greenhouse. There were five replications for each group of soil. All polythene bags were watered regularly to maintain adequate soil moisture. No artificial light was supplied and the temperature during the experimental period ranged between 24 and 34°C. Fourteen days after planting (DAP) seedling emergence was recorded and then the plants were thinned to two seedlings per polythene bag. The plants were harvested four weeks after planting and the average plant height and dry weight per bag was determined.

Aqueous legume extract study

This experiment was conducted to determine whether the legume debris contained any water soluble phytotoxic components. Ten grams each of the fresh root, stem and leaf tissues of the legume cover crops were cut into 2 to 4 cm lengths before extraction. Plant materials were kept in a flask containing 150 mL distilled water and agitated for 12 hours on an orbital shaker at room temperature (27 ± 3°C). The legume extract was strained through four layers of cheesecloth, then through two layers of Whatman No. 2 filter paper. The extract was kept refrigerated at 5°C for a maximum of 12 days before use. Three concentrations of legume cover crops aqueous extract were used for the experiment i.e., full-strength (66.6 g L^{-1}), half-trength (33.3 g L^{-1}) and quarter-strength (16.7 g L^{-1}). Dilution was made with distilled water. Three concentrations of polyethylene glycol (PEG) 6000 MW of 0, 5, 8 and 10% were included as controls for the possible osmotic effects of the legume extracts. A distilled water control was included with both the extract and PEG treatments. The pH of the five legume extract solutions and three PEG concentrations ranged from 5.2 to 6.2. The osmotic potentials of two leguminous cover crops (*M. cochinchinensis* and *C. caeruleum*) and PEG solutions were determined.

Twenty five seeds each of *A. intrusa* and *P. conjugatum* were placed in separate petri dishes fitted with 9 cm Whatman No. 2 filter papers. Ten millilitres each of legume cover crops extract, PEG, or distilled water for the controls, were used to wet the filter papers. The covered petri dishes were placed in an incubator at 30°C. Percent germination and growth parameters (radicle length and dry weight) were recorded after 14 days. Seeds with a 5 mm radicle length (RL) were considered germinated. RL (*P. conjugatum*) or radicle plus hypocotyl length (RH) for *A. intrusa* and dry weight of the seedlings were expressed as a percentage of the control (distilled water).

Legume debris bioassay

These studies were conducted to determine whether the legume debris would have an effect on the growth of *A. intrusa* and *P. conjugatum* under field-like

conditions. Legume plant tissue was cut into 2 to 4 cm lengths and added to polythene bags containing Serdang Series soil (pH 4.6, organic carbon 0.68%, sand 80.3, silt 5.8, clay 13.9%). Six concentrations of legume debris viz. 0, 2.2, 4.4, 6.6, 8.8 or 11 g per 1500 g soil were either placed on the soil surface or mixed thoroughly into the entire potting medium. A concentration of 2.2 g debris per 1500 g soil was comparable to legume dry matter level of 3220 kg ha^{-1} in the field (derivation based on 2.24×10^6 kg soil ha^{-1} per 15 cm depth). There were five replications for each treatment of each bioassay species.

Five seeds of *A. intrusa* and *P. conjugatum* were planted in each bag and the bags were watered as needed. Seedling emergence was recorded 14 days after planting and the plants were thinned to two seedlings per bag. The plants were harvested 4 weeks after planting and dry weight per bag was determined.

Statistical analysis

A complete randomized design with five replications for each concentration was used for the debris and extract studies. All data was subjected to an analysis of variance and Duncan's multiple range test to determine differences among treatments at 0.05 probability level.

Results

Soil-root core bioassay

Based on the statistical analysis of two independent samples of soil with and without legume cover crops it was shown that the emergence, plant height and dry weight of *A. intrusa* was increased by 8, 42 and 58% respectively, while for *P. conjugatum* the increase was 13, 30 and 35% respectively (Table 1).

Aqueous legume extract bioassay

The response of the bioassay species to the aqueous extracts varied between the two species of *A. intrusa* and *P. conjugatum* and differed significantly amongst the legume extracts for each bioassay species. Table 2 showed that full-strength of *C. caeruleum* and *M. cochinchinensis* extracts reduced the germination of *A. intrusa* by 19 and 23% respectively, while *C. pubescens* and *C. mucunoides* extracts showed no effect on the germination of *A. intrusa*. In contrast, *P. javanica* extract at full-strength increased the germination of *A. intrusa* by 10%. Full-strength of *C. caeruleum* and *M. cochinchinensis* extract reduced *P. conjugatum* germination by 34 and 27% respectively, while *C. pubescens* extracts showed no effect. On the contrary, germination of *P. conjugatum* increased significantly by 17 and 14% at 66.6 g L^{-1} of *C. mucunoides* and *P. javanica* , respectively (Table 2).

A. intrusa radicle length (RH) was

Table 1. Effect of soil from field–grown with or without legume cover crops on emergence, plant height and dry weight of *A. intrusa* and *P. conjugatum* (±S.E.).

Parameter	*A.intrusa*		*P.conjugatum*	
	soil with LCC[A]	without LCC	soil with LCC	without LCC
Emergence (%)	80 ± 6.32	68 ± 4.90	72 ± 4.90	64 ± 7.43
Plant height (cm)	17 ± 0.72	12 ± 0.81	13 ± 0.57	10 ± 0.46
Dry weight (g)	0.63 ± 0.06	0.40 ± 0.03	0.42 ± 0.04	0.31 ± 0.03

[A] Legume cover crops.

Table 2. Effect of aqueous legume debris extract on germination of bioassay species.

Extract conc. ($g L^{-1}$)	Legume species[A]									
	CC		MC		CP		CM		PJ	
	AI	PC[B]	AI	PC	AI	PC	AI	PC	AI	PC
	(% of control)									
0.0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{ab}	100^{b}	100^{b}	100^{c}
16.7	99^{a}	85^{b}	98^{a}	96^{a}	102^{a}	99^{a}	106^{a}	104^{ab}	106^{ab}	105^{bc}
33.3	99^{a}	73^{c}	93^{a}	75^{b}	103^{a}	106^{a}	103^{a}	113^{ab}	104^{ab}	118^{a}
66.6	81^{b}	66^{c}	77^{b}	73^{b}	105^{a}	111^{a}	93^{ab}	117^{a}	110^{a}	114^{ab}

Values are means of five replications. Column of means followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

[A] Legume species (CC = *C. caeruleum*; MC = *M. cochinchinensis*; CP = *C. pubescens*; CM = *C. mucunoides*; PJ = *P. javanica*).

[B] AI = *A. intrusa* and PC = *P. conjugatum*.

Table 3. Effect of aqueous legume debris extract on radicle length of bioassay species.

Extract conc. ($g L^{-1}$)	Legume species[A]									
	CC		MC		CP		CM		PJ	
	AI	PC[B]	AI	PC	AI	PC	AI	PC	AI	PC
	(% of control)									
0.0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{b}	100^{c}	100^{b}
16.7	83^{b}	94^{a}	91^{a}	92^{a}	106^{a}	100^{a}	112^{a}	126^{a}	106^{bc}	123^{ab}
33.3	75^{b}	77^{ab}	75^{b}	82^{ab}	104^{a}	117^{a}	101^{a}	133^{a}	121^{ab}	125^{a}
66.6	75^{b}	65^{b}	73^{b}	61^{b}	84^{b}	120^{a}	97^{a}	131^{a}	125^{a}	127^{a}

Values are means of five replications. Column of means followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

[A] Legume species (CC = *C. caeruleum*; MC = *M. cochinchinensis*; CP = *C. pusbescens*; CM = *C. mucunoides*; PJ = *P. javanica*).

[B] AI = *A. intrusa* and PC = *P. conjugatum*.

Table 4. Effect of aqueous legume debris extract on dry weight of bioassay species.

Extract conc. ($g L^{-1}$)	Legume species[A]									
	CC		MC		CP		CM		PJ	
	AI	PC[B]	AI	PC	AI	PC	AI	PC	AI	PC
	(% of control)									
0.0	100^{a}	100^{a}	100^{a}	100^{a}	100^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{b}
16.7	98^{a}	79^{ab}	96^{ab}	92^{a}	138^{a}	101^{a}	108^{a}	101^{a}	117^{a}	112^{ab}
33.3	87^{ab}	63^{b}	85^{ab}	66^{ab}	135^{a}	118^{a}	120^{a}	119^{a}	117^{a}	135^{a}
66.6	69^{b}	52^{b}	71^{b}	65^{b}	119^{ab}	116^{a}	105^{a}	119^{a}	128^{b}	135^{a}

Values are means of five replications. Column of means followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

[A] Legume species (CC = *C. caeruleum*; MC = *M. cochinchinensis*; CP = *C. pusbescens*; CM = *C. mucunoides*; PJ = *P. javanica*).

[B] AI = *A. intrusa* and PC = *P. conjugatum*.

reduced to 25, 27 and 16% of the control in the full-strength of *C. caeruleum*, *M. cochinchinensis* and *C. pubescens* extracts, respectively, but remained unaffected by the full-strength of *C. mucunoides*. However, *P. javanica* extract increased the RH of *A. intrusa* by 25% (Table 3). The radicle length of *P. conjugatum* was reduced to 35 and 39% in the full-strength *of C. caeruleum* and *M. cochinchinensis* extracts, respectively, but there was no significant difference in the radicle length of *P. conjugatum* in extract of *C. pubescens*. *P. javanica* and *C. mucunoides* extract increased the radicle length of *P. conjugatum* by 27 and 31% respectively (Table 3). The dry weight of *A. intrusa* was reduced by 31 and 29% of the control in full-strength of *C. caeruleum* and *M. cochinchinensis* extract solutions respectively (Table 4). The dry weight of *A. intrusa* increased by 38% in quarter-strength but showed no significant effect at full-strength of *C. pubescens*. *C. mucunoides* extract showed no effect on the dry weight of *A. intrusa*, while *P. javanica* increased 28% of *A. intrusa* dry weight. *C. caeruleum* and *M. cochin-chinensis* reduced dry weight of *P. conjugatum* by 48 and 35%, respectively (Table 4). There was no significant difference in dry weight of *P. conjugatum* due to *C. mucunoides* and *C. pubescens* extracts, while *P. javanica* increased 35% dry weight of *P. conjugatum* in full-strength of extract.

Table 5 shows the osmotic potentials of legume extract and PEG solutions. The osmotic potentials for each concentration did not differ much between the two species. The percentage germination of *A. intrusa* seeds, radicle length and dry weight remained unaffected by PEG solution, but tended to decrease with the increase of PEG concentrations. In contrast, *P. conjugatum* germination and radicle length was slightly reduced by 13 and 34% of the control in 10% PEG (Table 6).

Legume debris bioassay

The emergence of *A. intrusa* and *P. conjugatum* seedlings were not affected when *C. pubescens* debris remained on the soil surface. The emergence decreased progressively to 80 and 75% of that of the control, respectively, with an increase in the rate of incorporated *C. pubescens* debris (Figure 1A). Increasing the amount of *M. cochinchinensis* debris reduced the emergence of *A. intrusa* and *P. conjugatum* seedlings. Incorporated debris of *M. cochinchinensis* at 8.8 g per 1500 g soil showed a significant reduction on the emergence of *A. intrusa* (Figure 1B). *C. caeruleum* debris inhibited the emergence of *A. intrusa* and *P. conjugatum* seedlings when incorporated at the highest rate (Figure 1C). The emergence of *P. conjugatum* seedling was affected when the debris of *C. caeruleum* was placed on the soil surface.

The dry weight of *A. intrusa* and *P. conjugatum* responded positively to *C. pubescens* placed on the soil surface at the highest rates. In contrast, *C. pubescens* debris slightly reduced the dry weight of *A. intrusa* and *P. conjugatum* when *C. pubescens* debris was incorporated at the highest rate (Figure 2A). *A. intrusa* and *P. conjugatum* dry weight was slightly reduced by *M. cochinchinensis* debris placed on the soil surface. The dry weight of *A. intrusa* and *P. conjugatum* decreased progressively to 51 and 74% of the control with an increase in the concentration of incorporated *M. conchinchinensis* debris (Figure 2B). *C. caeruleum* reduced the dry weight of *A. intrusa* and *P. conjugatum* with high rate debris placed on the soil surface or incorporated into the soil. The dry weight of *A. intrusa* and *P. conjugatum* was reduced to 64 and 41% of the control at the highest rate of incorporated *C. caeruleum* debris (Figure 2C).

Table 5. Osmotic potentials of legume extract and PEG solutions.

Extract conc. ($g\ L^{-1}$)	Osmotic potential CC (mOsm)	MC (mOsm)
16.7	15	13
33.3	26	28
66.6	55	58
PEG conc.(%)	(mOsm)	
5	18	
8	35	
10	60	

CC = *C. caeruleum*.
MC = *M. cochinchinensis*.

Table 6. Effect of PEG solution on germination, radicle length and dry weight of *A. intrusa* and *P.conjugatum*.*

PEG conc.(%)	*A.intrusa* G	RL	DW	*P.conjugatum* G	RL	DW
0	78[a]	100[a]	100[a]	73[a]	100[ab]	100[ab]
5	77[a]	94[a]	97[a]	81[ab]	108[a]	121[a]
8	72[a]	89[a]	89[a]	71[bc]	88[b]	82[b]
10	70[a]	84[a]	82[a]	67[c]	66[c]	72[b]

Values are means of five replicates. Column of means followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range.

* G = germination in percentage, RL = Radicle length in percent of control, DW = Dry weight in percent of control.

Discussion

The soil, field-grown with legume cover crops, had no negative effects on the growth of *A. intrusa* and *P. conjugatum*. The results showed that *A. intrusa* and *P. conjugatum* slightly increased their germination and growth compared to those of the control. This enhancement was probably due to the higher N content in the soil where legume cover crops were grown. It has been reported that the yield of corn was increased when grown with intercropped legume cover crops compared to corn monoculture for several years in the same land (Scott *et al.* 1987). Legume cover crops such as Hairy vetch and Crimson clover supplied biologically fixed N to the corn (Ebelhar *et al.* 1984, Holderbaum *et al.* 1990). For long-term periods soil under legume cover crops were more stimulatory to the growth of neighbouring plants.

Moisture stress of the extract solution did not affect the germination and growth of *A. intrusa*. However, the germination and growth of *P. conjugatum* was reduced to 71 and 67% by 8 and 10% PEG concentrations, respectively. This germination rate was greater with full-strength of *C. caeruleum* (48%) and *M. cochinchinensis* (53%) extract on *P. conjugatum*. Therefore, the reduction in *Paspalum* germination may have been the result of the osmotic potential and allelochemical in the extracts. The osmotic potential of 10% PEG did not differ significantly with full-strength of *M. cochinchinensis* and *C. caeruleum* aqueous extracts (Table 5). Plant species may differ in their tolerance to osmotic pressure of the solutions (Bieber and Hoveland 1968). In this case *P. conjugatum* was found to be more sensitive to osmotic concentration than *A. intrusa*. Sahid (1985) reported the reduction of percentage germination, radicle length and wet weight of *P. conjugatum* with the increase of osmotic potential of the germinating medium. While germination and growth of *A. intrusa* was not affected by the osmotic potential, the pH of the leguminous extract was not the factor affecting germination of *A. intrusa* and *P. conjugatum* seeds (Sahid and Juraimi 1989). The optimum pH for germination of *A. intrusa* range from 4.0 to 8.0, while it was 5.0 to 7.0 for *P. conjugatum* (Sahid 1985, Sahid and Juraimi 1989).

Soil variables such as shading, aeration, water and nutrient infiltration, and water holding capacity may be modified by the addition of debris to the soil surface or

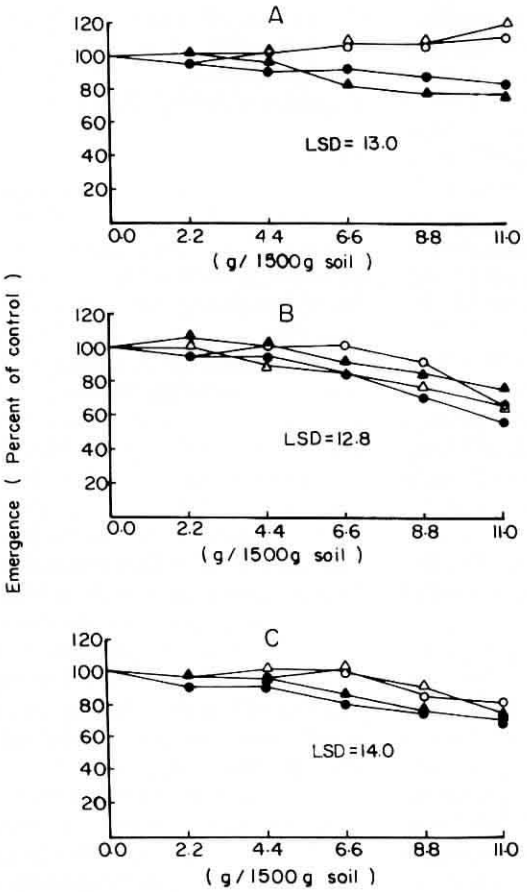

Figure 1. Effect of legume debris (A, *C. pubescens*; B, *M. cochinchinensis*; C, *C. caeruleum*) at two soil locations (Top = soil surface and Inc. = incorporated) on emergence of *A. intrusa* and *P. conjugatum*. o—o *A. intrusa* (Top), ●—● *A. intrusa* (Inc), Δ—Δ *P. conjugatum* (Top), ▲—▲ *P. conjugatum* (Inc).

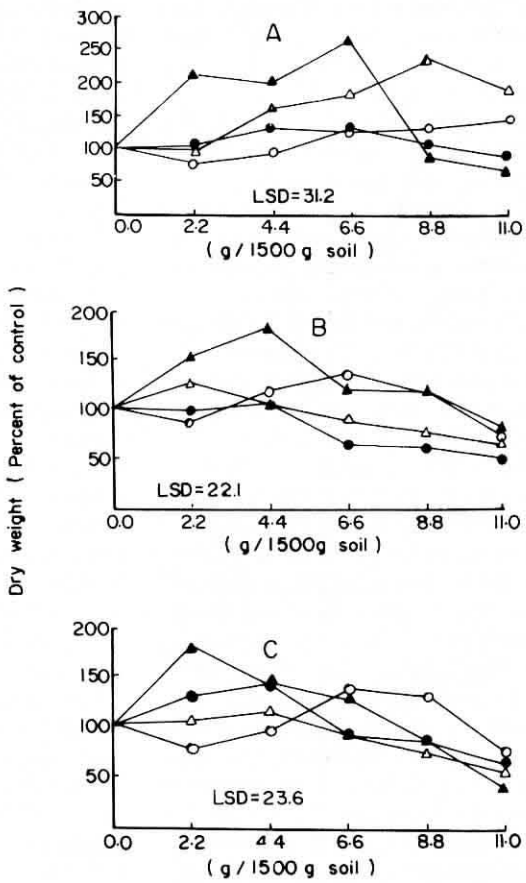

Figure 2. Effect of legume debris (A, *C. pubescens*; B, *M. cochinchinensis*; C, *C. caeruleum*) at two soil locations (Top = soil surface and Inc = incorporated) on dry weight of *A. intrusa* and *P. conjugatum*. o—o *A. intrusa* (Top), ●—● *A. intrusa* (Inc), Δ—Δ *P. conjugatum* (Top), ▲—▲ *P. conjugatum* (Inc).

incorporation into the soil. These factors could influence the biochemical or physical interaction occurring between the debris and test plants. In our experiments adequate amounts of water and nutrient solutions were supplied and seeds were covered with loose soil to avoid obstruction from the incorporated debris to minimize the effect of these factors. Seedling emergence appeared not to be physically impeded by debris at either location or at any concentration (White *et al.* 1989).

The test plants in the debris study responded not only to the amount of debris, but also to the change in debris location. Thus, the location of debris in relation to growing roots appears to be an important factor in the allelopathic interactions. Our results have shown that phytotoxicity was enhanced by soil incorporation of the legume cover crop debris into the soil. Incorporation of the debris into the soil may promote its chemical and microbial decomposition accompanied by the release of soluble organic constituents. Weed or crop seeds and roots in proximity would therefore have a greater probability of coming into contact with allelopathic compounds. Conversely, debris located on the soil surface may decompose at a lower rate and thus release allelochemicals in lower quantities, distant from expanding roots. Furthermore, seeds may be unable to germinate due to less light reaching the soil surface if it is covered with plant debris.

A. intrusa and *P. conjugatum* growth were reduced by certain legume cover crop species (*M. cochinchinensis* and *C. caeruleum*) only where quantities of debris were greater than normal field levels. These results were in line with those of White *et al.* (1989) who stated that legume cover crops such *Trifolium* sp. and *Vicia* sp. only affected the growth of certain crops and weeds at high rates. Evenari (1949) noted that certain plants contain alkaloids that inhibit the physiological processes of other plants at higher rates, but were stimulatory at lower level.

Therefore, successfully planted legume cover crops could suppress weed growth not only due to their high competitiveness for nutrients, light and water, but also through the possible release of allelochemicals to the environment. However, further studies are needed to identify the active chemical component of the inhibitors present in legume debris. Thus, implementing legume cover crop planting into weed control strategies in no-till crops would appear practical and of some potential benefit when coordinated with herbicide applications.

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