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# 34 The Allelopathic Effect of Dead and Living Mulches from Perennial Ryegrass (*Lolium perenne* L.) on *Calystegia sepium* (L.) R. Br

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## ABSTRACT

The allelopathic effects of dead and living mulches from perennial ryegrass on the growth of *Calystegia sepium* were investigated. Dead mulch led to a significant inhibition on the germination and growth of both lettuce and *C. sepium*. The inhibition was mulch rate-dependent. However, the rhizome bud sprouting of *C. sepium* was hardly influenced by the surface mulching. Shoot residues of ryegrass before acetone extraction was more allelopathic than acetone-extracted residues to the growth of lettuce and *C. sepium* developed from rhizome. The inhibitory effect of incorporated residues before extraction was rate dependent and it decreased along with the decomposition process. The inhibitory effect of residues after extraction increased with the decomposition course, indicating the occurrence of microbial transformation of non-toxic substances into toxic phytotoxins. Rhizome bud sprouting, seed germination, and growth of *C. sepium* were suppressed by the living mulch of ryegrass. The inhibition of the growth of this weed by ryegrass cover is not due to less light. The decaying residues and the continuous release of allelochemicals from living ryegrass account for the accumulation of sufficient level of phytotoxins to suppress the *C. sepium*. The present study showed that ryegrass allelopathy is an important factor inhibiting the growth of *C. sepium* in pasture.

the allelopathy of ryegrass in pastures. Uneven distribution of plant residues led to the localization of allelochemicals on the soil surface, suggesting that allelochemicals might be released from localized residues in sufficient quantity to affect plant growth.<sup>4,15</sup>

Original freeze-dried shoot residues of ryegrass (before acetone extraction) were more allelopathic than acetone-extracted residues on the root elongation and lettuce hypocotyls. It shows that the available phytotoxins from shoot residues of ryegrass were removed after acetone extraction. The original residues may immediately release its available phytotoxins into the soil to attack a target plant species after incorporation. The allelopathic potential of original residues decreased as the period of incorporation increased. The residues remain allelopathic even after 30 das of decomposition. In agreement with these results, Mallik<sup>16</sup> found that phytotoxins from lambsquarters shoots persisted even after a decomposition of 30 days. In contrast, Kimber<sup>17</sup> reported that the inhibition of aqueous extracts of several legumes and grasses, decomposed up to 21 days, was negligible. It is possible that the available phytotoxins in the original shoot residues may encounter biodegradation after incorporation, subsequently leaving no traces of toxicity. In ryegrass pasture, however, residue build-up is a continuous dynamic process, and the collected residue is always in a stage of early decomposition, which is inhibitory to the germination and growth of other species. It is interesting to note that the extracted residues also showed certain level of inhibition on germination and growth of lettuce. The allelopathic potential of the extracted residues increased slightly as the period of incorporation increased. It may be inferred that microbial transformation of non-toxic residues into toxic substances took place after the residue incorporation. Chase<sup>18</sup> reported that soil microorganisms can either release or produce phytotoxins directly from residues, or use the residues as substrates in the production of bioactive compounds.

Similar results were found in pot experiments for *C. sepium* developed from rhizomes. The inhibitory effect of residues before extraction, decreased along with the decomposition process up to 20 days. From 20 days to 30 days of decomposition, the inhibitory effect tended to increase, indicating the involvement of microbial transformation. The inhibitory effect of the residues before extraction was rate dependent. However, residues amended with over 6 grams per pot did not lead to a significant increase of its inhibitory effect. The inhibitory effect of the residues after extraction increased with the course of decomposition. It is clear that when the allelopathic residue is applied to the field, the available phytotoxins are immediately released to attack the growth of other plant species. The inhibitory feature will persist for about 15 days. After this, a least inhibitory period is approached. After the amendment of the residues for about 20 days, the microbial transformation of non-toxic substances into toxic phytotoxins occurred. However, this transformation process started right after the application of non-toxic residues.

Residues can interact with plants and potentially reduce growth. This interference can arise from allelochemicals released by the decaying residues<sup>3,19</sup> and/or from the change of C:N ratio and the immobilization of nitrogen.<sup>20,21</sup> Excessive fertilization in the present research provided sufficient nutrients, avoiding the nutrient competition between plant species. By using original and acetone-extracted residues in soil incorporation experiments, the same immobilization process occurs. The only difference between these two kinds of residues is their allelopathic potential. Therefore, the influence of immobilization on the allelopathic potential of ryegrass shoot residue is avoided in the present experiment by using acetone-extracted residues as controls. It would be better if residue incorporation and soil chemical analysis are coupled, which may lead to a better understanding of the allelopathic potential of residues.

## MULCH EXPERIMENT

Thirty seeds of lettuce, 10 rhizome buds, and 10 seeds of *C. sepium* were sown in pots filled with 1200 g of soil mixture with sandy soil and sand (2:1, v/v). Ground powder of freeze-dried shoots from 8-month-old ryegrass with four application rates (0, 4, 8, and 12 g per 1200 g sandy soil) were evenly mulched on the soil surface of each pot. The pots were arranged in a randomized complete block design with 3 replicates and placed in a greenhouse at 26 C/22 C in daily cycles of 12 hr light — 12 hr dark. Four fluorescent lamps (400 W, Philips) were placed 1 m above the supporting table (4 x 1.5 m<sup>2</sup>). After initial watering with 50 mL of nutrition solution, the pots were saturated with 100 mL of nutrition solution every week (alternation with solution A and B) in order to avoid nutrient stress caused by the microbial transformation of residues. Pots were subirrigated to maintain 80 % field capacity when necessary. The total numbers of seedlings were counted 2 weeks after sowing. At this time, the pots were thinned to 3 plants each. After continuous growth for another 2 weeks, the whole plant in each pot was harvested individually by carefully clearing the soil cores with pressurized tap water. The whole plant was divided into aboveground and underground parts. Plant materials were oven-dried at 70 C for 48 hours. The total dry weights of both aboveground and underground parts, and dry weights of whole plants were determined for each pot.

Solution A or B: Four hundred grams of fertilizer A or B was dissolved in 200 litres of deionized water mixed with 30 mL of Fe-EDTA (concentration, 3%) and 200 mL of H<sub>3</sub>BO<sub>3</sub> (concentration, 0.286%), respectively. Fertilizer A contained 18% N, 18% P<sub>2</sub>O<sub>5</sub>, 18% K<sub>2</sub>O, 0.04% B, 0.2% Mn, 0.001% Mo, 1.8% Mg, 0.06% Cu, 0.06% Zn, and 0.13% Fe. Fertilizer B contained 21% N, 7% P<sub>2</sub>O<sub>5</sub>, 22% K<sub>2</sub>O, 3% MgO, 0.04% B, 0.1% Mn, 0.0005% Mo, 0.002% Cu, 0.05% Zn, and 0.2% Fe.

## PREPARATION OF ACETONE-EXTRACTED RESIDUE

Four hundred and eighty grams of freeze-dried and pulverized shoot residues of 8-month-old ryegrass was first put into a container filled with 10 L of acetone, agitated and left to stand for 20 hours. The acetone mixture was filtered through 4 layers of cheesecloth, washed thoroughly with deionized water, agitated and extracted with 10 L of deionized water for 24 hours at room temperature. The residue was then filtered and washed completely with deionized water. The final extracted residue was collected, oven-dried and designated as shoot residue after acetone extraction.

## PARTIAL DECOMPOSITION OF RESIDUES

Petri dish experiments were carried out using extracts from soil incorporated with residues. Twelve grams of eight-month-old shoot residues (before and after extraction) were incorporated into 600 grams of sandy soil in polyethylene bags 0, 5, 10, 20, and 30 days prior to extraction. Two hundred and fifty grams of freeze-dried sandy soil samples amended with shoot residues were immediately shaken for 12 hours after the addition of 250 mL of deionized water and left to stand for 5 hours at room temperature. After centrifugation (8,000 g, 15 min.), the supernatants (extracts) were vacuum-filtered with 3 layers of filter papers and stored at 5 C before use. Extracts from sandy soil (without the amendment of residues) and deionized water were used as controls.

Lettuce was used for the bioassays. Twenty-five grams of water-washed, oven-dried (150 C, 24 hr) and sized sand were thoroughly mixed with 25 seeds of lettuce in each dish. After the addition of five mL of each extract or water (control), the dishes were sealed with a plastic film

level of incorporated residues increased (Table 4), showing the rate-dependent nature. There is almost no difference between 6 grams and 12 grams of residues amended to 1200 grams of soil, revealing that the amount of residue when amended over 6 gram per pot (1200 grams of soil) did not significantly enhance its inhibitory ability. A rate of 6 g residue per pot (1200 g soil) is equivalent to ryegrass dry matter of 15,000 - 24,000 kg ha<sup>-1</sup> in the field (assumed bulk density, 1.2-1.5 g cm<sup>-3</sup>).

**Table 4.** The level of incorporated residues on the growth of *C. sepium* developed from rhizome buds.\*

Level of residue incorporated (g/pot)	Length of new rhizome (cm)	Number of rhizome bud	Weight of new rhizome (mg)	Weight of root (mg)	Weight of shoot (mg)
3	21.97	12.31	0.30	0.033	0.16
6	18.73	10.28	0.28	0.031	0.15
12	18.72	10.39	0.28	0.030	0.13

\*: Average data per plant calculated on 3 plants per pot with three replicates.

#### EFFECT OF LIVING COVER FROM RYEGRASS

The growth of sprouts developed from rhizomes was significantly suppressed as the level of ryegrass coverage increased (Table 5). Results clearly showed that bud sprouting and seed germination were greatly inhibited by ryegrass cover. The inhibitions cannot be ascribed to the absence of light, because the bud developed quickly and freely under the cover of a black plastic film (data not shown). Data in Figure 2 revealed that a continuous grass cover had profound influence on the growth of *C. sepium* from rhizome buds. The continuous release of allelochemical(s) by living ryegrass cover enables continuous inhibition on seedling growth of *C. sepium*. The weights of roots, shoots and whole plants were greatly reduced. It seems plausible that the ryegrass roots are able to release inhibitory phytotoxins in the associated soil. The contaminated soil covered by ryegrass was also inhibitory to the growth of *C. sepium* when compared to uncontaminated soil covered by ryegrass. In this treatment, it took time for the ryegrass to develop its new root system to release allelochemicals. Its toxicity was, therefore, lower than the continuous ryegrass cover.

**Table 5.** Living mulch effect of ryegrass on *C. sepium*.

	Germination	Sprouting	Weight (mg/plant)***		
	%	%	Root	Shoot	Plant
Ryegrass cover 0%	31.1 <sup>a</sup>	67.2 <sup>a</sup>	4.7 <sup>a</sup>	48.3 <sup>a</sup>	53.0 <sup>a</sup>
Ryegrass cover 85%*	33.3 <sup>a</sup>	55.6 <sup>a</sup>	2.6 <sup>ab</sup>	13.2 <sup>b</sup>	15.9 <sup>b</sup>
Ryegrass cover 95%**	28.9 <sup>a</sup>	31.3 <sup>b</sup>	0.6 <sup>b</sup>	7.9 <sup>b</sup>	8.5 <sup>b</sup>
Ryegrass cover 100%	6.7 <sup>b</sup>	13.3 <sup>c</sup>	2.2 <sup>ab</sup>	11.8 <sup>b</sup>	14.0 <sup>b</sup>

\*: 5 holes of 2 x 2 cm<sup>2</sup>;

\*\* : 5 holes of 1 x 1 cm<sup>2</sup>;

\*\*\*: Data from seedlings developed from rhizome buds;

Means not followed by the same letters are significantly different at 5% level within a column.

were also covered by the film right after the buds/seeds of *C. sepium* were inserted 0.5 cm deep into soil.

A randomized complete block design with 3 replicates was used with, three treatments (A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>) and five subtreatments (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub> and B<sub>5</sub>).

#### STATISTICAL ANALYSIS

A randomized complete block design with 3 or 4 replicates was normally used for each experiment. Data was statistically analyzed using Genstat 5.1 for analysis of variance and treatment means were tested separately with least significant difference (LSD) at a 5% or 1% level of probability.

## RESULTS

#### ALLELOPATHIC POTENTIAL FROM SURFACE MULCH OF RYEGRASS SHOOT RESIDUE

Ryegrass mulch caused a significant reduction in seed germination of both lettuce and *C. sepium*. Inhibition of germination was enhanced as the mulch rate of ryegrass shoot residue increased (Table 1). The growth of lettuce was more sensitive than *C. sepium* to ryegrass mulch. Higher mulch rate (up to 0.12 g cm<sup>-2</sup>) is required to inhibit the growth of *C. sepium* seedlings. As for the *C. sepium* developed from rhizomes, the root weight was remarkably reduced only at mulch rates of 0.12 g cm<sup>-2</sup> of residue, compared to no mulch. The growth of shoots was stimulated at 0.04 g mulch rate and then sharply decreased to a significant level at the rate of 0.12 g. However, the surface mulching was not inhibitory to rhizome bud sprouting of *C. sepium* over the range of mulch rates. These results indicate that rhizome sprouting is hardly influenced by the mulching effect of ryegrass shoot residues.

**Table 1.** Allelopathic mulching effect of ryegrass on the germination/sprouting and growth of tested species

Mulch rate (g/cm <sup>2</sup> )	Lettuce			Seed of <i>C. sepium</i>			Rhizome of <i>C. sepium</i>		
	Germination (%)	Weight (mg)*		Germination (%)	Weight (mg)		Sprouting (%)	Weight (mg)	
		Root	Shoot		Root	Shoot		Root	Shoot
0.00	91.11 <sup>a</sup>	10.17 <sup>a</sup>	113.7 <sup>a</sup>	96.7 <sup>a</sup>	87.6 <sup>a</sup>	531 <sup>a</sup>	100 <sup>a</sup>	55.0 <sup>a</sup>	185 <sup>ab</sup>
0.04	16.67 <sup>b</sup>	7.13 <sup>b</sup>	103.9 <sup>a</sup>	50.0 <sup>b</sup>	64.2 <sup>a</sup>	423 <sup>a</sup>	93 <sup>a</sup>	46.5 <sup>b</sup>	253 <sup>b</sup>
0.08	18.89 <sup>b</sup>	6.17 <sup>bc</sup>	80.2 <sup>b</sup>	40.0 <sup>b</sup>	62.8 <sup>a</sup>	421 <sup>a</sup>	100 <sup>a</sup>	48.2 <sup>ab</sup>	162 <sup>a</sup>
0.12	3.33 <sup>c</sup>	4.17 <sup>c</sup>	33.9 <sup>c</sup>	43.0 <sup>b</sup>	39.1 <sup>b</sup>	270 <sup>b</sup>	93 <sup>a</sup>	43.1 <sup>b</sup>	157 <sup>a</sup>

\*: Average weight per plant calculated on 3 plants per pot with three replicates;

Means not followed by the same letters are significantly different at 5% level within a column

#### EFFECT OF DECOMPOSING PERIODS ON THE INHIBITORY ACTIVITY OF DECAYING RYEGRASS RESIDUE

The inhibition of decaying shoot residues before extraction on the growth of lettuce slightly decreased over time (Figure 1A). The inhibitory ability was found even after 30 days of decomposition when compared with soil and water controls (Figure 1B). After the amendment of acetone-extracted shoot residues, it seems that the inhibition on germination and growth increased slightly over the time course of decomposition, but no significant differences were found.