ALLELOPATHY OF SHOWY RATTLEBOX DECOMPOSITION ON THE COMMON BEAN IN NO-TILL

ALELOPATIA DA DECOMPOSIÇÃO DE CROTALÁRIA NO FEIJOEIRO EM PLANTIO DIRETO

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Abstract

This study aimed to identify and quantify the phenolic compounds in soil covered with showy rattlebox (*Crotalaria spectabilis* Roth) at different decomposition times, followed by the sowing of common bean (*Phaseolus vulgaris* L.) and to determine the effects of these times on the emergence and initial growth of the subsequent crops. Common bean was sown at intervals of 0, 10, and 20days after the deposition of showy rattlebox biomass. Phenolic compounds in the soil were analyzed using ultra-performance liquid chromatography (UPLC). The evaluated parameters for common bean included emergence, emergence speed index (ESI), chlorophyll *a* and *b*, plant height, leaf area, and dry mass. Results showed that *C. spectabilis* decomposition increased the concentration of epicatechin and resveratrol in the soil, and catechin levels decreased. The showy rattlebox presence, independent of decomposition time, increased the plant height and dry mass of common bean. The shortest interval (0 days) between showy rattlebox biomass deposition and common bean sowing resulted in the highest rates of emergence, ESI, and chlorophyll *a.* Meanwhile, the common bean leaf area was greater when sown after 10 and 20 days of *C. spectabilis* decomposition.
Keywords: Allelochemicals, *Crotalaria spectabilis*, *Phaseolus vulgaris*, Phenolic compounds.

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Resumo

Este estudo buscou identificar e quantificar os compostos fenólicos no solo coberto com biomassa de crotalária (*Crotalaria spectabilis* Roth.) em diferentes tempos de decomposição, seguido pela semeadura de feijão (*Phaseolus vulgaris* L.) e determinar o efeito destes tempos na emergência e crescimento inicial do cultivo em sucessão. O feijão foi semeado nos períodos de 0, 10 e 20 dias após a deposição da biomassa de crotalária. Os compostos fenólicos do solo foram identificados e quantificados por cromatografia liquida de alta eficiência (HPLC). As variáveis avaliadas no feijão foram emergência, índice de velocidade de emergência (IVE), clorofila *a* e *b*, altura, área foliar e massa seca. Os resultados demonstraram que a decomposição de *C. spectabilis* liberou epicatequina e resveratrol no solo e, diminuiu a quantidade de catequina. A presença da cobertura de crotalária, independente do tempo de decomposição, aumentou a altura e a massa seca de feijão. O menor intervalo (0 dias) entre a deposição da biomassa de crotalária e a semeadura de feijão resultou nos maiores valores de emergência, IVE e clorofila *a*. Enquanto, a área foliar do feijão foi maior quando semeado após 10 e 20 dias de decomposição da *C. spectabilis*.
Palavras-chave: Aleloquímicos, Compostos fenólicos, *Crotalaria spectabilis, Phaseolus vulgaris.*

1. INTRODUCTION

Intensive soil mobilization, which is a hallmark of conventional farming systems, degrades the soil's physical, chemical, and biological properties, ultimately compromising productivity (Quintarelli *et al*., 2022). In this context, the search for sustainable practices for food production becomes relevant, such as the no-till system, which improves soil quality and system resilience using plant rotations, minimal soil disturbance, and cover crops (Koudahe; Allen; Djaman, 2022).

The no-till system for common-bean (*Phaseolus vulgaris* L.) is employed across various regions in Brazil. In 2021, Brazil was the second-largest producer of common bean globally (FAO, 2023). The interactions between cover crops and this grain crop are significant, as these plants can impact the growth of subsequent crops (Formigheiri *et al*., 2018; Hartmann *et al*., 2019).

During their growth and subsequent biomass decomposition, cover crops can release various chemical compounds into the soil (Koehler-cole *et al*., 2020). These substances may influence the performance of successive crops through a process known as allelopathy, where ecological interactions between plants impact each other's growth (Reginatto *et al*., 2020).

Allelopathy refers to the effects that one plant can have on another or on the microorganisms in the surrounding environment. This occurs through the release of chemical compounds, known as allelochemicals, produced by the plants' specialized (secondary) metabolism (Rice, 1984).

Showy rattlebox (*Crotalaria spectabilis* Roth.), a member of the Fabaceae family, is commonly used as a cover crop. It has the potential to produce over nine Mg ha^{-1} of dry mass and contribute up to 260 kg N ha^{-1} to the soil through its symbiotic association with bacteria (Cherubin, 2022). While the allelopathic effects of *C. juncea* L. are well-documented for weed control (Bundit; Ostlieband; Prom-U-Thai, 2021), there is limited information on the allelopathic effects of *C. spectabilis*. This species, which has a short stature, is easily managed in no-till systems and releases phenylpropanoids and flavonoids into the soil, which may also impact subsequent crops (Gomes; Souza; Oliveira, 2020; Scupinari *et al*., 2020).

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This study aimed to identify and quantify the phenolic compounds released into the soil from the decomposition of showy rattlebox (*C. spectabilis*), and to assess how this cover crop affects the emergence and initial growth of common bean (*P. vulgaris*).

2. MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Fronteira Sul (UFFS), in Laranjeiras do Sul, Parana, Brazil, from April to May 2019, with an average temperature of 25 ± 2 ºC.

C. spectabilis was grown between rows in the UFFS organic orchard, located in a region classified as humid subtropical mesothermal (Cfb), with average monthly temperatures and rainfall of 18.9 ºC and 166 mm, respectively.

A completely randomized experimental design (CRD) was employed, in a 2x3 factorial scheme with four replications. This design included two levels of *C. spectabilis* biomass densities (0 and 30 Mg ha^{-1}) and three biomass decomposition periods (0, 10, and 20 days) before sowing common bean. For the analysis of phenolic compounds, the 2x3 factorial scheme was used with an additional factor: the amended soil that served as the substrate (control).

C. spectabilis was harvested at full bloom, and the aerial parts were cut into 5 cm sections. These sections were then placed in pots measuring 52.5 x 31 x 16.5 cm (length x width x depth, respectively). The substrate used consisted of a mixture of soil and sand in a 2:1 ratio. The chemical characteristics of this substrate are detailed in Table 1.

Table 1. Physical-chemical characterization of the soil used in the pot substrate.

pH	OM	P	K	Ca	Mg	H+Al	SB	CEC	V
	g dm^{-3}	mg dm^{-3}	cmol dm^{-3}						%
4.2	38.1	2.7	0.05	1.2	1.0	8.6	2.5	11.1	22.4

pH ($CaCl_2$): hydrogen-potential; OM: organic matter; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of bases; CEC: cation-exchange capacity; V: base saturation.
Source: The authors, 2024.

The soil was fertilized according to the recommendations for common bean (SBCS, 2019), using calcitic limestone, boiler ash, and a mixture of cattle and poultry manure in a 4:1 (v:v) ratio. The amount of water applied was determined according to the guidelines provided by Monteiro and Frighetto (2000), based on soil samples collected at the beginning of the experiment.

Common bean (*P. vulgaris* cv. IPR Uirapuru) was sown at a density of 25 seeds per plot after various decomposition periods of the cover crop (0, 10, and 20 days). Phenolic compounds were identified and quantified (µg kg^{-1} of soil) both in the initial corrected substrate and in the substrate after 25 days of common bean growth. This analysis was conducted for plots where common bean was sown at 0, 10, and 20 days following the deposition of the showy rattlebox.

The extraction method for phenolic compounds from the soil was adapted from Bittencourt *et al*. (2018). A 0.15 kg soil sample was dried in a forced-circulation oven at 40 °C heating to constant mass. The dried soil was then ground with a pestle, sieved to a 2 mm particle size, and stored at -20 °C. For the extraction, the sample was divided into three 50 g sub-samples. Each sub-sample was threatened with 150 mL of analytical-grade methyl alcohol and homogenized at 25 °C and 200 rpm for 48 h using an orbital shaker. Following homogenization, the samples were centrifuged at 1,200 g for 15 min at 25 °C. The supernatant was filtered through 25 µm filter paper, and then the solvent was evaporated using a rotary evaporator at 40 °C under vacuum. The

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residue was then dissolved in 2 mL of methyl alcohol, and the final extracts were filtered through a 0.22 µm PTFE syringe filter and stored in vials.

Phenolic compounds were identified and quantified using standard solutions of (+) catechin, (-) epicatechin, caffeic acid, gallic acid, vanillic acid, *p*-coumaric acid, trans-iso-ferulic acid, (-) resveratrol, myricetin, and quercetin.

The analysis was performed on a Shimadzu UFLC liquid chromatograph equipped with a diode array detector and an NST-C18 column (25 cm x 4.6 mm, 5 µm particles) maintained at 40 °C. Using an automatic sampler, 1 µL of each extract was injected into the column with a mobile phase flow rate of 1.2 mL min^{-1}. Mobile phase B consisted of 99.9% methanol and 0.1% formic acid, while mobile phase A was 99.9% water and 0.1% formic acid (Table 2).

Table 2. Gradient elution program for phenol analysis by ultra-performance liquid chromatography (UPLC)

Stage	Time (min)	B mobile phase concentration (%)
1	0.01	14
2	16	55
3	16.01	100
4	17	100
5	17.01	14
6	20	14

Source: The authors, 2024.

The following parameters of the common bean were evaluated: emergence percentage, emergence speed index (ESI), chlorophyll content (*a* and *b*), plant height (cm), leaf area (cm^2), and dry mass (g).

Emergence was determined by counting the number of normal seedlings (≥ 2 cm, free of damage, deformities, or signs of deterioration) on the 25th day after sowing. Concurrently, the emergence speed index (ESI) was calculated following the method described by Maguire (1962) (Equation 1):

$$ESI = (E1/N1) + (E2/N2) + (E3/N3) + ... + (En/Nn) \quad [Equation\ 1]$$

Where ESI represents the emergence speed index, E denotes the number of normal seedlings that have emerged, and N indicates the number of days.

Measurements for the other variables were taken from 10 randomly selected plants per pot 25 days after sowing. Chlorophyll *a* and *b* levels were measured using the Chlorophyllog® device, with the analysis performed on the fully expanded third leaf.

To measure height, the common bean plants were cut close to the ground and measured from the base to the apex of the stem. The leaves were removed and assessed for leaf area using a leaf area meter (Bio Science CI 203®). The remaining aerial parts of the plants were dried in a forced air circulation oven at 62°C heating to constant mass to determine the shoot dry mass.

Data were analyzed for normality and homogeneity of variance using Shapiro-Wilk and Bartlett tests, respectively. Epicatechin and resveratrol data were transformed using $\sqrt{(x+1)}$ and subsequently analyzed by analysis of variance (ANOVA), with post hoc comparisons performed using F-test or Tukey's test, as appropriate. All statistical analyses were conducted using R software version 4.2.0 ($p < 0.05$).

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3. RESULTS AND DISCUSSION

Sowing common bean in soil with showy rattlebox biomass at various decomposition stages resulted in the presence of catechin in the substrate, which was not detected in control (Table 3). The catechin concentration ranged from 100.9 to 101.4 µg kg^{-1} when common bean was sown up to 10 days after biomass deposition. However, this concentration decreased by 57% in the substrate where common bean was sown 20 days after showy rattlebox decomposition. This reduction in catechin levels over time may be attributed to its susceptibility to volatilization (Jiang *et al*., 2019), oxidation (Tanaka; Kouno, 2003), and biological degradation (Wang *et al*., 2013).

Table 3. Catechin levels in the substrate with or without showy rattlebox biomass and common bean cultivation (control) at various biomass decomposition intervals.

Showy rattlebox decomposition times (days)	Catechin (µg kg^{-1})
Control	0 c
0	100.9 a
10	101.4 a
20	43.8 b
CV (%)	17.9

Letters indicate differences according to Tukey test ($p < 0.05$). The minimum detection level was 5 µg kg^{-1}.
Source: The authors, 2024.

Catechin is a polyphenol known for its antioxidant activity (Gadkari; Balaraman, 2015) and is found in various fruits, vegetables, and legumes (Arts; Putte; Hollman, 2000). Within the *Crotalaria* genus, catechin has been identified in extracts of *C. retusa* (Sinan *et al*., 2020), root exudates of *C. burhia* (Anwar *et al.,* 2022), and seeds of *C. juncea* (Rawat; Saini, 2022). However, to our knowledge, there are no reports of catechin synthesis by *C. spectabilis.*

It is not possible to confirm that catechin was released only due to the decomposition *of C. spectabilis* biomass. Notably, a biomass application of *C. spectabilis* (30 Mg ha^{-1}) resulted in a 30% reduction in catechin levels in the soil compared to soil where beans were grown without the cover crop (0 Mg ha^{-1}) (Table 4). In contrast, the synthesis of catechin by common bean has already been documented in previous studies (Yang *et al*., 2020).

Table 4. Catechin levels in the substrate with or without showy rattlebox biomass and subsequent common bean cultivation.

Showy rattlebox biomass (Mg ha^{-1})	Catechin (µg kg^{-1})
0	72.3*
30	50.7
CV (%)	17.9

* Indicate differences according to the F-test ($p < 0.05$). The minimum detection level was 5 µg kg^{-1}.
Source: The authors, 2024.

The reduction in catechin levels with the addition of showy rattlebox biomass (Table 4) may be related to increased biological activity resulting from decomposition. Microorganisms in the soil can utilize catechin as a carbon source, converting it into organic acids (Molen *et al*., 2023). Another possibility for catechin reduction is its adsorption to soil colloidal particles and newly added organic matter, making it less available or undetectable by the analytical methods used in this study. Furthermore, abiotic transformations, such as oxidation or polymerization, can reduce the concentration of free catechin over time.

In contrast, epicatechin and resveratrol were detected in the soil only when common bean were grown with the presence of showy rattlebox biomass. Their concentration ranged from 23.1 to 35.6 for epicatechin and from 2.7 to 6.4 µg kg^{-1} for resveratrol (Table 5). This pattern, unlike the reduction observed with catechin, indicates that these allelochemicals are likely to originate from showy rattlebox.

Table 5. Epicatechin and Resveratrol levels in the substrate with or without showy rattlebox biomass and common bean cultivation (control) at various biomass decomposition intervals.

Showy rattlebox decomposition (days)	Epicatechin[1]		Resveratrol[1]	
	μg kg^{-1}			
	Biomass (Mg ha^{-1})			
	0	30	0	30
Control	0 A	0 C	0 A	0 D
0	0 A	26.8 AB*	0 A	6.4 A*
10	0 A	23.1 B*	0 A	3.6 B
20	0 A	35.6 A*	0 A	2.7 C*
CV (%)	16.7		3.1	

[1]Data were transformed using √(x+1). Within each column, values with the same uppercase letter are not significantly different according to Tukey test ($p < 0.05$). * Denotes differences according to the F-test (p < 0;05) between different rattlebox biomass amounts for the same substrate. Both in each allelochemical. Minimum detection levels were 7 μg kg^{-1} for epicatechin and 2 μg kg^{-1} for resveratrol. **Source**: The authors, 2024.

Epicatechin is a flavonoid known for its antioxidant properties (Zhang *et al.,* 2022), herbicidal activities (Kato-Noguchi; Kurniadie, 2022), and its ability to inhibit seed germination and plant growth (Serniak, 2016). It is synthesized by fruit trees such as grapes, kiwifruit and apples, as well as shrubs like *Camellia sinensis* (Shukla *et al*., 2019). While epicatechin has been identified in *C. burhia* (Anwar *et al*., 2022) and *C. calycina* (Mazumder *et al*., 2022), this study represents the first report of its presence in *C. spectabilis.* This may also indicate the use of these Fabaceae in weed control, through the production of these allelochemicals, as already noted for *C. anagyroides* (Carvalho; Malaquias; Wanderley, 2021).

Resveratrol is a polyphenol with noted antioxidant and anti-inflammatory effects, has been documented in over 72 plant species (Meng *et al*., 2021). In plants, it can protect against diseases but is rapidly degraded by microbial activity (Kurt; Minoia; Spain, 2018) and is easily volatilized (Olson *et al*., 2021). This volatility may account for the 58% reduction in resveratrol concentration observed when common bean was sown 20 days after showy rattlebox decomposition (Table 5). Although resveratrol production in the *Crotalaria* genus has not been reported, it has been identified in other Fabaceae, such as soybean and common bean (Xu *et al*., 2024).

Both resveratrol and epicatechin, detected in soil containing *C. spectabilis* residues, have been associated with reduced seedling growth of cultivated species such as *Raphanus sativus*, *Lactuca sativa* and *Lepidium sativum* (Fan; Hostettmann; Lou, 2010). Studies indicate that these phenolic compounds exert a more pronounced allelopathic effect on the root system than on the hypocotyl of seedlings, possibly by interfering with root cell division and elongation processes (Politycka, 1999). This differential inhibition of root growth suggests a direct impact on the initial establishment capacity of plants, reinforcing the potential of these metabolites for the selective suppression of sensitive species.

Common bean emergence and emergence speed index (ESI) were 22% and 30% higher, respectively, when common bean was sown immediately after the deposition of showy rattlebox (0 days, 30 Mg ha^{-1}) compared to 0 Mg ha^{-1} (Table 6). This enhancement is attributed to the improved thermal insulation and increased moisture retention provided by the soil cover (Fayad *et al*., 2019; Adetunji *et al*., 2020).

Table 6. Common bean emergence, emergence speed index (ESI), Chlorophyll *a* and *b,* and leaf area at different times with or without showy rattlebox biomass.

Showy rattlebox decomposition (days)	Emergence		ESI		Chlorophyll *a*		Chlorophyll *b*		Leaf area	
	%				SPAD				cm²	
	Biomass (Mg ha^{-1})									
	0	30	0	30	0	30	0	30	0	30
0	72A	92A*	21A	30A*	27A	29A*	9A	9A	145A	152B
10	78A	76B	26A*	21B	26A	26B	7B	7B	110A	172A*
20	78A*	55C	23A*	16B	27A*	23C	9A*	6B	124A	214A*
CV (%)	8.0		13.3		2.8		9.5		20.8	

Values with the same uppercase letters within a column (decomposition days) are not different according to Tukey test ($p < 0.05$). * Indicates differences according to the F-test ($p < 0.05$) within the same variable in each row between different rattlebox biomass amounts. Both in each variable. **Source**: The authors, 2024.

The presence of the showy rattlebox biomass decomposed for 10 and 20 days reduced both the emergence and ESI of the common bean compared to the 0-day biomass condition (Table 6). The lower allelopathic effect observed when common bean was sown immediately after the deposition of showy rattlebox can be attributed to the dynamic release of secondary metabolites present in the straw. At the time of cutting, most allelochemicals remain retained in plant tissues, exhibiting low availability in the soil due to the absence of significant cellular degradation. As the straw decomposes (10 to 20 days after handling), microbial and enzymatic processes intensify cell lysis and promote the gradual release of phenolic compounds and other bioactive metabolites, increasing their concentration in the soil solution and enhancing the inhibitory effects on common bean emergence and emergence speed index. Subsequently, the continuous action of microorganisms, combined with the processes of oxidation, volatilization, and adsorption on soil particles, tends to reduce the phytotoxicity of these compounds, attenuating allelopathic interference over time (Hoang Anh *et al*., 2021).

It can not be ruled out that the hypothesis of allelopathic compounds not detected by the analytical methods employed in this study contributed to the observed results. Monocrotaline, for example, an alkaloid characteristic of *Crotalaria* genus, is widely documented in the literature for its phytotoxic potential (Rech *et al*., 2024). Monocrotaline can inhibit both seedling emergence and vigor (Kostina-Bednarz; Plonka; Barchanska, 2023), reinforcing the hypothesis that alkaloids released during biomass decomposition are key agents driving the inhibitory effects observed in the present study.

Furthermore, it is important to consider the interaction between the chemical and biological processes associated with biomass. The activity of microorganisms not only accelerates the decomposition of plant material but can also transform precursor compounds into more active allelopathic derivatives, amplifying their inhibitory potential (Cheng; Cheng, 2015; Swapnil *et al.*, 2023). Therefore, the reduced emergence and vigor of common bean seedlings may result from a combined effect of allelochemicals from showy rattlebox biomass decomposed and the microbially mediated transformation of these compounds in the soil environment.

The reduction in ESI (Table 6) may also be attributed to the temporary immobilization of nitrogen, which can occur due to increased biological activity from cover crop decomposition (Engedal *et al*., 2023). This is consistent with the observed decrease in chlorophyll *a* and *b* levels for common bean over 20 days, as nitrogen is a crucial component of chlorophyll (Taiz; Zeiger, 2009). When nitrogen is immobilized, its availability for plant absorption diminishes, leading to reduced chlorophyll content. Moreover, with decomposition advances, however, mineralization tends to surpass immobilization and can eventually restore chlorophyll content and photosynthetic activity (Yang *et al*., 2022). However, the leaf area of common bean grown with *C. spectabilis* biomass was greater after 10 days compared to common bean grown in bare soil (Table 6). This improvement is likely due to the enhanced nutrient availability from biomass decomposition, with emphasis on nitrogen and phosphorus, essential macronutrients, and increased microbial activity, like beneficial bacterial population (Adetunji *et al*., 2020; Arone *et al*., 2024).

Common bean plant height and shoot dry mass were 23 and 41% higher, respectively, when grown with showy rattlebox biomass (Table 7). Common bean height increased by 28-31% when sown after 10 days of showy rattlebox decomposition (Table 8). These observations align with the well-documented benefits of cover crops, including nutrient cycling and availability (Nascente; Stone; Crusciol, 2015), moisture retention (Mendis *et al*., 2022), and enhanced biological activity and diversity (Kim *et al*., 2020), all of which contribute to improving soil quality (Weerasekara *et al*., 2017) and increased productivity of subsequent crops (Peng *et al*., 2024).

Table 7. Plant height and shoot dry mass of common bean sown with or without (0 Mg ha^{-1}) showy rattlebox biomass.

Showy rattlebox biomass (Mg ha^{-1})	Height (cm)	Dry mass (g)
0	13.7	0.47
30	17.7*	0.80*
CV (%)	12.6	16.8

* Indicates differences according to the F-test ($p < 0.05$) between biomass amounts. **Source**: The authors, 2024.

Table 8. Plant height of common bean at different intervals with or without (0 days) showy rattlebox biomass.

Showy rattlebox decomposition (days)	Stature (cm)
0	13.1 b
10	17.2 a
20	16.8 a
CV (%)	12.6

Values followed by the same letter are not different according to Tukey's test ($p < 0.05$). **Source**: The authors, 2024.

The decomposition of showy rattlebox releases epicatechin and resveratrol into the soil (Table 5), while simultaneously reducing catechin levels after initial common bean cultivation (Table 4). Additionally, as decomposition progresses, there is a noticeable decline in common bean emergence and chlorophyll concentrations (Table 6), potentially due to other unidentified chemical compounds. Despite these effects, the presence of showy rattlebox biomass enhances leaf area (Table 6), plant height, and shoot dry mass of common bean (Tables 7 and 8). This suggests that the use of *C. spectabilis* is beneficial for improving certain growth parameters of common bean. However, further field trials are recommended to fully understand these relationships and their implications for crop management under uncontrolled conditions, as well to determine its effect over productivity-related variables. This could lead to more sustainable farming practices and improved crop management strategies, ultimately enhancing agricultural productivity and soil conservation.

4. FINAL CONSIDERATIONS

The decomposition of *C. spectabilis* resulted in the release of epicatechin and resveratrol into the substrate, with concentrations ranging from 23.1 to 35.6 μg kg^{-1} for epicatechin and 2.7 to 6.4 μg kg^{-1} for resveratrol. Notably, the concentration of resveratrol decreased as the decomposition interval increased. Conversely, common bean grown without the biomass of *C.*

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spectabilis had a catechin concentration of 72.3 μg kg^{-1}, which was reduced by 30% in the presence of *C. spectabilis* biomass, with a further decline as decomposition progressed.

Common bean sown immediately after *C. spectabilis* deposition exhibited enhanced emergence, emergence speed index (ESI), and chlorophyll concentration. Despite these benefits, the presence of cover crop biomass, regardless of the decomposition time, consistently led to increased plant height and shoot dry mass.

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