Spring 4-29-2024

Developing a novel assay for quantifying allelopathy tolerance in soybeans to pennycress glucosinolates in a controlled environment

A. Jonaus
Macalester College, ac.jonaus@gmail.com

Developing a novel assay for quantifying allelopathy tolerance in soybeans to pennycress glucosinolates in a controlled environment

Devon Jonaus

An honors thesis submitted to the Biology Department at Macalester College, Saint Paul, Minnesota, USA

Advisors: Lucas Roberts, University of Minnesota;
Mary Heskel, Macalester College

April 28, 2024

ABSTRACT

In a pennycress (*Thlaspi arvense* L.) and soybean (*Glycine max* L.) relay-cropping system, one concern for farmers is the effects of pennycress allelochemicals on soybean growth and yield. Pennycress root exudates are known to contain sinigrin, a glucosinolate which hydrolyzes to form the harmful compound allyl isothiocyanate (AITC). In addition to serving as defense against herbivory, glucosinolates are also known to have allelopathic effects on surrounding plants by possibly affecting traits like germination, biomass accumulation, nutrient uptake, mycorrhizal symbioses, and nitrogen fixation. This is not ideal in a relay cropping system, where the presence of pennycress can decrease soybean yields by nearly half after only 6 to 8 weeks of resource competition. It may be possible to develop soybeans that are more tolerant to this system if researchers can identify the genetic architecture of this allelopathy tolerance. Here I demonstrate the efficacy of using an aqueous solution of sinigrin or AITC to assess juvenile allelopathy tolerance of different soybean genotypes, while also determining phenotypes potentially impacted by glucosinolate presence. I found that biomass accumulation and chlorophyll were significantly negatively affected by treatments, whereas height, internode distances, and developmental stages were not consistently affected. By determining which phenotypes are most affected by treatment, researchers can use this protocol to breed soybeans that are tolerant to these allelochemicals.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

A massive thank you to Lucas Roberts for being my primary advisor on this project. I am so lucky to have had the opportunity to work with him on his Ph.D. research and learn from him. His passion for plant science is incredible and I can only hope to know as much as him one day. I will forever be grateful to him for advocating for me and helping me become a better scientist!

Thank you to Dr. Mary Heskel for being my Macalester thesis advisor and helping me through every step of the process. I am so grateful to her for inspiring me to continue working on this project and helping with all of the little details of writing this.

Thank you also to Dr. Mike Anderson for being a committee member and helping with data analysis. I don't think I could've figured out everything in R without him and his knowledge of statistics!

Dr. Paul Overvoorde deserves a big thank you for agreeing to be part of my thesis committee despite his packed schedule as an administrator. Your perspectives as a plant physiologist were much appreciated and helped balance out the group.

I would like to thank Dr. Aaron Lorenz and the members of the Lorenz Lab who helped me with data collection for this research – it would not have been possible without their help! I have greatly enjoyed working with them and have loved learning more about the many aspects of soybean breeding by spending time with everyone.

Thank you to IPREFER and the USDA-NIFA for making this project possible through funding my summer internship. A huge thank you to the University of Minnesota CFANS for supporting my research this past school year and to Macalester for giving me the opportunity to present my research at the Midstates URS and the ASPB Midwest meeting.

1. INTRODUCTION

A large portion of the American Midwest is colloquially known as the Corn Belt due to the large amount of maize cultivated in the region (CEC 2011). Most commonly, farmers alternate growing corn and soybeans each summer due to the environmental and economic benefits of this rotation as well as its simplicity (USDA 1997). This cropping system allows for herbicide use while providing benefits not present in repeated corn monocultures, such as lowering disease carryover and somewhat reducing the need for nitrogen (N) application through soybean nitrogen fixation (Alberti et al. 2021). However, this "land simplification" for the production of cash crops can also have negative effects on both yield and climate when compared to more diverse crop rotations (Socolar et al. 2021). Tradeoffs include a need for nitrogen application that could eventually lead to runoff and eutrophication (Stanger & Lauer 2008), higher pest pressure and insecticide requirements (Meehan et al. 2011), and steady yield decreases with soil degradation (Bowles et al. 2020). Because of these issues, some farmers are turning to alternative cropping systems like diverse crop rotations and intercropping (Yang et al. 2021).

Intercropping is defined as the process of planting of two or more crops in the same field at the same time (Alberti et al. 2021). This cropping system can provide benefits in addition to greater biodiversity such as increased yields, reduced pests, weed suppression, reduced fertilizer needs, and improved soil health (Moore et al. 2022). However, many choose not to adopt this system because it is considered more labor-intensive (Moore et al. 2022). Within this broader definition of intercropping is relay cropping, a system in which crops are planted at different times and grow together for only part of their seasons (Alberti et al. 2021). Relay cropping can be particularly valuable in systems where it is possible for one crop to grow in a field that would normally be left fallow.

1.1. Pennycress Intercropping

In the past decade, field pennycress (*Thlaspi arvense* L.) and its use as a crop have been the subject of over 1000 research articles (Digital Science 2024). Often considered a weed – it is even colloquially known as 'stinkweed' – pennycress shows promise as a winter annual to be added to low-diversity, high fertilizer summer annual farming systems (Cubins et al. 2019). In addition to reducing excess nutrients from fertilizer (Johnson et al. 2017), providing forage resources for pollinators in early spring (Eberle et al. 2015), reducing weed cover (Johnson et al. 2015), and being a possible food source for humans and animals (Chopra et al. 2020), domesticated pennycress shows strong potential as biodiesel for aviation – a much-needed resource following the recent creation of sustainable aviation fuel goals in the US (Fan et al. 2012).

In the upper Midwest, field pennycress seems to have potential in a soybean (*Glycine max* L.) intercropping system, where pennycress is grown in the winter following maize harvest and relay cropped with soy in the spring (Johnson et al. 2015). The timeline in Figure 1 shows that since Northern states have a shorter summer growing season, soybeans must be planted into existing pennycress prior to senescence. However, many issues could arise with this coexistence of almost mature pennycress with seedling and juvenile soybeans. Those most likely to induce stress include resource competition, increased pest pressure, shading, and allelopathy (Moore et al. 2022). Previous studies indicate that weed pressure during the earliest stages of soybean development has the greatest potential to negatively impact yield, and as pennycress is only a recently domesticated weed one would imagine that this would hold true in this intercropping system (Horvath et al. 2023). Additionally, the early shading caused by the existing pennycress canopy could have effects on plant architecture by causing a decrease in branching (Hussain et al. 2020). As soybean plants with greater branching tend to have higher yields, a decrease in branching would likely negatively affect yield (Carpenter & Board 1997). For this research, the focus will primarily be on the potential effects of pennycress' allelochemicals on early soybean growth.

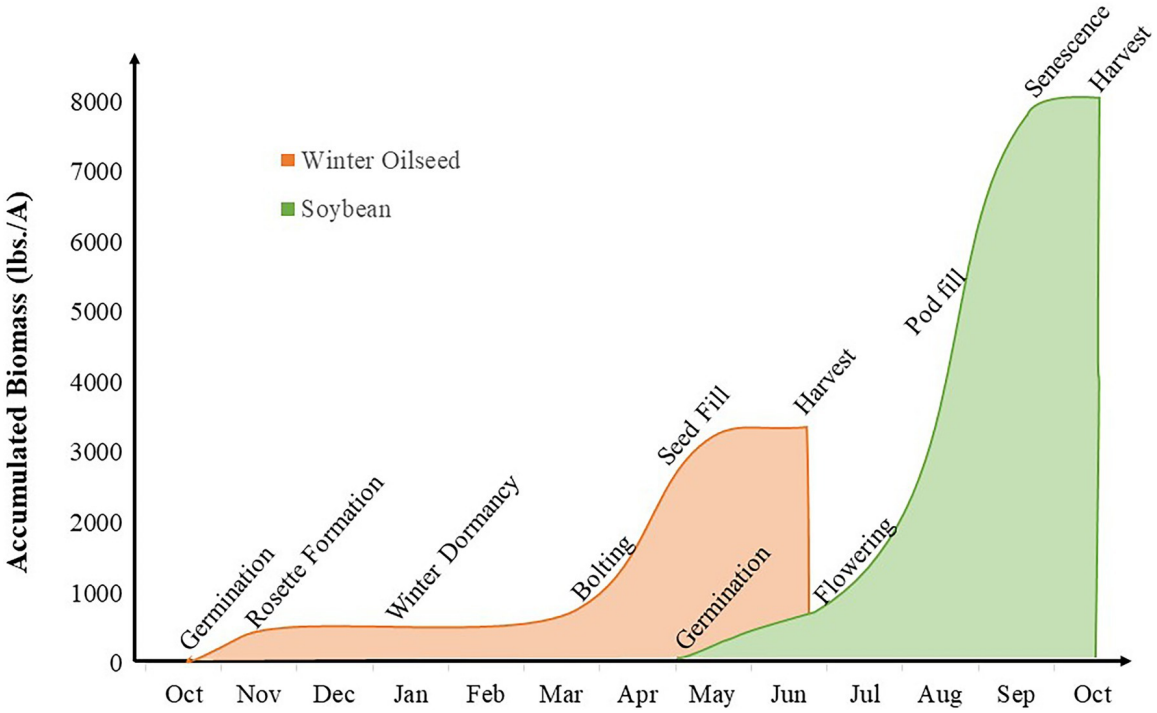

Figure 1: Accumulated biomass in a pennycress-soybean intercropping system. Image from Moore et al. 2022.

1.2. Glucosinolates

The glucosinolate-myrosinase complex, informally called the 'mustard oil bomb', is a plant defense mechanism present in all members of the family *Brassicaceae* whose primary function is to deter herbivory (Lüthy & Matile 1984; Kliebenstein et al. 2005). Minimally harmful compounds known as glucosinolates are stored separately from myrosinases, either in separate organelles of the same cell or in different cells entirely, most often adjacent to myrosinase-expressing phloem cells (Kissen et al. 2008). The two generally only interact when plant tissue is damaged, which occurs with herbivory (Kliebenstein et al. 2005). When no longer separated, myrosinase will hydrolyze glucosinolates into a number of more volatile and harmful compounds in an attempt to deter further damage (Kissen et al. 2008).

Glucosinolates are one class of compounds known to have allelochemical properties. Allelopathy can be defined as any direct or indirect deleterious effect by one plant on another caused by the production and release of chemical compounds referred to as allelochemicals (Rice 1984). The mechanisms of plant allelochemical release most pertinent to this research include root exudation, leaching, volatilization, and biomass decay (Lalljee et al. 1998). These compounds can be taken up by plant roots with water from the soil, transformed by soil microorganisms, decomposed by sunlight, and leached by water from the rhizosphere (Lalljee et al. 1998). Allelochemicals also have the potential to impact germination, biomass accumulation, mineralization of nutrients, production of phytohormones, mycorrhizal symbioses, and nitrogen fixation among other processes (Lalljee et al. 1998).

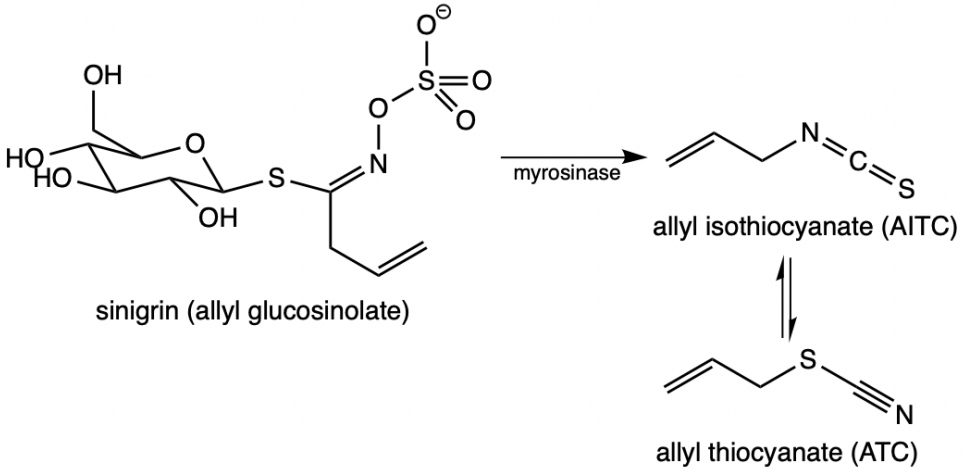

Figure 2: Sinigrin decomposition to allyl isothiocyanate, which can spontaneously convert to allyl thiocyanate.

The only glucosinolate found in pennycress was previously identified as sinigrin (SG), an aliphatic glucosinolate also called allyl glucosinolate shown in Figure 2 (Gmelin & Virtanen 1959). Its most common decomposition products include allyl isothiocyanate (AITC), allyl thiocyanate (ATC), and allylnitrile (Borek et al. 1994; Warwick et al. 2002). Hayes et al. (1999) reported that this process is pH dependent, and above a pH of 4, ATC and AITC are functionally the exclusive products. Since soil pH varies greatly across Minnesota, the University of Minnesota Crops Extension recommends adding lime to soil as needed to increase pH to between 6.0 and 6.5 (Kaiser & Rosen 2023). ATC can spontaneously convert to AITC, so it is the sole hydrolysis product of sinigrin evaluated in my study (Smith and Emerson 1971). Pure AITC, known as mustard oil, can severely irritate skin, eyes, and lungs and has a dermal LD50 of only 88 mg/kg (NCBI 2024). Glucosinolates and isothiocyanates have also been long-recognized for their antifungal, antibacterial, and antinutritional qualities (Fahey et al. 2001). Given this potential danger, it is possible that sinigrin itself imposes no immediate stress upon plants and instead its decomposition products have the strongest effects (Patra 2012).

1.3. Breeding soybeans for allelopathy tolerance

The overall goal of this research is to determine how best to assess soybean allelopathy tolerance for successful soybean breeding. Studies found soybean yield reductions between 12 and 47% when grown in a pennycress relay cropping system, so by determining the main causes of yield reductions in this system plant breeders can prioritize specific goals (Hoerning et al. 2020; Johnson et al. 2017; Moore et al. 2022). Genetic diversity in response to this system has been observed, implying that there may be genes correlated with allelopathy tolerance. By creating a method of assessing allelopathy tolerance, the genetic architecture of allelopathy tolerance can be characterized using a genome-wide association study (GWAS). The objectives of this study are to (i) determine whether sinigrin and/or AITC presence affects juvenile soybean growth and if so, (ii) is there a predictable dose response to these compounds and (iii) how can data from this study be used to screen soybean genotypes in a full GWAS. In this study, I will attempt to answer these questions by dosing plants with several concentrations of either compound weekly and collecting many phenotypes.

2. METHODS

2.1. Soil

Due to the pH dependency of sinigrin hydrolysis products, the pH of Sungro Sunshine Mix #1 soil was experimentally estimated prior to transplanting soybeans. The pH was estimated to be 6.5-7.1, which was a favorable level for this experiment. This pH range can promote hydrolysis of sinigrin to AITC and ATC rather than allyl cyanide or allylnitrile, the more prevalent products at pH levels below 6 (Hayes et al. 1999; Borek et al. 1994). *Bradyrhizobium* and Osmocote slow release fertilizer were also added to the soil to encourage root nodule formation and minimize other factors that could cause stress. Approximately 200 g of soil were then added to each container without compaction.

2.2. Soybean genotypes

Four genotypes of soy were selected for this experiment, specifically PI438381, M13-264055, BS1146, and AG17X8. The first two genotypes listed are relatively low-yield, but M13-264055 has been recently shown to have a stable yield under pennycress intercropping stress (Unpublished data from L. Roberts). M13-264055 is a genotype developed by University of Minnesota Soybean Breeding Program for aphid resistance, whereas PI438381 was shown to have low yields but moderate tolerance to pennycress intercropping. BS1146 is a promising genotype that is not very strongly impacted by the presence of pennycress and still has a relatively average yield. It was created by Brushvale Seed and is a commercial variety for organic systems with soybean cyst nematode (SCN) resistance. On the other hand, AG17X8 was chosen as an elite variety with high yields and high susceptibility to pennycress intercropping stress when grown in the field. This glyphosate and dicamba resistant variety from Asgrow was genetically modified to perform best in a monoculture system with the use of herbicides.

2.3. Experiment setup

In this study, each genotype had four replicates of each treatment, one planted each day. In addition to a control, four sinigrin and four AITC treatments were selected. Previous research from Gimsing et al. (2005) found that surrounding two other plants from the family Brassicaceae, soil glucosinolate concentrations ranged from 0.11 to 21.7 nanomoles per gram of soil. As glucosinolate concentrations in soil from pennycress root exudates have currently not been explored, we decided to look at a somewhat broader range of concentrations between 0.1 and 100 nmol per gram of soil. These concentrations may not be entirely biologically relevant, so further research may be needed to

characterize glucosinolate concentrations given distance from the allelopathic plant and soil permeability. For the purpose of this experiment, these are assumed to be the same for every plant due to consistent variation. The nine treatments should give researchers a better understanding of if the possible effects of sinigrin and its hydrolysis product follow a dose-response relationship. After finding the average mass of soil in fifteen random pots to be 201.3 g, total AITC and sinigrin needed for five weekly treatments were calculated to be 177.4 mg and 643.0 mg. One issue with this procedure is that previous studies indicate the presence of myrosinase in soils even when no glucosinolate-containing plants have grown in that soil (Gimsing & Kirkegaard 2009). This would imply that sinigrin treatments may quickly hydrolyze to AITC, resulting in no differences between the two treatments. As a previously unused soilless growing medium was used for this project, it is unclear whether or not myrosinase was present.

144 total plants were separated into four replicate groups for planting, dosing, and eventually harvesting. Each block is named depending on the day it was planted, with one being planted on the first day and four on the fourth. Plants were grown and treated in a greenhouse after germination with placements randomly selected to minimize impacts of variance in temperature and sunlight. Previous research supports the idea that glucosinolates from pennycress are highly inhibitory to plant germination, and as this study seeks to only examine plant growth, soybeans were not exposed to glucosinolates prior to germination (Vaughn et al. 2006). To dose plants weekly, AITC and sinigrin were greatly diluted in H_2O since individual amounts of the two were too small to individually measure using available equipment. Additionally, AITC is only slightly soluble in water, making it preferable to use small quantities in larger amounts of water (Jiang 2020). First, 0.032 g of sinigrin hydrate and 8.76 µL of AITC were separately added to 1L H_2O and thoroughly mixed. The following doses were then each added to a 500 mL graduated cylinder, which was then filled with DI H_2O to dilute the solution to approximately 230 mL so that all plants were given equal amounts of solution.

Glucosinolate concentration (nmol/g soil)	Solution per dose (L)	Solution per dose (mL)
0.1	0.00023	0.225
1.0	0.00225	2.25
10.0	0.02250	22.5
100.0	0.22502	225

Table 1: Glucosinolate treatment concentrations with the corresponding amount of solution per dose.

Solutions were applied weekly from the top according to each plant's randomly selected treatment. This method, previously used by other researchers, aims to simulate an ongoing release of glucosinolates into soil (Portales-Reyes et al. 2015). All plants including controls were watered from the bottom with DI H_2O as needed throughout the five-week experiment. Timing of developmental stages was also tracked to see if the treatments had any significant effect.

As this experiment was conducted in a greenhouse with no cooling system during summer, a shade cloth was necessary to reduce the amount of heat entering the greenhouse. However, this greatly decreased light availability for experimental plants and led to etiolation. All plants were affected by this shading so comparisons between control and treated plants should still be considered valuable. Temperatures were also higher and relatively consistent inside of the greenhouse, so plants were frequently watered to eliminate drought stress.

2.4. Data collection

After five weeks, plants were harvested in groups of 36 according to which day they were planted. Leaf greenness was evaluated four days prior to termination of the experiment using an atLEAF CHL STD (FT Green LLC, Wilmington, DE). To standardize this process, the youngest open leaf was analyzed whenever possible. This experiment opted to use an atLEAF light transmittance meter rather than a SPAD 502 meter to minimize expenses in chlorophyll estimation. Previous work by Zhu et al. (2012) supports the idea that both provide similarly accurate and precise estimations of leaf chlorophyll content. Prior to biomass measurement, photographs were taken with a ruler for scale for internode length analysis and roots were cleaned to assess whether nodules had developed. Wet biomasses were also measured for preliminary data analysis. Following cleaning, above and belowground biomasses were dried for three days at approximately 40 ºC before re-measuring. Internode distances were analyzed using ImageJ and were also used to estimate height.

2.5. Statistical analysis

This experiment was a factorial Randomized Complete Block Design (RCBD) with four replicates. A three-way analysis of variance (ANOVA) was performed using R Statistical Software (v4.3.3; R Core Team 2022) to determine the effects of treatment, block, and genotype on biomass, chlorophyll concentration estimates, height, development, and internode lengths. Treatment and genotype were treated as fixed effects, whereas block was treated as a random effect. The packages "tidyverse" (v1.3.0; Wickham et al., 2019),

"readr" (v2.1.5; Wickham et al., 2024), and "DT" (v0.33; Xie et al., 2024) were used for data analysis and "ggplot2" (v3.5.0; Wickham, 2016) was used for visualization.

3. RESULTS

3.1. Biomass

As seen in Tables S1 and S2 and Figure 3, treatment ($p < 0.0001$), genotype ($p < 0.0001$), and block ($p < 0.0001$) all significantly affect aboveground wet and dry biomass ($n = 115$). Treatment had a negative effect and genotype effects varied in directionality. As many roots were very small with wet biomasses below 1 g, their dry biomass was measured to be 0 g due to imprecise measurements. Using dry biomass data shown in Table S3, it appears that only treatment ($p < 0.05$) and block ($p < 0.0001$) had any potential significant effect on belowground biomass ($n = 115$). However, looking back at wet biomass in Table 2 shows that treatment ($p < 0.0001$), genotype ($p < 0.0001$), and block ($p < 0.0001$) had significant effects on belowground biomass with significant interactions between treatment and genotype ($p < 0.0001$) and treatment and block ($p < 0.0001$) ($n = 144$). A Tukey HSD test shows that PI438381 was the only significantly different genotype. It generally had a greater biomass, whereas other genotypes were quite similar.

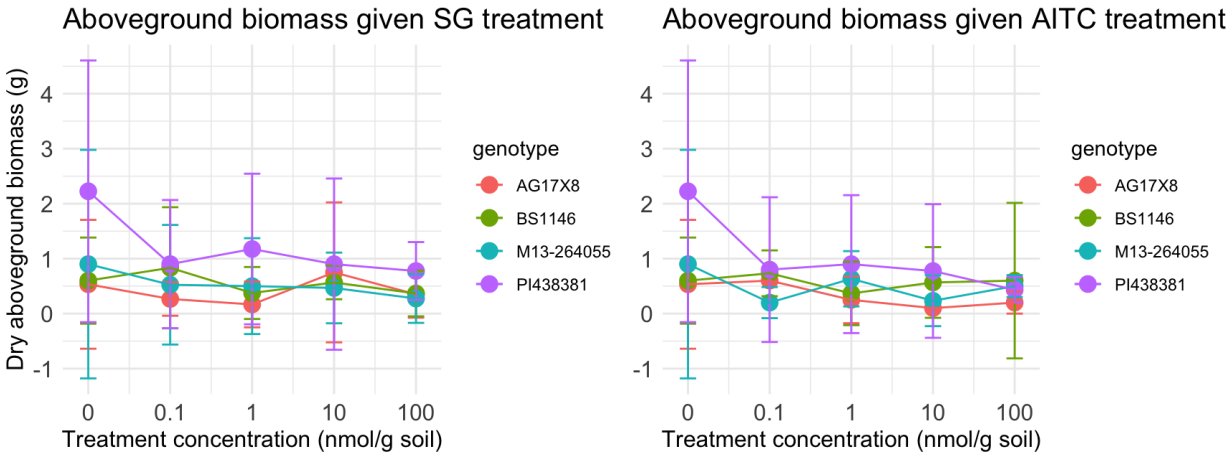

Figure 3: Aboveground biomass as affected by treatment strength, with error bars showing means and standard errors. Both treatments are included in this graph and genotype is shown with color.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	2.525	2.525	0.793	n.s.
Treatment	1	43.707	43.707	13.732	3.17×10^{-4} ***
Genotype	3	130.186	43.395	13.634	9.78×10^{-8} ***
Block	3	236.321	78.774	24.749	1.35×10^{-12} ***
Treatment:Genotype	3	37.685	12.562	3.947	9.98×10^{-3} ***
Treatment:Block	3	49.607	16.536	5.195	2.06×10^{-3} ***
Genotype:Block	9	39.317	4.369	1.373	n.s.
Residuals	123	391.496	3.183		

Table 2: ANOVA table of wet belowground biomass given treatment, genotype, block, and treatment:genotype interaction. *** indicates significance at the 99.9% level, with n.s. indicating no significance.

Root:shoot ratio was also briefly examined, but due to many dry belowground biomass values being zero or near zero, this phenotype was not further investigated.

A severe thrip infestation occurred approximately two weeks into this five week experiment. Pest pressure varied depending on block, with later blocks seeing an increased number of thrips and severity of damage. In addition to spatial variations, this may have contributed to the significant biomass differences between blocks.

3.2. Height

Results shown in Table 3 show that differences in height were most highly correlated with different genotypes ($p < 0.0001$), though block ($p < 0.01$) and treatment ($p < 0.05$) also appear to have had some effects ($n = 144$). Table S4 shows a linear model of height given treatment compound type, treatment concentration, genotype, and block to evaluate effect size.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	6.34	6.34	0.29	n.s.
Treatment	1	131.57	131.57	6.02	0.0156 *
Genotype	3	2927.65	975.88	44.62	1.389×10^{-16} ***
Block	3	282.53	94.18	4.31	0.0063 **
Treatment:Genotype	3	56.82	18.94	0.87	n.s.
Treatment:Block	3	78.88	26.29	1.20	n.s.
Genotype:Block	9	177.47	19.72	0.90	n.s.
Residuals	123	2689.88	21.87		

Table 3: An ANOVA of height given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

Based on the data in Table S4, it seems as though treatment had a general, non-significant negative effect on height. Genotype had the strongest effect on height, though blocks also affected this variable. Tukey's HSD test showed that all genotypes were significantly different from each other except for M13-264055 and BS1146. PI438381 was the tallest, followed by M13-264055, BS1146, and AG17X8.

3.3. Development

As some plants didn't get to later developmental stages, it was determined that the date at which plants reached V3 using the Fehr/Caviness method would be used to compare plants (Wisconsin Soybean Extension Program 2017). This stage can be defined as when a plant has three nodes on the main stem with fully developed leaves, including the first unifoliate leaves (Wisconsin Soybean Extension Program 2017). Table 4 shows that when compared to control plants of the same genotype, treatment plants did not have any significant differences in time to the developmental stage V3 ($p = 0.0739$). Only genotype ($p < 0.0001$) and block ($p = 0.0587$) seemed to significantly impact plant development (n = 111). More specifically, post-hoc analysis showed that PI438381 was

the only genotype where timing of this developmental stage was significantly different as it reached this stage earlier.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	1.700	1.700	0.215	n.s.
Treatment	1	25.816	25.816	3.271	0.0739
Genotype	3	663.289	221.096	28.012	6.94×10^{-13} ***
Block	3	61.014	20.338	2.577	0.0587
Treatment:Genotype	3	7.804	2.601	0.330	n.s.
Treatment:Block	3	4.648	1.549	0.196	n.s.
Genotype:Block	9	74.770	8.308	1.053	n.s.
Residuals	90	710.354	7.893		

Table 4: Days after planting to V3 development stage given treatment, genotype, block, and interactions. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

On the other hand, stage at death was strongly affected by treatment ($p < 0.01$), genotype ($p < 0.0001$), and block ($p < 0.0001$), shown in Table S5 ($n = 111$). As developmental stages over time were impacted by genotype and block, premature plant death could be the main contributor to the significance of treatment. Put more simply, treated plants died earlier and therefore couldn't live to later developmental stages. This is shown in Figure 4.

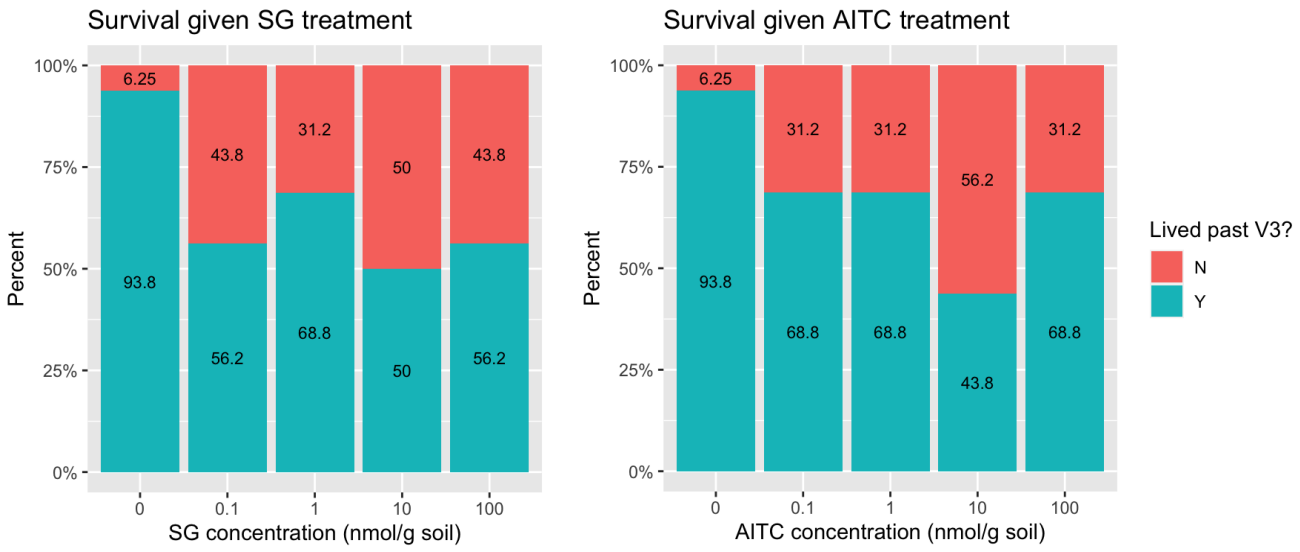

Figure 4: Plant survival given different treatment concentrations. If a plant was at or below development stage V3 when harvested, it likely died prior to harvesting or had damage leading to delays in development.

3.4. Leaf greenness as a proxy for chlorophyll concentration

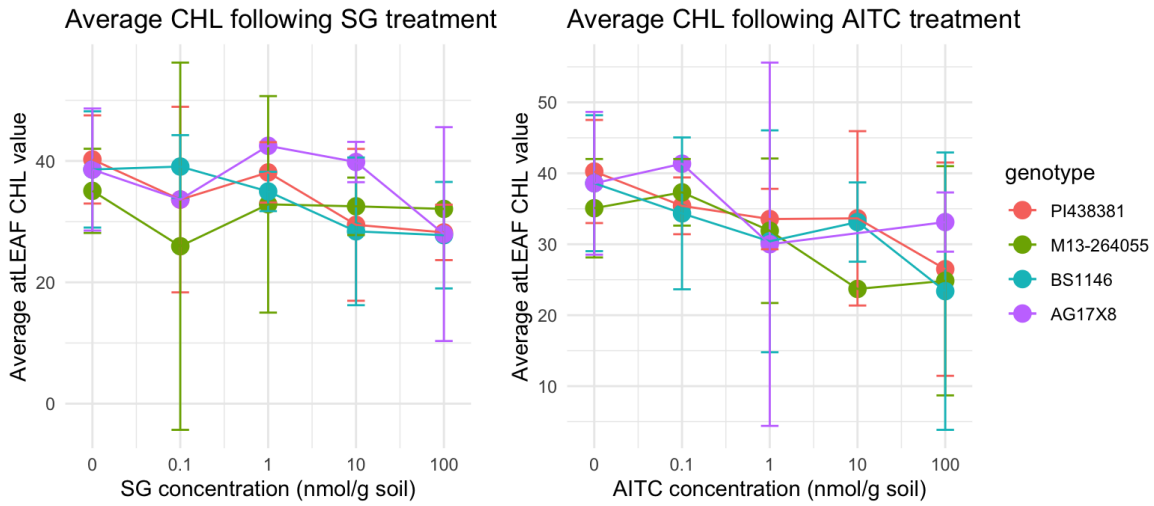

Figure 5: Mean estimated chlorophyll content measurements given all concentrations of both sinigrin and AITC treatments. Genotypes are shown with colors and bars indicate standard errors.

AtLEAF chlorophyll estimates suggest that leaf chlorophyll content is strongly affected by treatment ($p < 0.0001$) and less so by genotype ($p = 0.0427$) as seen in Table S6 ($n = 144$). Chlorophyll estimates within each treatment compound grouped by genotype can be seen in Figure S1. Table 5 shows the effect sizes of each treatment using estimated regression coefficients and standard errors and Figure 5 shows the direct effects of treatment on chlorophyll concentrations. Visually, it was clear that treated plants consistently had more interveinal chlorosis than their untreated counterparts. This is seen in Figure 6, where there is a clear color gradient from control plants to treated plants.

Treatment	**Estimated Coefficient**	**Standard Error**	**t-Statistic**	**Probability**
(Intercept)	38.9314	2.5200	15.4488	$< 2 \times 10^{-16}$ ***
SG	1.5001	3.0923	0.4851	n.s.
Treatment	-1.9090	0.9427	-2.0249	0.046 *
Genotype M13-264055	-4.9664	3.2661	-1.5206	n.s.
Genotype BS1146	-5.2645	3.0934	-1.7018	n.s.
Genotype AG17X8	-1.1373	3.3990	-0.3346	n.s.
Block 2	-4.1937	3.2118	-1.3057	n.s.
Block 3	-1.7032	3.1944	-0.5332	n.s.
Block 4	-0.2654	3.2830	-0.0808	n.s.

Table 5: A linear model of chlorophyll concentrations given each treatment. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

Figure 6: Block 1 AITC treatments. Plants are arranged with controls on the left and treatments increasing by orders of magnitude to the right. Rows are grouped by genotype. A1 = 0.1 nmol/g soil, A2 = 1 nmol/g soil, A3 = 10 nmol/g soil, and A4 = 100 nmol/g soil.

Unsurprisingly, a Dunnett's test revealed that the treatments with the most significantly negative effects were the highest strength sinigrin and AITC treatments. A linear model comparing each individual treatment with control plants is shown in Table 6.

	Estimate	Standard Error	*t* value	Pr (>F)
0.1 nmol/g soil SG – control	-4.393	2.051	-2.142	n.s.
0.1 nmol/g soil AITC – control	-2.099	1.916	-1.095	n.s.
1 nmol/g soil SG – control	-2.011	1.916	-1.050	n.s.
1 nmol/g soil AITC – control	-6.491	1.916	-3.387	0.00764 **
10 nmol/g soil SG – control	-6.403	2.051	-3.122	0.01755 *
10 nmol/g soil AITC – control	-6.050	2.362	-2.561	0.08407
100 nmol/g soil SG – control	-9.751	1.978	-4.928	$< 1 \times 10^{-4}$ ***
100 nmol/g soil AITC – control	-11.951	1.862	-6.419	$< 1 \times 10^{-4}$ ***

Table 6: A Dunnett's test comparing each treatment with controls. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

3.5. Internode lengths

While all internode distances above the cotyledon were analyzed following plant harvest, only the first three were analyzed in-depth to avoid having a smaller dataset. More specifically, the distances measured were from the cotyledon to the unifoliate leaves (first internode), the unifoliate leaves to the first trifoliate leaf (second internode), and the first to the second trifoliate leaf (third internode). Within all four genotypes, a ratio was created to compare the mean internode length of each treatment with the control for that genotype. Table S7 shows that significance of variables changes with each internode. Treatment compound is only a significant factor in the first internode distance ($p = 0.00287$, and genotype is significant for all ($p < 0.0001$). Treatment strength affected the second ($p = 0.0251$) and third ($p = 0.00302$) internode distances. There was some significant interaction between the treatment compound and concentration in the first internode length ($p = 0.0005$) and block did not have a significant effect on any internode length.

While these initial analyses suggest significant differences caused by treatments in the first internode, there is no clear trend or dose response curve. In the first internode, there is some reduction in treatment plants, though not for every treatment in every genotype.

This is seen in Figure S2, where treatments within each genotype fall both above and below a 1:1 treatment to control internode distance ratio in most genotypes. The only possible exception to this would be PI438381, where all treatments had shorter internode distances but no dose response curve.

Between the unifoliate leaves and the first trifoliate leaf, there is somewhat greater consistency within each genotype, shown in Figure 7. AG17X8, M13-264055, and PI438381 have treatment to control internode distance ratios that are consistently below 1, indicating a reduction in second internode distance with glucosinolate treatment.

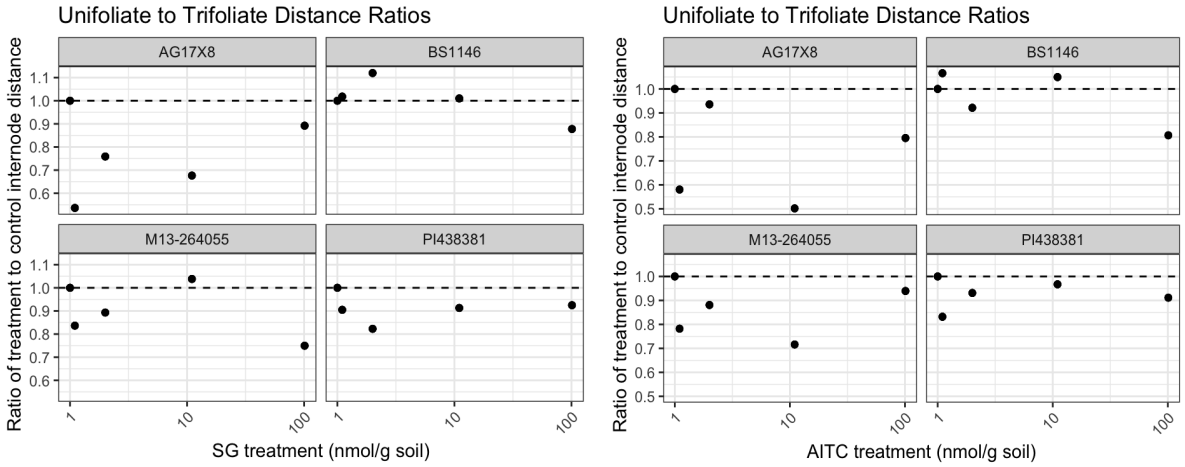

Figure 7: Ratios of the second internode distance in treated plants to the first internode distance in control plants averaged over four blocks. Treatment concentration of both compounds increases to the right, but there is no clear trend of ratio changing with increased treatment.

The third internode distance shows no obvious trend with genotype or treatment, shown in Figure S3. Overall, minimal trends in internode distance are visible across four genotypes.

4. DISCUSSION

4.1 Summary

Overall, the results of this experiment indicate that exposure to sinigrin or its hydrolysis product AITC can have negative effects on juvenile soybean growth. There does seem to be a dose response curve since plants with higher concentrations of sinigrin and AITC had lower aboveground and belowground biomass, greater amounts of interveinal chlorosis, and lower developmental stages at experiment termination.

This research differs from most previous work on pennycress intercropping in that it looks at the effects of allelopathy alone and that it occurs in a greenhouse environment. While omitting other stresses related to intercropping such as shading or resource competition does make it more difficult to look at the bigger picture, it is necessary in order to pick out individual effects of allelopathic compounds to selectively breed for allelopathy tolerance.

Additionally, only juvenile development is examined in this research rather than seed yield or quality. Previous research suggests that in conventional cropping systems, the most critical time period for controlling weeds to prevent soybean yield loss is from germination to approximately the V4 development stage (Van Acker et al. 1993). As plants likely cannot differentiate between intercropping with domesticated pennycress and the presence of weeds, it stands to reason that this period is also the most critical for reducing yield loss related to intercropping stresses. Therefore, soybeans that display juvenile tolerance to various stresses caused by pennycress may have greater yields (Horvath et al. 2023).

4.2 Biomass

Seed yield is generally positively correlated with biomass (biological yield), though harvest yield (seed yield/dry aboveground plant biomass) of mature plants can be a more accurate way of measuring yield improvements (Cui & Yu 2005). My research shows that pennycress allelochemicals reduce juvenile soybean biomass accumulation, so it is possible that juvenile soybean plants with lower biomass will end up with lower yields compared to conventionally cropped plants. Lower soybean yields could result in a profit loss for farmers, though that could be offset by money earned from growing pennycress. Because of this tradeoff, it is possible that this cropping system could increase overall seed yield and be more profitable than a monoculture, despite the soybean yield losses (Johnson et al. 2015). More research is needed to precisely quantify the economic

benefits and drawbacks of switching to this intercropping system from a traditional corn-soybean monoculture rotation.

4.3 Height and internode distance

There were no consistent dose-response trends with height or internode distances in this research, though treated plants were somewhat shorter than control plants. This suggests that changes in height and internode distances seen in field experiments could be due to other factors such as shading stress and competition for light. Previous research has found that soybean height and first internode distance are negatively correlated with yield, so shorter plants with smaller internode distances and more branching should have higher yields (Hussain et al. 2020). This is because only nodes are able to produce pods, so plants with shorter internode distances can have more nodes and therefore more soybeans. Relay cropped soybeans begin growing underneath a pennycress canopy with lower light quality and quantity, so one would expect a shade avoidance response which is increased heights and internode distances (Hussain et al. 2020).

Anecdotal reports from L. Roberts note that soybean plants intercropped with pennycress have more branches than their conventionally grown counterparts, contrary to the etiolation one would expect with sun-deprived plants. This could be partly due to isothiocyanate's role as an auxin antagonist, more specifically as a competitive binder to the major auxin receptor TIR1 (Vik et al. 2018). By strongly antagonizing auxin signaling, ITCs can affect plant growth and defense strategies (Vik et al. 2018). This means that the presence of glucosinolates could potentially affect apical dominance and stimulate the formation of axillary buds, explaining the shorter plants with greater branching seen in fields.

4.4 Development

While biomass accumulation was reduced, the transition between different vegetative growth stages did not appear to be delayed. As previously mentioned, plants within the same genotype and block with a significantly different number of days to reach a certain developmental stage often had other factors affecting development besides treatment. Growth of these plants was most often delayed due to insect damage or damage from handling while still juvenile and fragile. Because of these causes of delayed growth, one could propose that plants with significantly late development when compared to other plants within the same genotype should be excluded from data analysis in future studies. This was not done in this study to keep sample sizes larger.

4.5 Chlorophyll concentration

It was interesting to see that there were reductions in chlorophyll even though sufficient fertilizer was applied three times throughout this experiment. Chlorosis can be caused by many stressors, including nitrogen deficiency, but is most commonly associated with iron deficiency in soybeans when grown in a Minnesota field environment (Kaiser & Naeve 2023). While low chlorophyll levels alone may not cause yield reductions, they do indicate stress that could negatively affect yield (Slattery et al. 2017). When soybeans have iron deficiency chlorosis, farmers tend to see significant reductions in yield (Froehlich & Fehr 1981). It is unclear if the chlorosis seen in this research would have led to yield reductions, but either way it is a valuable phenotype for assessing allelopathy tolerance. Damage from thrips and two-spotted spider mites also caused greater leaf loss, leading to possible errors in chlorophyll estimates.

4.6 Future considerations

Controlled growth experiments in greenhouses provide important insights into the growth of crops and are essential for studying responses to chemical compounds on growth. Translating controlled growth chamber or greenhouse experiments to field growth conditions can entail challenges given the heterogeneity of weather and soil conditions. Drought conditions were observed in nearby fields in the duration of this experiment and had drastic effects on the surrounding crops, but this was not the case for the plants in this study. Additionally, allelochemical concentrations used in this study may be greater than what is observed in field conditions. As this is a relatively unknown component of allelopathy research, it is a valuable screening tool. To enhance the applicability of this type of research, a future question to consider is the concentration of allelochemical root exudates in soil surrounding plants given factors such as distance and soil permeability.

Pest pressure was a surprising element in this study, though it may make the experiment more relevant to the real-world situation it attempts to replicate. Nearby field studies had a greater diversity of pests, but seemed to be less affected than the plants in this study. A cause for this could be the Plant Stress Hypothesis (PSH), a theory which hypothesizes that plants under stress attract more pests (Larsson 1989). While this is not universally observed and highly dependent on the insect's mode of feeding, it may be applicable for sap-sucking insects like thrips (Koricheva et al. 1998). If stress from glucosinolate addition is related to an increased presence of thrips, it would add additional complexity to this study. It is also possible that greater insect attraction due to plant stress is an indicator of lower allelopathy tolerance.

An outstanding question for future research is the effect of pennycress intercropping on soybean root nodules. Root nodules, formed by the bacteria *Bradyrhizobium japonicum*, were originally going to be phenotyped as an additional variable of interest for this experiment, but as they were only found on four plants these measurements were not included in the analysis. As all plants had consistently water-saturated soil to try and combat the high temperatures, it is likely that this anoxic environment combined with consistent fertilization of nitrogen prevented the formation of nodules (Buttery 1986). However, evidence from other studies suggests that glucosinolate presence in soil has a negative effect on soil mycorrhizae and soybean root nodulation (Valetti et al. 2016; Hossain et al. 2015; Portales-Reyes et al. 2015). One recent study found that previous cropping of rapeseed (*Brassica napus* L.) had little to no effect on soybean nodulation but significantly decreased arbuscular mycorrhizal fungi colonization while others comparing different Brassicas and legumes found consistently fewer root nodules on legumes grown in soil with glucosinolates (Valetti et al. 2016; Hossain et al. 2015; Portales-Reyes et al. 2015). Other research looking into effects of glucosinolates on a wide variety of soil bacteria and fungi found that glucosinolate hydrolysis products were highly toxic to many species of symbiotic microbiota, which could lead one to the conclusion that even if root rhizobia could colonize nodules there may not be enough living to do so (Mancini et al. 1997; Tierens et al. 2001). Either way, this lack of nodules could have led to reduced nitrogen, causing leaf chlorosis.

4.7 Implications for future research

Since glucosinolate treatments had significant effects on soybean growth that varied with genotype, one can conclude that the method used to assess allelopathy tolerance in this research could be aligned with a GWAS to uncover the genetic architecture underlying allelopathy tolerance. For efficiency in cost and time, it would make sense to use one high concentration of AITC to screen genotypes instead of using several concentrations as in a dose-response experiment or using sinigrin. For other types of experiments, a lower dose may be more appropriate to ensure biological relevance as it could induce different responses.

In this experiment, the genotype PI438381 consistently was damaged least by glucosinolate addition. The minimally damaging response of PI438381 suggests it could be well-suited for pennycress intercropping due to a relatively high allelochemical tolerance. In practice, genotype PI438381 tends to have extreme lodging, regardless of whether or not it is intercropped with pennycress (Unpublished data from L. Roberts), which results in extremely low yields when compared to other genotypes. Thus, while genotype PI438381 may exhibit high allelopathy tolerance, it would not be a profitable variety in terms of yield. The genotypes BS1146 and M13-264055 have previously done

well in this intercropping system, though they did not seem much more tolerant to the individual allelochemicals (Unpublished data from L. Roberts). There are likely other factors contributing to their success in a relay cropping system. The diversity in responses to allelochemicals and yield shows that while some genotypes may have better yields overall in this system, there are important individual characteristics that could be targeted for improvement through plant breeding.

4.8 Conclusion

Allelopathy is an often-overlooked and complex element of plant-plant interactions that needs to be considered in intercropping systems. Resource competition is the focus of much intercropping research, though it is not the only biotic stressor present. My research shows that glucosinolates found in a pennycress-soybean intercropping system can have negative effects on several soybean phenotypes, impacting potential yield and field success. Biomass, which can often be correlated with yield, was significantly decreased with the addition of sinigrin or AITC. Height and internode lengths were not consistently significantly affected, indicating that differences seen in field studies are likely due to differences in light quality and quantity. Chlorophyll content was significantly negatively affected, indicating stress but not necessarily leading to yield reductions. Vegetative developmental stages were not affected and observing normal growth and development is encouraging given the stress that plants were under. Additionally, my research demonstrates that weekly application of an allelopathic aqueous solution can be an effective approach for screening diverse germplasm for allelopathy tolerance. Further investigation of stresses besides competition is also needed to understand how this stress interacts with other stresses like drought or pest pressure. In summary, presence of an aqueous solution of sinigrin or allyl isothiocyanate negatively affected juvenile soybean growth by decreasing biomass and chlorophyll content but had no direct effects on height, internode distances, or developmental stage. Pennycress allelopathy likely has negative effects on soybean early growth, and therefore should be further investigated to develop varieties that are tolerant to this system.

LITERATURE CITED

Alberti, P., Becker, T., Jones, J., & Johanning, N. (2021). *Cropping Systems and Alternative Crops*.

Borek, V., Morra, M. J., Brown, P. D., & McCaffrey, J. P. (1994). Allelochemicals Produced during Sinigrin Decomposition in Soil. *Journal of Agricultural and Food Chemistry*, *42*(4), 1030–1034. https://doi.org/10.1021/jf00040a037

Bowles, T. M., Mooshammer, M., Socolar, Y., Calderón, F., Cavigelli, M. A., Culman, S. W., Deen, W., Drury, C. F., Garcia y Garcia, A., Gaudin, A. C. M., Harkcom, W. S., Lehman, R. M., Osborne, S. L., Robertson, G. P., Salerno, J., Schmer, M. R., Strock, J., & Grandy, A. S. (2020). Long-Term Evidence Shows that Crop-Rotation Diversification Increases Agricultural Resilience to Adverse Growing Conditions in North America. *One Earth*, *2*(3), 284–293. https://doi.org/10.1016/j.oneear.2020.02.007

Buttery, B. R. (1987). SOME EFFECTS OF WATERLOGGING AND SUPPLY OF COMBINED NITROGEN ON SOYBEAN GROWTH. *Canadian Journal of Plant Science*, *67*(1), 69–77. https://doi.org/10.4141/cjps87-008

Carpenter, A. C., & Board, J. E. (1997). Branch Yield Components Controlling Soybean Yield Stability across Plant Populations. *Crop Science*, *37*(3), 885-891. https://doi.org/10.2135/cropsci1997.0011183X003700030031x

Chopra, R., Johnson, E. B., Emenecker, R., Cahoon, E. B., Lyons, J., Kliebenstein, D. J., Daniels, E., Dorn, K. M., Esfahanian, M., Folstad, N., Frels, K., McGinn, M., Ott, M., Gallaher, C., Altendorf, K., Berroyer, A., Ismail, B., Anderson, J. A., Wyse, D. L., … David Marks, M. (2020). Identification and stacking of

crucial traits required for the domestication of pennycress. *Nature Food*, *1*(1), 84–91. https://doi.org/10.1038/s43016-019-0007-z

Cubins, J. A., Wells, M. S., Frels, K., Ott, M. A., Forcella, F., Johnson, G. A., Walia, M. K., Becker, R. L., & Gesch, R. W. (2019). Management of pennycress as a winter annual cash cover crop. A review. *Agronomy for Sustainable Development*, *39*(5), 46. https://doi.org/10.1007/s13593-019-0592-0

Cui, S. Y., & Yu, D. Y. (2005). Estimates of relative contribution of biomass, harvest index and yield components to soybean yield improvements in China. *Plant Breeding*, *124*(5), 473–476. https://doi.org/10.1111/j.1439-0523.2005.01112.x

Digital Science. (2018-) Dimensions [Software] available from https://app.dimensions.ai. Accessed on March 30, 2024.

Eberle, C. A., Thom, M. D., Nemec, K. T., Forcella, F., Lundgren, J. G., Gesch, R. W., Riedell, W. E., Papiernik, S. K., Wagner, A., Peterson, D. H., & Eklund, J. J. (2015). Using pennycress, camelina, and canola cash cover crops to provision pollinators. *Industrial Crops and Products*, *75*, 20–25. https://doi.org/10.1016/j.indcrop.2015.06.026

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, *56*(1), 5–51. https://doi.org/10.1016/S0031-9422(00)00316-2

Fan, J., Shonnard, D. R., Kalnes, T. N., Johnsen, P. B., & Rao, S. (2013). A life cycle assessment of pennycress (Thlaspi arvense L.) -derived jet fuel and diesel.

Biomass and Bioenergy, *55*, 87–100. https://doi.org/10.1016/j.biombioe.2012.12.040

Gimsing, A. L., & Kirkegaard, J. A. (2009). Glucosinolates and biofumigation: Fate of glucosinolates and their hydrolysis products in soil. *Phytochemistry Reviews*, *8*(1), 299–310. https://doi.org/10.1007/s11101-008-9105-5

Gimsing, A. L., Kirkegaard, J. A., & Bruun Hansen, H. C. (2005). Extraction and Determination of Glucosinolates from Soil. *Journal of Agricultural and Food Chemistry*, *53*(25), 9663–9667. https://doi.org/10.1021/jf051812n

Gmelin, R., Virtanen, A. I., Eriksson, S., Hedén, C.-G., Malmgren, B., & Palmstierna, H. (1959). A New Type of Enzymatic Cleavage of Mustard Oil Glucosides. Formation of Allylthiocyanate in Thlaspi arvense L. and Benzylthiocyanate in Lepidium ruderale L. and Lepidium Sativum L. *Acta Chemica Scandinavica*, *13*, 1474–1475. https://doi.org/10.3891/acta.chem.scand.13-1474

Hayes, J., Kelleher, M., & Eggleston, I. (1999). *Anticarcinogenic effects of glucosinolate breakdown products*.

Hoerning, C., Wells, M. S., Gesch, R., Forcella, F., & Wyse, D. (2020). Yield tradeoffs and weed suppression in a winter annual oilseed relay-cropping system. *Agronomy Journal*, *112*(4), 2485–2495. https://doi.org/10.1002/agj2.20160

Horvath, D. P., Clay, S. A., Swanton, C. J., Anderson, J. V., & Chao, W. S. (2023). Weed-induced crop yield loss: A new paradigm and new challenges. *Trends in Plant Science*, *28*(5), 567–582. https://doi.org/10.1016/j.tplants.2022.12.014

Hossain, S., Bergkvist, G., Glinwood, R., Berglund, K., Mårtensson, A., Hallin, S., & Persson, P. (2015). Brassicaceae cover crops reduce Aphanomyces pea root rot without suppressing genetic potential of microbial nitrogen cycling. *Plant and Soil*, *392*(1), 227–238. https://doi.org/10.1007/s11104-015-2456-y

Hussain, S., Pang, T., Iqbal, N., Shafiq, I., Skalicky, M., Brestic, M., Safdar, M., Mumtaz, M., Ahmad, A., Asghar, M., Raza, A., Allakhverdiev, S., Wang, Y., Wang, X., Yang, F., Yong, T., Weiguo, L., & Yang, W. (2020). Acclimation strategy and plasticity of different soybean genotypes in intercropping. *Functional Plant Biology*, *47*. https://doi.org/10.1071/FP19161

Johnson, G. A., Kantar, M. B., Betts, K. J., & Wyse, D. L. (2015). Field Pennycress Production and Weed Control in a Double Crop System with Soybean in Minnesota. *Agronomy Journal*, *107*(2), 532–540. https://doi.org/10.2134/agronj14.0292

Johnson, G. A., Wells, M. S., Anderson, K., Gesch, R. W., Forcella, F., & Wyse, D. L. (2017). Yield Tradeoffs and Nitrogen between Pennycress, Camelina, and Soybean in Relay- and Double-Crop Systems. *Agronomy Journal*, *109*(5), 2128–2135. https://doi.org/10.2134/agronj2017.02.0065

Kaiser, D. E., & Naeve, S. L. (2023). *Managing iron deficiency chlorosis in soybean*. https://extension.umn.edu/crop-specific-needs/managing-iron-deficiency-chlorosis-soybean

Kaiser, D. E., & Rosen, C. J. (2023). *Lime needs in Minnesota*. University of Minnesota Extension. https://extension.umn.edu/liming/lime-needs-minnesota

Kissen, R., Rossiter, J. T., & Bones, A. M. (2008). The 'mustard oil bomb': Not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry Reviews*, *8*(1), 69–86. https://doi.org/10.1007/s11101-008-9109-1

Kliebenstein, D. J., Kroymann, J., & Mitchell-Olds, T. (2005). The glucosinolate–myrosinase system in an ecological and evolutionary context. *Current Opinion in Plant Biology*, *8*(3), 264–271. https://doi.org/10.1016/j.pbi.2005.03.002

Koricheva, J., Larsson, S., & Haukioja, E. (1998). Insect Performance on Experimentally Stressed Woody Plants: A Meta-Analysis. *Annual Review of Entomology*, *43*(1), 195–216. https://doi.org/10.1146/annurev.ento.43.1.195

Lalljee, B., & Facknath, S. (2000). Allelopathic interactions in soil. In S. S. Narwal, R. E. Hoagland, R. H. Dilday, & M. J. Reigosa (Eds.), *Allelopathy in Ecological Agriculture and Forestry: Proceedings of the III International Congress on Allelopathy in Ecological Agriculture and Forestry, Dharwad, India, 18–21 August 1998* (pp. 47–58). Springer Netherlands. https://doi.org/10.1007/978-94-011-4173-4_4

Larsson, S. (1989). Stressful Times for the Plant Stress: Insect Performance Hypothesis. *Oikos*, *56*(2), 277–283. https://doi.org/10.2307/3565348

Lüthy, B., & Matile, P. (1984). The mustard oil bomb: Rectified analysis of the subcellular organisation of the myrosinase system. *Biochemie Und Physiologie Der Pflanzen*, *179*(1–2), 5–12. https://doi.org/10.1016/S0015-3796(84)80059-1

Meehan, T. D., Werling, B. P., Landis, D. A., & Gratton, C. (2011). Agricultural landscape simplification and insecticide use in the Midwestern United States.

Proceedings of the National Academy of Sciences, *108*(28), 11500–11505. https://doi.org/10.1073/pnas.1100751108

Moore, V. M., Schlautman, B., Fei, S., Roberts, L. M., Wolfe, M., Ryan, M. R., Wells, S., & Lorenz, A. J. (2022). Plant Breeding for Intercropping in Temperate Field Crop Systems: A Review. *Frontiers in Plant Science*, *13*, 843065. https://doi.org/10.3389/fpls.2022.843065

National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 5971, Allyl isothiocyanate. Retrieved March 30, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Allyl-isothiocyanate.

Patra, A. K. (2012). An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. *Dietary Phytochemicals and Microbes*, 1–32. https://doi.org/10.1007/978-94-007-3926-0_1

Portales-Reyes, C., Van Doornik, T., Schultheis, E. H., & Suwa, T. (2015). A novel impact of a novel weapon: Allelochemicals in Alliaria petiolata disrupt the legume-rhizobia mutualism. *Biological Invasions*, *17*(9), 2779–2791. https://doi.org/10.1007/s10530-015-0913-4

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rice, E. L. (1984). 1—Introduction. In E. L. Rice (Ed.), *Allelopathy (Second Edition)* (pp. 1–7). Academic Press. https://doi.org/10.1016/B978-0-08-092539-4.50005-8

Smith, P. A. S., & Emerson, D. W. (1960). The Isomerization of Alkyl Thiocyanates to Isothiocyanates. *Journal of the American Chemical Society*, *82*(12), 3076–3082. https://doi.org/10.1021/ja01497a025

Stanger, T. F., & Lauer, J. G. (2008). Corn Grain Yield Response to Crop Rotation and Nitrogen over 35 Years. *Agronomy Journal*, *100*(3), AGJ2AGRONJ20070280. https://doi.org/10.2134/agronj2007.0280

Tierens, K. F. M.-J., Thomma, B. P. H. J., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., Mauch-Mani, B., Cammue, B. P. A., & Broekaert, W. F. (2001). Study of the Role of Antimicrobial Glucosinolate-Derived Isothiocyanates in Resistance of Arabidopsis to Microbial Pathogens. *Plant Physiology*, *125*(4), 1688–1699.

Valetti, L., Iriarte, L., & Fabra, A. (2016). Effect of previous cropping of rapeseed (Brassica napus L.) on soybean (Glycine max) root mycorrhization, nodulation, and plant growth. *European Journal of Soil Biology*, *76*, 103–106. https://doi.org/10.1016/j.ejsobi.2016.08.005

Vaughn, S. F., Palmquist, D. E., Duval, S. M., & Berhow, M. A. (2006). Herbicidal activity of glucosinolate-containing seedmeals. *Weed Science*, *54*(4), 743–748. https://doi.org/10.1614/WS-06-007R.1

Vik, D., Mitarai, N., Wulff, N., Halkier, B. A., & Burow, M. (2018). Dynamic Modeling of Indole Glucosinolate Hydrolysis and Its Impact on Auxin Signaling. *Frontiers in Plant Science*, *9*, 550. https://doi.org/10.3389/fpls.2018.00550

Warwick, S. I., Francis, A., & Susko, D. J. (2002). The biology of Canadian weeds. 9. *Thlaspi arvense* L. (updated). *Canadian Journal of Plant Science*, *82*(4), 803–823. https://doi.org/10.4141/P01-159

Wisconsin Soybean Extension Program. (2017). *A Visual Guide to Soybean Growth Stages*. Cool Bean. https://coolbean.info/library/documents/2017_Soybean_GrowthDev_Guide_FINAL.pdf

Yang, H., Zhang, W., & Li, L. (2021). INTERCROPPING: FEED MORE PEOPLE AND BUILD MORE SUSTAINABLE AGROECOSYSTEMS. *Frontiers of Agricultural Science and Engineering*, *8*(3), Article 3. https://doi.org/10.15302/J-FASE-2021398

Zhu, J., Tremblay, N., & Liang, Y. (2012). Comparing SPAD and atLEAF values for chlorophyll assessment in crop species. *Canadian Journal of Soil Science*, *92*(4), 645–648. https://doi.org/10.4141/cjss2011-100

SUPPLEMENTAL FIGURES

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	0.075	0.075	0.338	0.56217
Treatment	1	3.048	3.048	13.824	3.42×10^{-4} ***
Genotype	3	11.796	3.932	17.837	3.16×10^{-9} ***
Block	3	15.771	5.257	23.847	1.56×10^{-11} ***
Treatment:Genotype	3	1.229	0.410	1.859	0.14202
Treatment:Block	3	2.179	0.726	3.294	0.02396 *
Genotype:Block	9	2.972	0.330	1.498	0.16011
Residuals	93	20.502	0.220		

Table S1: An ANOVA of dry aboveground biomass given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	4.2	4.22	0.875	n.s.
Treatment	1	81.1	81.07	16.798	7.49×10^{-5} ***
Genotype	3	389.7	129.90	26.918	1.88×10^{-13} ***
Block	3	437.6	145.86	30.224	1.04×10^{-14} ***
Treatment:Genotype	3	47.8	15.93	3.300	0.0227 *
Treatment:Block	3	56.5	18.82	3.899	0.0106 *
Genotype:Block	9	73.2	8.13	1.684	n.s.
Residuals	123	593.6	4.83		

Table S2: An ANOVA of wet aboveground biomass given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	0.053	0.0532	0.982	n.s.
Treatment	1	0.294	0.2939	5.421	0.0221 *
Genotype	3	0.319	0.1062	1.958	n.s.
Block	3	1.382	0.4607	8.497	4.78×10^{-5} ***
Treatment:Genotype	3	0.064	0.0213	0.392	n.s.
Treatment:Block	3	0.507	0.1688	3.114	0.0300 *
Genotype:Block	9	0.312	0.0346	0.638	n.s.
Residuals	93	5.042	0.0542		

Table S3: An ANOVA of dry belowground biomass given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

Treatment	Estimated Coefficient	Standard Error	t-Statistic	Probability
Intercept	18.45351	2.15859	8.549	4.06×10^{-14} ***
SG	0.44088	2.61892	0.168	n.s.
Treatment	-0.01960	0.78908	-0.025	n.s.
Genotype BS1146	0.28196	2.68321	0.105	n.s.
Genotype M13-264055	1.84782	2.68321	0.689	n.s.
Genotype PI438381	12.00410	2.68321	4.474	1.73×10^{-5} ***
Block 2	-6.16662	2.68321	-2.298	0.0232 *
Block 3	-6.45094	2.68321	-2.404	0.0177 *
Block 4	-5.42222	2.68321	-2.021	0.0455 *

Table S4: Coefficients from a linear model of height given treatment, genotype, and block. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	0.26	0.263	0.379	n.s.
Treatment	1	5.75	5.755	8.295	0.00497 **
Genotype	3	42.26	14.085	20.305	3.89×10^{-10} ***
Block	3	22.34	7.445	10.733	4.25×10^{-6} ***
Treatment:Genotype	3	1.76	0.587	0.847	n.s.
Residuals	90	62.43	0.694		

Table S5: Developmental stage at death given treatment, genotype, block, and interactions. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Treatment compound	1	27.2	27.2	0.946	n.s.
Treatment	1	1577.5	1577.5	54.846	1.04×10^{-10} ***
Genotype	3	245.4	81.8	2.844	0.0427
Block	3	153.0	51.0	1.773	n.s.
Treatment:Genotype	3	73.7	24.6	0.854	n.s.
Treatment:Block	3	130.9	43.6	1.517	n.s.
Genotype:Block	9	523.0	58.1	2.020	0.0471 *
Residuals	82	2358.5	28.8		

Table S6: An ANOVA of atLEAF chlorophyll estimates given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

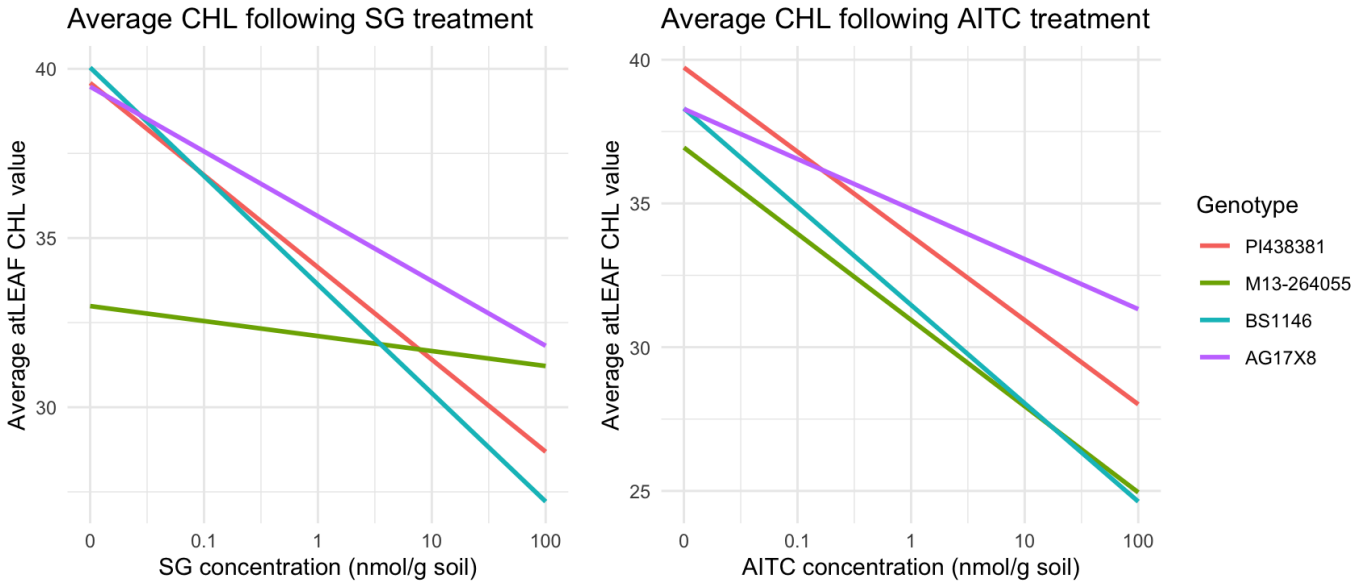

Figure S1: atLEAF chlorophyll estimates grouped by treatment compound and genotype. Standard error is not shown.

		Df	Sum Sq.	Mean Sq.	F value	Pr (>F)
Cotyledon to first unifoliate	Treatment compound	1	0.0624	0.06241	9.253	0.002874 **
	Treatment	1	0.0099	0.00994	1.473	n.s.
	Genotype	3	0.4732	0.15772	23.384	4.81×10^{-12} ***
	Block	3	0.0000	0.00000	0.000	n.s.
	Treatment compound:Treatment	1	0.0862	0.08621	12.781	0.000501 ***
	Residuals	123	0.8296	0.00674		
First unifoliate to first trifoliate	Treatment compound	1	0.0126	0.0126	0.821	n.s.
	Treatment	1	0.0791	0.0791	5.143	0.0251 *
	Genotype	3	1.0130	0.3377	21.944	1.9×10^{-11} ***
	Block	3	0.0000	0.0000	0.000	n.s.
	Residuals	123	1.8928	0.0154		
First trifoliate to second trifoliate	Treatment compound	1	0.1335	0.1335	1.5909	n.s.
	Treatment	1	0.7684	0.7684	9.1538	0.00302 ***
	Genotype	3	5.0052	1.6684	19.8743	1.44×10^{-10} ***
	Block	3	0.0000	0.0000	0.0000	n.s.
	Treatment compound:Treatment	3	0.6761	0.2254	2.6845	0.04961 *
	Treatment:Genotype	3	0.8272	0.2757	3.2847	0.02315 *

	Treatment:Block	3	0.0000	0.0000	0.0000	n.s.
	Genotype:Block	9	0.0000	0.0000	0.0000	n.s.
	Treatment compound:Treatment: Genotype	3	1.2531	0.4177	4.9759	0.00271 **
	Residuals	123	10.3256	0.0839		

Table S7: ANOVAs of internode distances given treatment, genotype, block, and interaction terms. *, **, and *** indicate significance at the 95%, 99%, and 99.9% levels respectively, with n.s. indicating no significance.

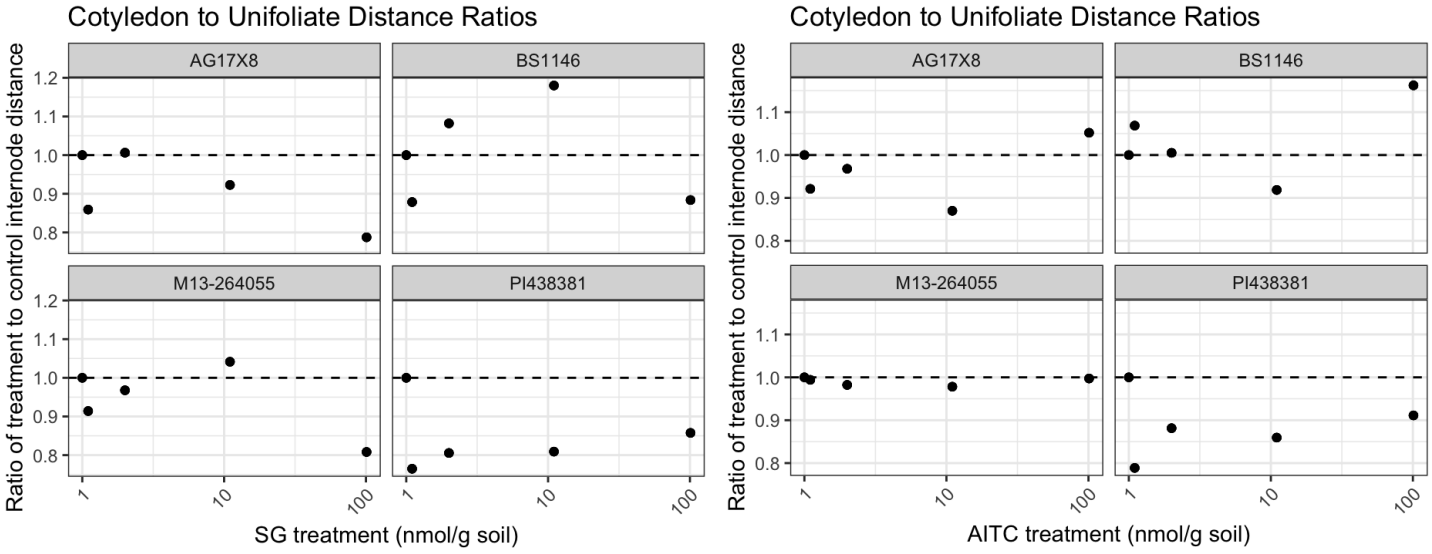

Figure S2: Ratios comparing the first internode distance of treatment plants to control plants. Values are averaged for each treatment within each genotype.

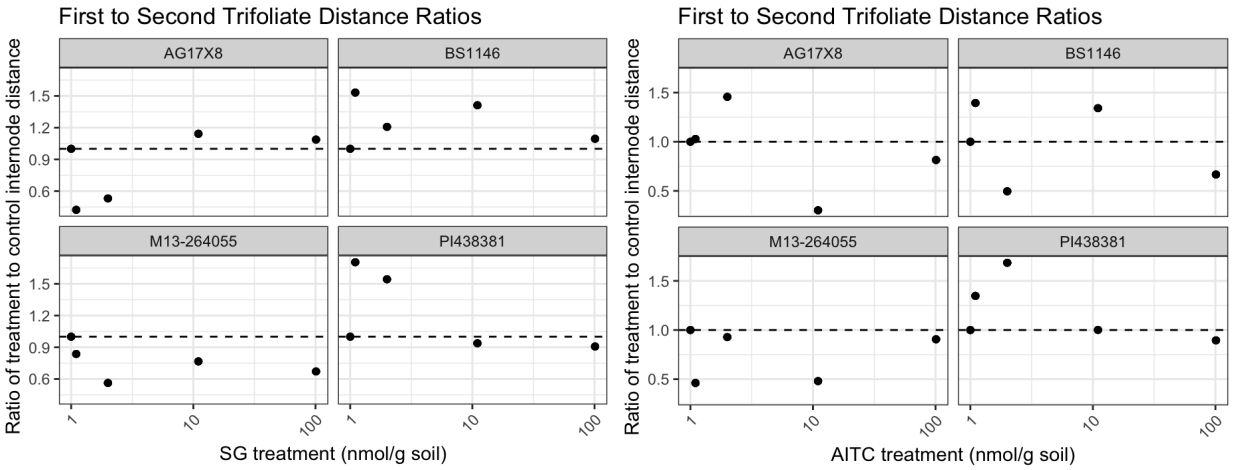

Figure S3: Ratios comparing the third internode distance of treatment plants to control plants. Values are averaged for each treatment within each genotype.