Allelopathic effects of kudzu (Pueraria montana) on seed germination and their potential use as a natural herbicide

Shreya Mathur, Dr. Sunil Mathur

Oxford High School, Oxford, MS

Summary

Allelopathy is the inhibition of growth of one species of plants by chemicals produced by another, typically competing, species. The chemical compounds released in this phenomenon have the potential to be used as natural weed control agents. In this study, we tested kudzu (Pueraria montana) to determine its allelopathic potential for weed control and suppression. The aqueous extracts of different kudzu organs (leaf, stem, and root) were assessed for allelopathic properties on the seeds of several competing species: white clover, dandelion, bermudagrass, and ryegrass. Remarkably, both the kudzu leaf and root extracts significantly changed the majority of the measured germination indices, comprising total germination and the speed of germination. The total germination of white clover seeds was 25% less than the control in both the kudzu leaf and root extract bioassays, while speed of germination was reduced by 77% and 72%, respectively. Likewise, dandelion seeds demonstrated 53% and 73% reduced speed of germination when treated with kudzu leaf extract and root extract, respectively. Both leaf extract and root extract also reduced total germination in ryegrass and bermudagrass seeds by a minimum of 25%. These results suggest that the kudzu leaves and roots can be considered to be allelopathic and show potential as a weed control agent.

Introduction

Allelopathy, the inhibition of growth in one species of plants by chemicals produced by another species, has been used in the past to explain how some invasive species outcompete native ones (1). In this phenomenon, the allelopathic species will release phenolic compounds into the soil in order to reduce the population of other plant species that are directly competing for similar resources (2). Phenolic compounds, chemical compounds which contain a hydroxyl group directly bonded to an aromatic hydrocarbon group, are known to act as a plant's natural defense in preventing herbivory, and are currently the accepted mechanism for allelopathic inhibition (2). These phenolic compounds have acidic properties and can alter the pH of the surrounding soil or water to inhibit the growth of competing species. Although many studies on the allelopathic potential of other plants have been published, neither the allelopathy of Pueraria montana, or kudzu nor its potential phenolic compounds have been identified or examined for the specific purpose of weed control research (3,4,5).

Kudzu is prolific in the Southeastern United States, where it is classified as a noxious weed. Kudzu can survive in a wide variety of habitats but is most abundant in undisturbed open areas. We observed that few plant species coexist with kudzu in nature. Those that do coexist are usually found in small amounts. Therefore, we hypothesized that phenolic compounds released by kudzu plants cause allelopathic interference amongst its competitors. We rationalized that if the kudzu plants were indeed allelopathic, then their phenolic compounds must be specialized to inhibit the growth of competing species: white clovers, dandelions, ryegrass, and bermudagrass.

To test this hypothesis, we organized this study around two main objectives. First, we sought to determine whether kudzu was allelopathic by testing the effects of various kudzu-organ extracts on ryegrass, a competitor species, under laboratory conditions (4,6,7). Furthermore, to determine if kudzu extracts could potentially be used as a weed killer in a bermudagrass lawn, we tested the effects of the different kudzu extracts on white clover, dandelion, and bermudagrass. Since allelochemicals typically reduce competition by preventing the germination of the seeds of other species (2), we decided to test kudzu allelopathy on the seeds of these four plant species.

After conducting the experiment, we found that the extracts of kudzu leaves and roots had allelopathic effects on all species tested, suggesting that these parts of the kudzu vine could potentially be involved in the plant's allelopathic strategy.

Results

We calculated and compared the speed of germination and total germination of each seed and extract combination in order to interpret the data collected during the experiment (Figure 1).

Germination Index	Formula	Reference
Total Germination (TG)	$\mathrm{TG} = \frac{N_T \times 100}{N}$, where N_T is the number of germinated seeds found for the last time measurement. N is the number of seeds used in the bioassay.	Anjum and Bajwa 2005
Speed of Germination (S)	$S = (N_1 \times 1) + \frac{1}{2}(N_2 - N_1) + \frac{1}{3}(N_3 - N_2) + \cdots + \frac{1}{n}(N_n - N_{n-1})$, where $N_1, N_2, N_3, \ldots N_{n-1}, N_n$ is the number of germinated seeds obtained on the first (1), second (2), third (3),…,$(n-1)$, and (n) days.	Anjum and Bajwa 2005

Figure 1: Formulas that were used to calculate germination indices. The first formula, total germination, was used to calculate percentage of seeds which germinated over the course of experimentation. The second formula, speed of germination, permits calculation of a weighted sum of the number of germinated seeds observed each day.

The total germination and the speed of germination are compared across all seed types in Figures 2 and 3.

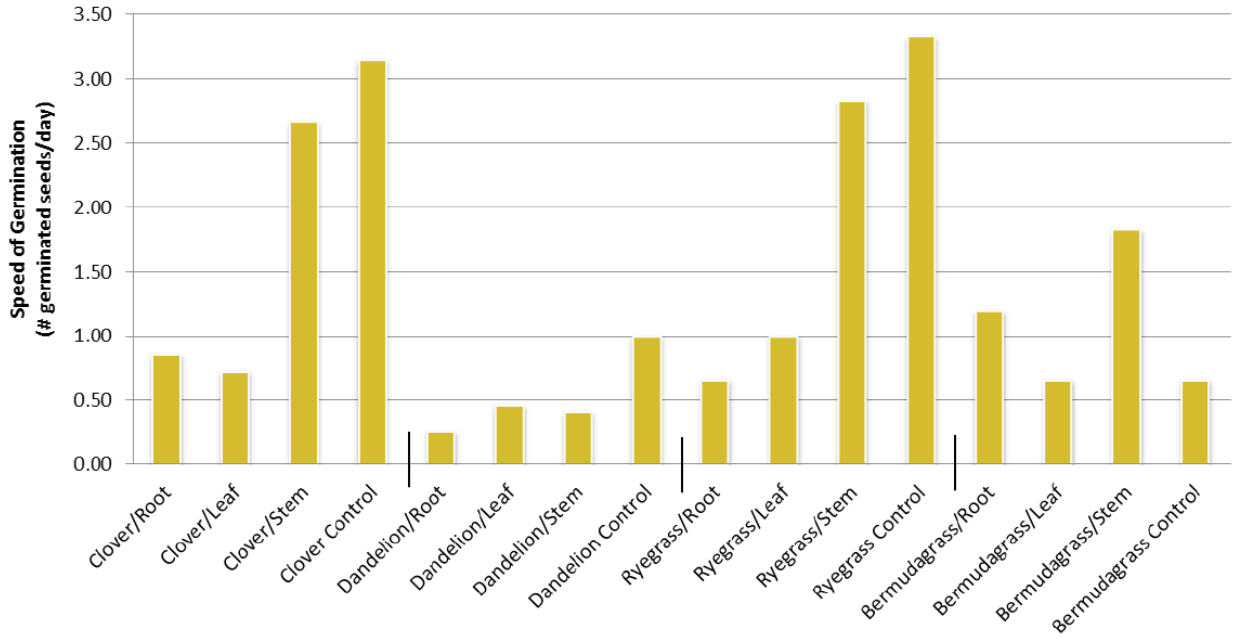

Figure 2. Speed of germination of the samples across all seed types. The X-axis lists each sample type, with the first word being the sample seed type and the second word being the kudzu extract type. Note that comparison between seed types would be inaccurate as some seed types inherently germinate more slowly than others.

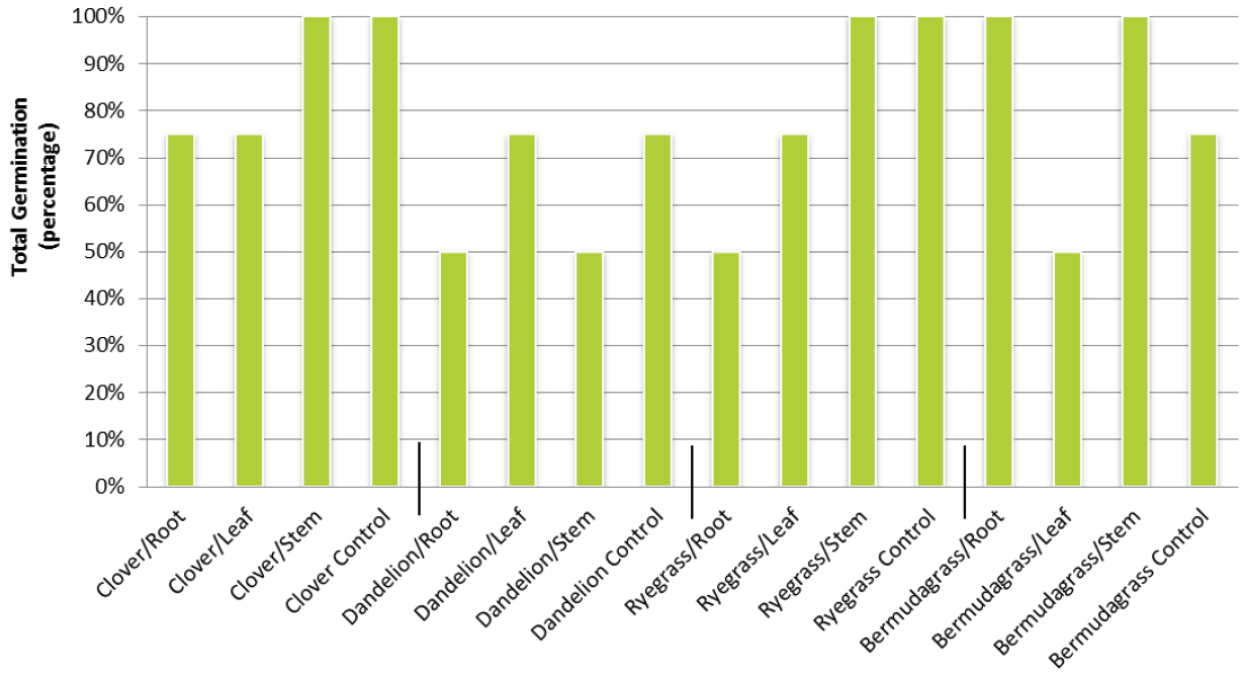

Figure 3. Total germination of the samples across all seed types. The X-axis lists each sample type, with the first word being the sample seed type and the second word being the kudzu extract type.

We found that the root and leaf extracts reduced the total germination (TG) of the white clover seeds by 25% as compared to the control (Figure 3). However, the stem extract did not reduce the total germination of the white clover seeds (Figure 3). The root extract reduced the speed of germination (S) of the white clover seeds by ~72%, the leaf extract reduced S in white clover seeds by ~77%, and in the stem extract condition, reduced S in white clover seeds and by ~15% in the leaf extract condition, comparative to the control (Figure 2).

Both the root and stem extracts reduced the TG of the dandelion seeds by 50% (Figure 3). The leaf extract reduced TG in dandelion seeds by only 25% (Figure 3). The speed of germination showed a 73% reduction by root extract, 53% reduction by leaf extract, and 58% reduction by stem extract, compared to the control condition (Figure 3).

In the ryegrass seeds, the TG by 50% and 25% in root extract and leaf extract conditions, respectively (Figure 3). The stem extract did not decrease TG in ryegrass seeds (Figure 3). The root extract lowered S by 80%, the leaf extract lowered S by 70%, and the stem extract lowered S by 15% (Figure 2).

In the bermudagrass seeds, the root and stem extracts increased TG by 25% and the leaf extract lowered TG by 25% (Figure 3). The root extract increased S by 79%, and the stem extract increased S by 173%, but the leaf extract did not affect S, compared to the control (Figure 2).

All differences between control seeds and seeds treated with extract demonstrated significance by t-test ($p<0.001$).

Discussion

Our data show that the kudzu leaf and root extracts sufficiently lowered the total growth and the speed of germination of the dandelion, white clover seeds, and ryegrass seeds but had a less profound effect on bermudagrass seeds. The kudzu stem extract had a less profound effect on the speed of germination and the total growth of each of the seed types, except for dandelion seeds. In the dandelion seed bioassay, the stem extract reduced both S and TG by ~50% (Figures 2 and 3). As a result, we theorize that the kudzu plant must not use the stem as the main organ for allelopathic inhibition.

Furthermore, the root and stem extracts enhanced the TG and S of bermudagrass seeds as compared to the control. Kudzu root and stem are a rich source of polyphenolic compounds, including isoflavones, isoflavonoid glycosides, coumarins, and puerarols (11). We hypothesize that the interactions between these kudzu polyphenolic compounds and the compounds contained in the bermudagrass seeds may be responsible for the enhancement of the TG and S of bermudagrass, but further studies are required to better understand any potential relationship. Our observation that the kudzu leaf and root extracts sufficiently lowered the TG and S of the weed seeds (dandelion and white clover), while benefiting the bermudagrass seeds, provides support for our theory that kudzu allelochemicals can potentially be used as a natural weed control agent in a bermudagrass lawn (9).

Of all germination indices used, the most appropriate for testing the allelochemical effects on germination was the speed of germination because it was the most sensitive to the data than total germination (8). Our statistical analysis on the average speed of germination shows that each of our experimental results, except for that of the leaf extract on bermudagrass, was statistically significant. In the case of the kudzu leaf extract on bermudagrass, our statistical analysis found that these results were not significantly different from the control, suggesting that the kudzu leaf extract had little or no effect on the speed of germination of the bermudagrass seeds.

Though we attempted to control the experiment as much as possible, some factors were beyond our control, such as the quality of each individual seed. Furthermore, it was difficult to check whether the concentration of allelopathic compounds was consistent across each individual kudzu clipping. There is also the possibility of slight human interference, such as shaking the petri dish while sealing it or while placing it for germination.

Further research should be conducted to determine the specific chemical compounds that give Pueraria montana its allelopathic properties. Research also needs to be done to determine the means by which these chemicals interact with their targets and how allelochemicals interact with one another. Because the release of phenolic compounds is the currently accepted mechanism for allelopathy (2), it is possible that the kudzu plant may produce phenolic compounds in its roots and leaves, which it releases to inhibit the growth of its competitors.

Moreover, though it seems that the current concentration of kudzu extract was successful in the allelopathic inhibition of weeds, more research needs to be done to determine the ideal concentration of kudzu extract that should be used as a weed killer, as higher concentrations may have an adverse effect on bermudagrass. Kudzu allelochemicals should also be tested on a larger variety of seed and plant types. Finally, field research should be conducted to determine their potential to be used as a natural weed killer and their effects on humans and animals.

Methods

Extract Preparation

Mature kudzu green leaves, stems, and roots were collected from a secluded naturally growing population in Oxford, MS. The kudzu organs were washed using distilled water and then oven dried at 63°C for 72 hours. The dried kudzu was then ground into a fine powder using a mortar and pestle. 10 g of each type of kudzu organ powder was placed in one of three beakers and then dissolved in 300 mL of boiling water to make a 33 mg/ml solution. The kudzu powders were stirred in the water every hour for 24 hours and filtered through four sheets of filter paper (Fisher Scientific) to remove excess plant matter. This final extract was used immediately in experimentation.

Seed Treatment with Kudzu Extract

Commercially available white clover, dandelion, bermudagrass, and ryegrass seeds (Outsidepride Inc.) were used in the bioassays. Sixteen petri dishes (Fisher Scientific) were aligned into four rows with four petri dishes each. Each row contained a different type of seed and each column contained a different

type of extract. The bottom of each petri dish was lined with two sheets of 10 cm filter paper. Then, four seeds of each type were placed in each petri dish of succeeding rows, maintaining a minimum of 1 cm of space between each seed.

Each petri dish within a column was treated with 5mL of the kudzu extract corresponding to that particular column. The pipette used to administer treatments was washed with distilled water after each use. In the last column, 5mL of distilled water was used as a control treatment. The top of each petri dish was then closed and taped shut.

Each petri dish was then placed inside its own labeled polyethylene bag. Each bag was placed in a controlled storage area at 25°C until the germination period was over.

Data Collection

Germination was deemed to have occurred when the radicle protruded outside the seed coat by at least 1 mm and the germination period was deemed to be over when no change was seen in the number of germinated seeds for three consecutive days. The number of germinated seeds in each petri dish was recorded daily (10). These recordings were used to determine the speed of germination and total germination (8). The formulas used to calculate the germination indices are presented in Figure 1.

Statistics

For statistical analysis, we applied the t-test using the null hypothesis that the mean difference in the speed of germination between the treatment samples and control samples is zero. A 95% confidence interval was used, with p-values less than 0.05 corresponding to the rejection of the null hypothesis. The total germination and speed of germination for each seed type was calculated using already proposed formulas. These formulas are delineated in Figure 1.

References

1. Hierro JL and Callaway RM (2003). Allelopathy and Exotic Plant Invasion. Plant and Soil 256: 29-39

2. Westra EP (2003). Can Allelopathy Be Incorporated into Agriculture for Weed Suppression? Insect Behavior Review Articles. Colorado State University, Spring 2010

3. Al-Humaid AI, and Warrag MOA (1998). Allelopathic Effects of Mesquite (Prosopis Juliflora) Foliage on Seed Germination and Seedling Growth of Bermudagrass (Cynodon Dactylon). Journal of Arid Environments 38(2): 237-43.

4. Burgos NR and Talbert RE (2000). Differential Activity of Allelochemicals from Secale Cereale. Weed Science 48: 302-10.

5. Ferguson JJ, Rathinasabapathi B, and Chase CA (2003). Allelopathy: How Plants Suppress Other Plants. University of Florida IFAS Extension.: HS944

6. Barnes JP and Putnam AR (1986). Evidence for Allelopathy by Residues and Aqueous Extracts of Rye. Weed Science 34(3): 384-90

7. Anderson M and Magleby R (1997). Agricultural Resources and Environmental Indicators, 1996-97. Economic Research Service: AH712.

8. Anjum T and Bajwa R (2005). Importance of Germination Indices in Interpretation of Allelochemical Effects. International Journal of Agriculture & Biology 7(3): 417-19

9. Naqvi A. Green Economy Initiative of the United Nations Environment Programme (UNEP). United Nations Environment Programme (UNEP). Web. 11 Feb. 2011.

10. Bradbeer JW. Seed Dormancy and Germination. New York: Chapman and Hall, 1988. Print.

11. Wong KH, Li GQ, Li KM, Razmovski-Naumovski V, and Chan K. (2011). Kudzu root: traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases. Journal of Ethnopharmacology, 134(3), 584-607.