Pennycress (*Thlaspi arvense L.*) Seed Glucosinolate Levels Are Impacted by Growing Location

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ABSTRACT

Researchers have been working toward making pennycress a commercially available cover crop that can be used as sustainable biofuel for aviation and animal meal for livestock. To make pennycress into a palatable food product for animals, lower levels of glucosinolate concentrations are desirable to reduce the bitter taste and minimize toxicity in the seed meal. Researchers have identified sinigrin as the glucosinolate in pennycress seeds. This study investigates the effects of growing location and environmental temperatures on sinigrin levels in golden pennycress seed from line 'tt8-t/ARV1'. The seeds were collected from ten separate locations across the Midwestern United States: Hoyleton, IL; Macomb, IL; Mount Pulaski, IL; Normal, IL; Morris, MN; Rosemont, MN; St Paul, MN; Custar, OH; Lancaster, WI; and Madison, WI. Statistical analyses revealed that seeds grown in Hoyleton, IL had the highest average sinigrin levels of 50 μmol/g for golden pennycress line 'tt8-t/ARV1' across all locations. When comparing temperature and precipitation levels to sinigrin levels at three locations, there were no noticeable patterns in Macomb, IL, Normal, IL, or in Custar, OH. Further studies on crops preceding pennycress sowing, soil characteristics, and biotic factors would need to be examined to determine the environmental impacts on sinigrin concentrations in pennycress seeds.

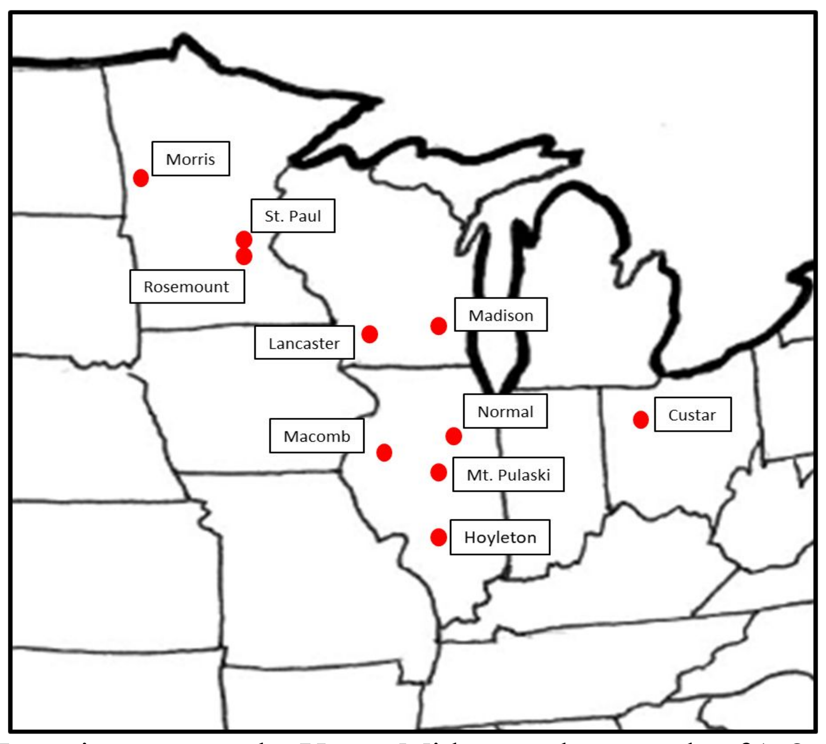


Figure 1. Locations across the Upper Midwest where seeds of 'tt8-t/ARV1' were harvested for glucosinolate analyses.

INTRODUCTION

Researchers are interested in making pennycress a commercially available cover crop that can be used as a sustainable biofuel for aviation and as a source of food product for animals, particularly livestock. Pennycress is a member of the Brassica (*Brassicaceae*) family which includes broccoli, cabbage, kale, and other oilseed crops such as rapeseed and canola. Plants in this family contain bioactive chemicals known as glucosinolates that can be both beneficial and harmful to the plants (Bischoff, 2016). HPLC/MS analyses have shown that the predominant glucosinolate in pennycress seed is sinigrin. Sinigrin is a strong, pungent, and bitter tasting when ingested; but it serves as a repellent and acts as a defense mechanism against insects, herbivores, and pathogens in plants.

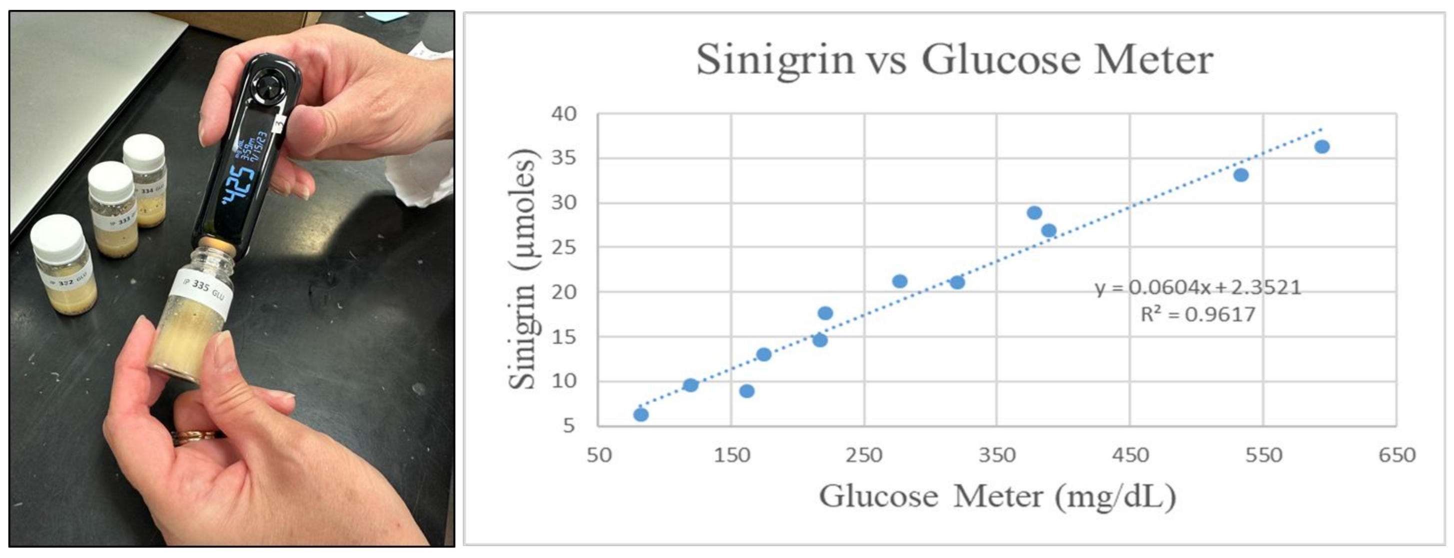
In respect to animal feed, lower glucosinolate concentrations are desirable because higher concentrations can have severe effects on certain livestock animals such as pigs. When comparing a pig's tolerance to other animals like cows, pigs have a smaller range of tolerance. Higher concentrations of glucosinolates have shown to induce iodine deficiency, hypertrophy of liver, kidney, and thyroid, and in extreme concentrations mortality (Tripathi et al., 2007).

Extreme weather events and increased temperatures have shown to increase the glucosinolate concentrations for Brassicas such as arugula (*Eruca sativa*). While most plants have detrimental effects at 40° C, there are some *E. sativa* cultivars that can withstand those temperatures. However, these temperatures have been shown to increase glucosinolate levels within the plants post-harvest (Wagstaff et al., 2020). Spring season Brassica plants that are grown in intermediate temperatures with high light intensity, shorter days, and high-water availability appear to have higher glucosinolate concentrations. Meanwhile, autumn/winter crops show to have lower glucosinolate concentrations due to shorter days with lower light intensity, less water availability, and lower temperatures (Bjorkman et al., 2011).

This study compared glucosinolate levels to environmental temperatures at three different locations across the Midwest. To investigate the variability of glucosinolate levels in pennycress seeds, glucosinolate levels were analyzed from ten separate locations across the Midwestern United States (**Figure 1**).

A B

Figure 2. A. Pennycress seeds in vial with potassium phosphate buffer. **B.** Seeds being ground with homogenizer for glucose testing.



A B

Figure 3. A. Glucose levels measured using a blood glucose meter and test strips. **B.** Calibration curve for Sinigrin conversion

MATERIALS AND METHODS

Planting and harvesting: Golden pennycress seeds from line 'tt8-t/ARV1' were collected from ten locations across the Midwestern United States: Hoyleton, IL; Macomb, IL; Mount Pulaski, IL; Normal, IL; Morris, MN; Rosemont, MN; St Paul, MN; Custar, OH; Lancaster, WI; and Madison, WI. Seeds were collected and stored between 2020-2023. Typically, the seeds were planted in the fall, around October 1st, and harvested during spring of the following year, in late May or early June. In order to harvest the seeds, a combine was used to bag the seed by individual plots for further processing and testing.

Seed preparation: Seed samples were processed through a Yankee Clipper office tester, a seed blower, and a VMEK optical seed sorter to remove any excess debris. Cleaned seed was further analyzed by a Marvin Seed Analyzer for seed area, seed size, and TD-NMR for total oil content.

Glucosinolate readings: Sinigrin has been identified by HPLC/MS as the only glucosinolate present in pennycress seeds. Since sinigrin is readily hydrolyzed to glucose in the presence of endogenous myrosinase in the seed, a glucose meter was used to determine the amount of sinigrin converted to glucose. Approximately 450 mg of seed was weighed out, recorded, and placed into a scintillation vial with 5 mL potassium phosphate buffer (pH 7.0) (**Figure 2. A**). To hydrolyze the sinigrin in the seeds to glucose, the seed was ground using an Ingenieurburo CAT X 120 homogenizer (**Figure 2. B**). The vials were covered and incubated at room temperature (21° C) for 24 hours. Glucose levels were measured using a Contour Next One blood glucose meter and test strips (**Figure 3. A**). To calculate sinigrin using the glucose levels, a calibration curve was constructed from seeds with known amounts of sinigrin (**Figure 3. B**).

Data analyses: Weather data was collected at each site for each year from archived weather (Weather Underground.com). Sinigrin, location, and weather data measurements were analyzed by running ANOVA and Tukey (HSD) tests in RStudio. All graphs were created using RStudio.

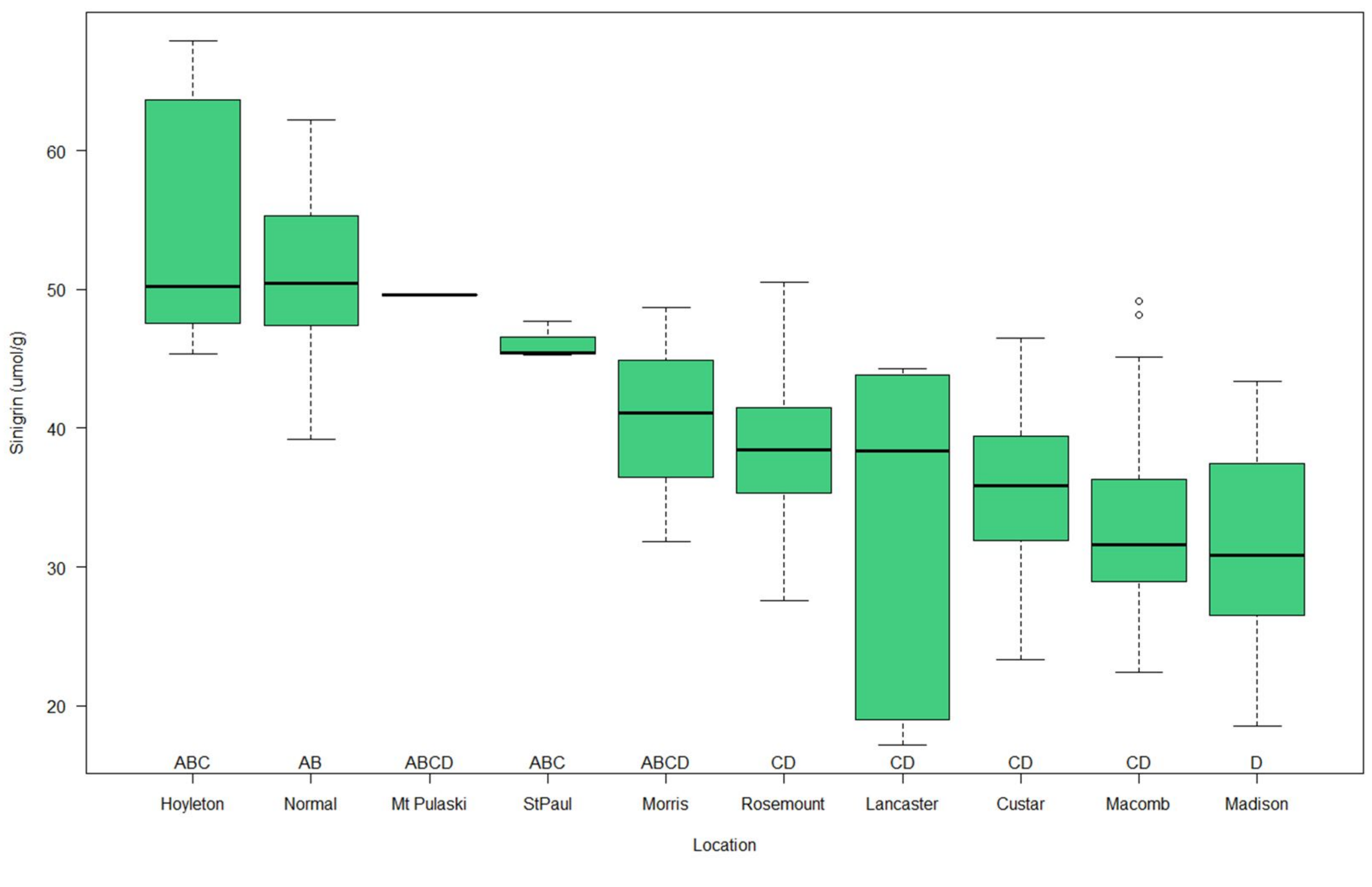


Figure 4. Sinigrin levels by location: Hoyleton, IL; Normal, IL; Mt Pulaski, IL; St Paul, MN; Morris, MN; Rosemount, MN; Lancaster, WI; Custar, OH; Macomb, IL; Madison, WI. A difference in letters indicates statistical significance.

RESULTS

Figure 4 examines the differences that each location had in sinigrin concentrations within the pennycress seeds. Sinigrin concentrations were significantly different in Hoyleton, IL, Normal, IL, and St. Paul, MN.

Figure 5 examines the differences that three locations, Macomb, IL; Normal, IL; and Custar, OH have between the years 2020-2023. Graphs illustrate the averages for sinigrin levels, precipitation, and temperatures for the month of May at each location for years 2020-2023. There does not appear to be a correlation between sinigrin levels and temperature or precipitation. Macomb, IL had a significant difference in sinigrin levels between 2020 to 2023 ($p = 6.01 \times 10^{-10}$) appearing to have lower concentrations through the years. Normal, IL had a significant difference in sinigrin levels from 2020 to 2023 ($p = 0.000676$). Custar, OH had a significant difference in sinigrin levels from 2020 to 2023 ($p = 0.00321$).

Lancaster, WI had a significant difference in sinigrin levels between 2020 to 2022 ($p = 1.16 \times 10\text{-}5$). However, Madison, WI did not show a significant difference in sinigrin levels between 2021 to 2022.

Glucosinolate levels across locations and years demonstrated no significant correlation to thousand seed weight or total seed oil.

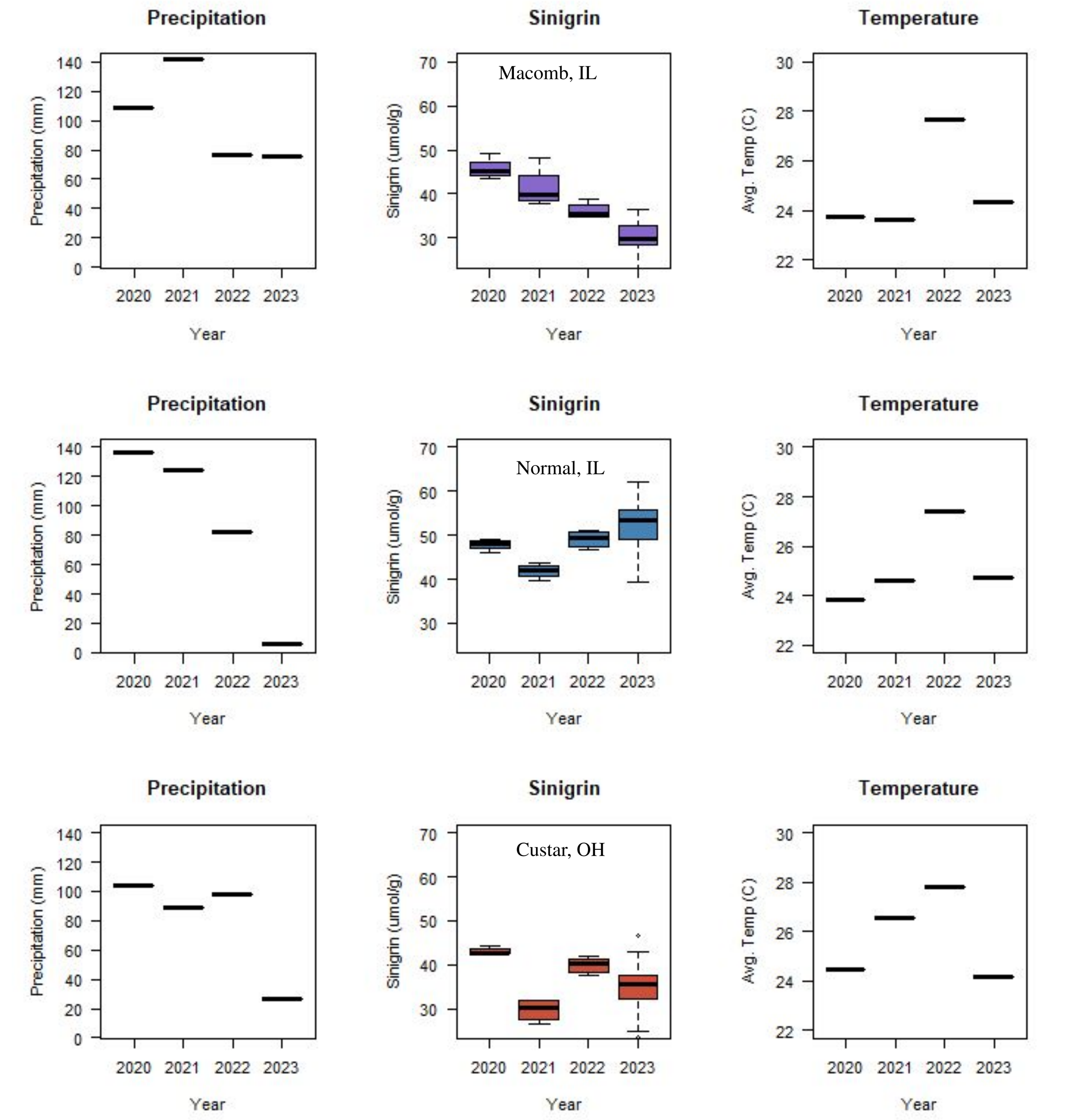


Figure 5. Sinigrin levels (μmol/g), average May temperatures (°C), and average May precipitation rates for Macomb, IL (purple), Normal, IL (blue), and Custar, OH (red) for the years 2020, 2021, 2022, and 2023.

CONCLUSIONS

Glucosinolate concentrations in the form of sinigrin can vary based on the location pennycress is grown. Seeds that were grown in Hoyleton, IL appear to have the most significant differences between the other locations (**Figure 4**). When temperature and precipitation rates were compared at three locations (Macomb, IL, Normal, IL, and Cluster, OH,) there were no significant correlations to sinigrin levels (**Figure 5).** Seeds grown in Lancaster, WI had a significant difference in sinigrin levels between 2020 to 2022. Seeds grown in Madison, WI had no significant differences in 2021 and 2022. Glucosinolate levels across locations and years had no significant correlation to thousand seed weight or total seed oil. Further studies are needed to determine the cause of the differences in sinigrin levels. Soil characteristics, crops preceding pennycress sowing, biotic factors, or other environmental factors at separate locations must be considered to determine what could be causing difference in sinigrin levels within pennycress seeds.

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LITERATURE CITED

Bischoff, K. 2016. Chapter 40 – Glucosinolates *In* Nutraceuticals-Efficacy, Safety and Toxicity, ed. R.C. Gupta, Academic Press. pgs 551-554. doi: 10.1016/B978-0-12- 802147-7.00040-1

Tripathi, M., A. Mishra. 2007. Glucosinolates in animal nutrition *In* Animal Feed Science and Technology. pgs 1-27. doi: 10.1016/j.anifeedsci.2006.03.003

Jasper J., C. Wagstaff, and L. Bell. 2020. Growth temperature influences postharvest glucosinolate concentrations and hydrolysis product formation in first and second cuts of rocket salad *In* Postharvest Biol Technol. doi: 10.1016/j.postharvbio.2020.111157.

Björkman, M., I. Klingen, A.N.E. Birch, A.M. Bones, T.J.A. Bruce, T.J. Johansen, R. Meadow, J. Mølmann, R. Seljåsen, L. E. Smart, and D. Stewart. 2011. Phytochemicals of Brassicaceae in plant protection and human health *In* Influences of climate, environment, and agronomic practice, Phytochemistry. pgs 538-556. doi: 10.1016/j.phytochem.2011.01.014

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