

A Simplified Procedure for Glucosinolates Quantification

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Introduction

- ① The challenge in plant protection programs is to seek management alternative that mitigate environmental degradation while maintaining agricultural productivity and profitability.
- ① Materials of natural origin are biodegradable and could serve as environmentally safe pesticide.
- ① Glucosinolates (GSLs) is a group of naturally occurring thioglucosides in Cruciferous vegetables that can be used as biofumigants.

- **Biofumigation** is the incorporation of plant residues that release pesticidal compounds into the soil as they decompose. Cruciferous crops (e.g. mustard, cabbage, turnip, canola) can be used as biofumigants because they contain GSL, long recognized as powerful natural pesticides.
- GSL-containing plants inhibit weed seed germination and some soil pathogens. They can be used as cover crops to reduce dependence on synthetic pesticides.
- *Sclerotinia sclerotiorum* & *Phytophthora capsici* are two broad-spectrum soil-borne diseases that cause severe damage to vegetable production.



Sclerotinia rot of carrot caused by *Sclerotinia sclerotiorum*

**Stem Lesion in pepper
caused by
*Phytophthora capsici***



Background

- *Sclerotinia sclerotiorum* is found worldwide on a wide range of host plants: 64 plant families - 225 genera and 361 species.
- The infected areas has a bleached appearance.
- >120 different GSL have been described - most have cancer preventing activities in human diet.
- On the contrary, GSLs develop goiter in animal food.
- GSLs by themselves are not biologically active. The volatile hydrolysis products isothiocyanates are toxic to some soil-borne plant pathogens.

- GSLs and their hydrolysis products are responsible for the sharp or biting taste of condiments (horseradish or mustard) and contribute to the characteristic flavors of plants whose leaves (brussels sprouts, cabbage), floral buds (broccoli, cauliflower), stems (Kohlarabi) or roots (radish, turnips) are consumed by humans.

The present study is a continuation of our previous work on natural products for pest control.

Objectives

1- To develop and validate a simplified procedure for quantification glucosinolates in *Brassica* accessions.

2- To identify *Brassica* species and/or accessions with high levels of glucosinolates for future research on fungicidal efficiency of their crude extracts.

Materials & Methods

Ten accessions that demonstrated relative cold tolerance, rapid maturity, and superior biomass production were selected from 48 accessions of the National Germplasm Repository:

Ames 8660

Brassica juncea

Ames 8674

Brassica juncea

Ames 8709

Brassica juncea

Ames 8887

Brassica juncea

PI-120923

Brassica juncea

PI-603015

Brassica juncea

Pacific gold

Brassica juncea

PI-169083

Brassica napus

PI-633215

Eruca sativa

Ida gold

Brassica campestris

Indian mustard

Oil seed rape

Arugula

Field mustard

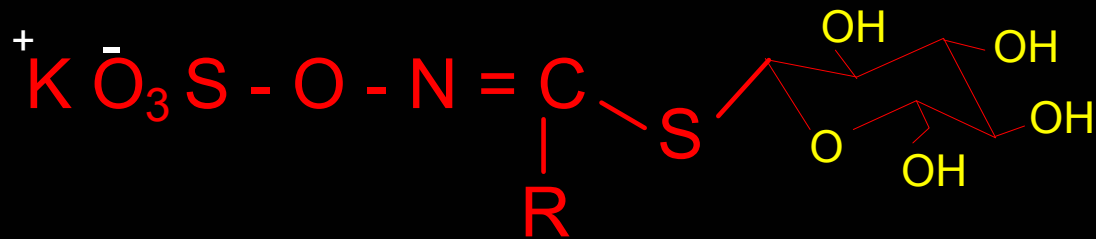
Greenhouse

High Tunnels

Field

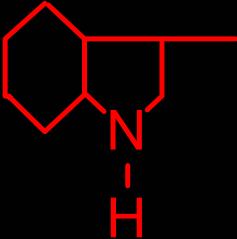
Analysis of Glucosinolates

- ① Extraction using boiling methanol
- ① Clean-up using celite column
- ① Separation by anion-exchange resin “Sephadex”
- ① Hydrolysis with Thioglucosidase
- ① Quantification

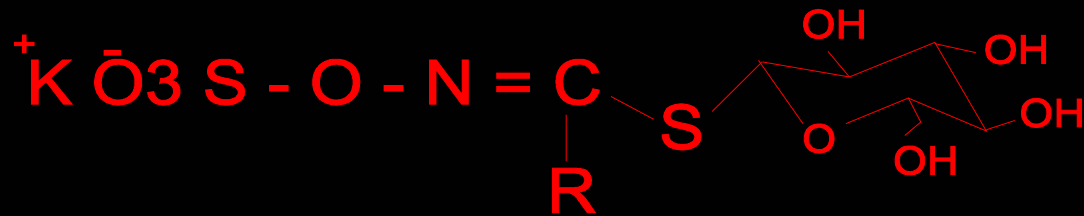


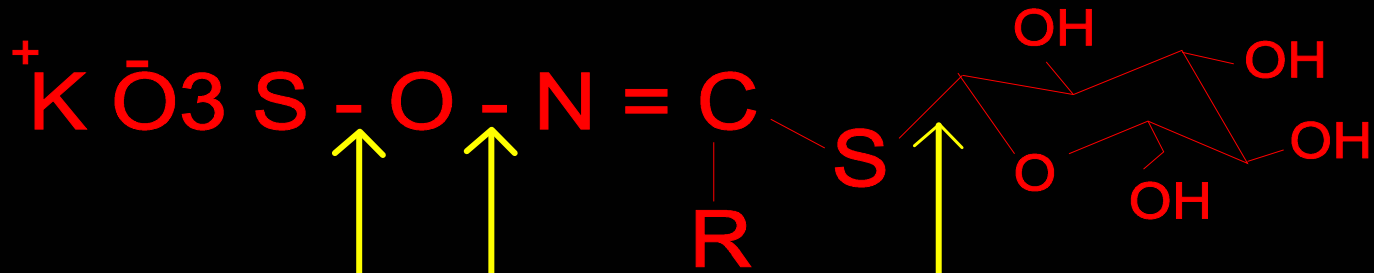
R = 2-hydroxy-3-butenyl; $\text{CH}_2=\text{CHCHOHCH}_2-$; Progoitrin

R = 2-phenylethyl; $\text{C}_6\text{H}_5-\text{CH}_2\text{CH}_2-$; Gluconasturtiin

R = 3-indolylmethyl;  ; Glucobrassicin

- GC analysis is not amendable to some GSLs containing polar side chains (Lee et al. 2006).
- Total sulfur determination by wet digestion and ICP analysis (Bloem et al. 2005).
- High-performance liquid chromatography (Tolra et al. 2000).

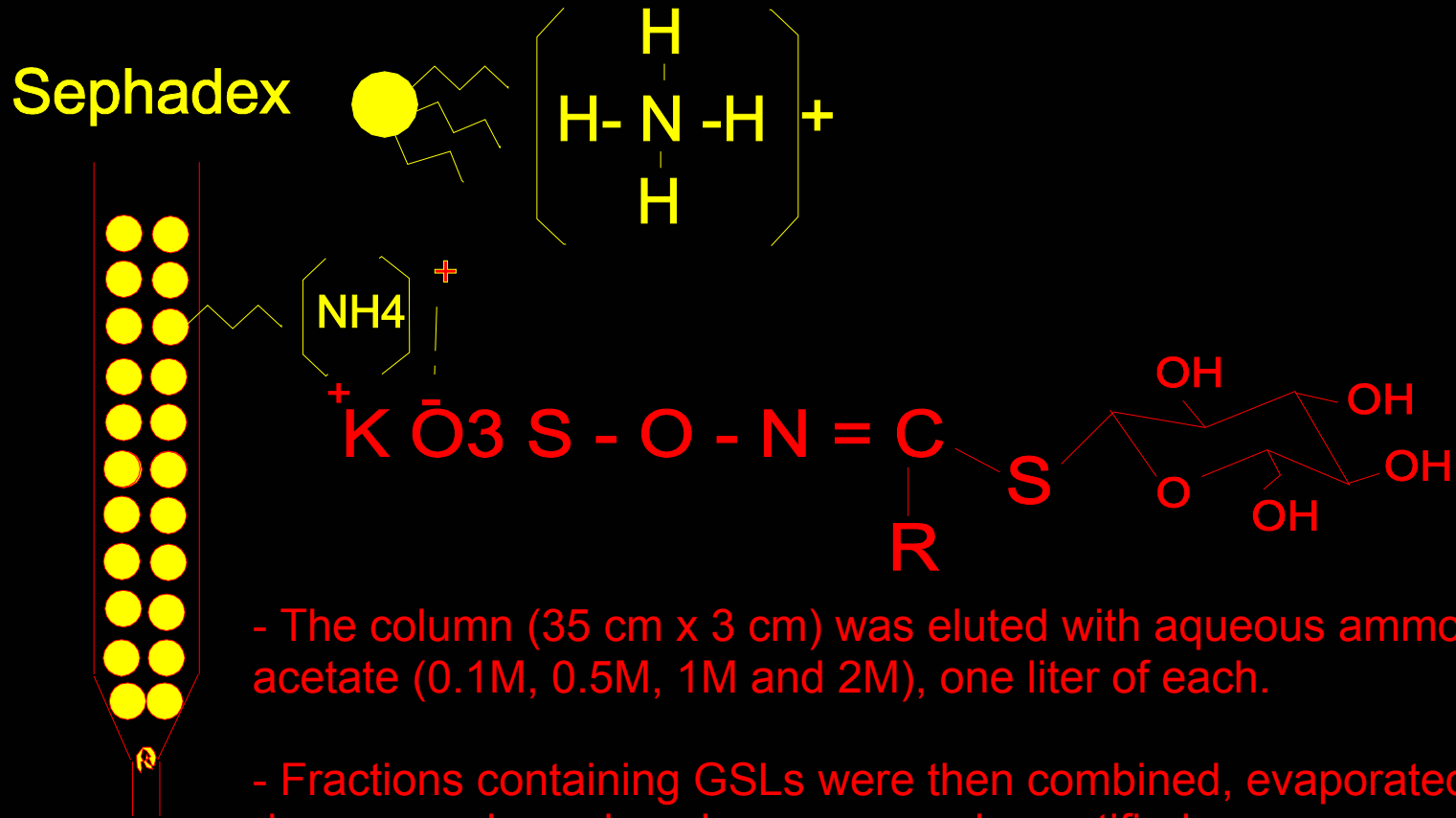




Sulfatase

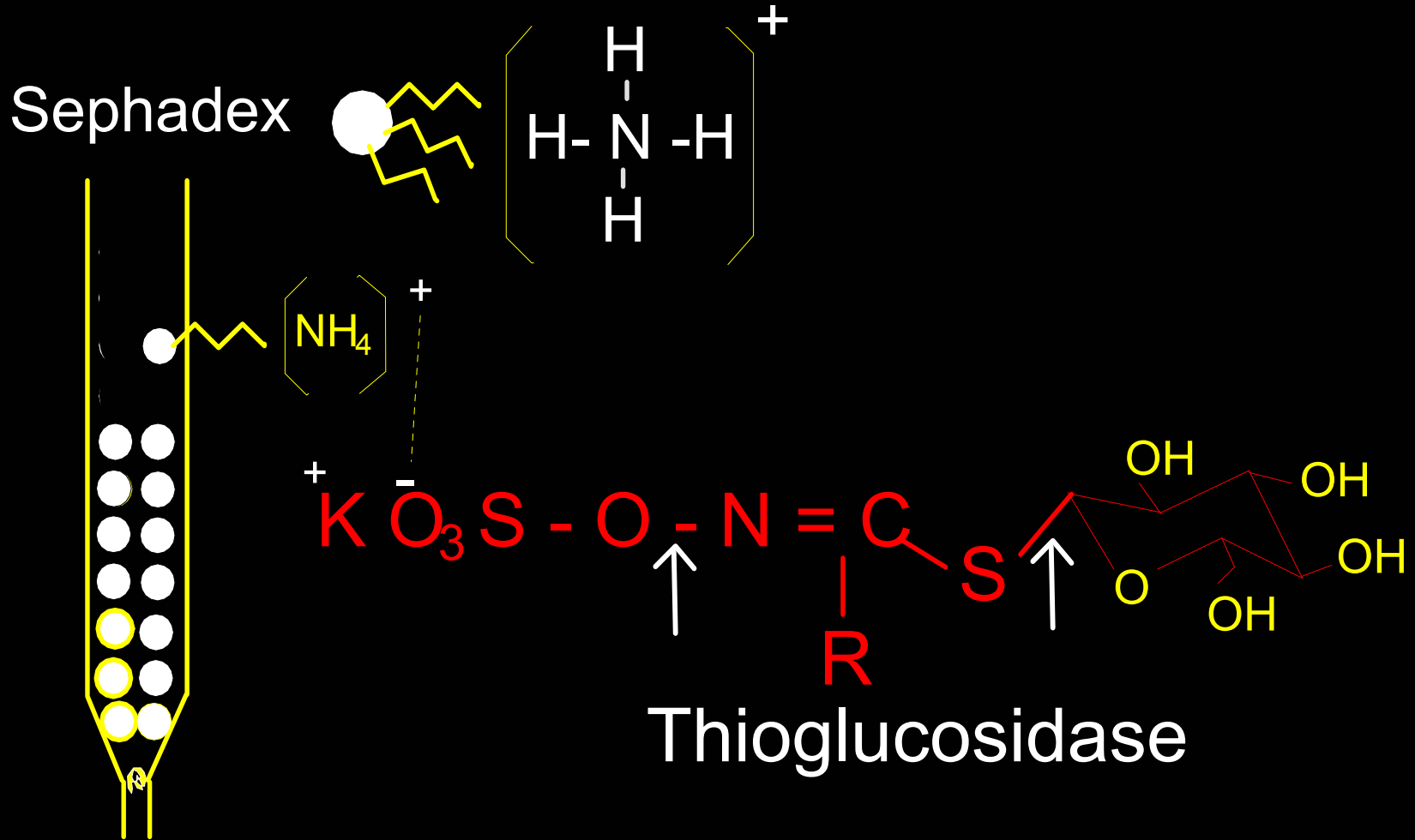
Thioglucosidase

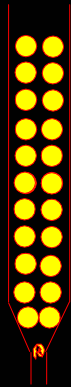
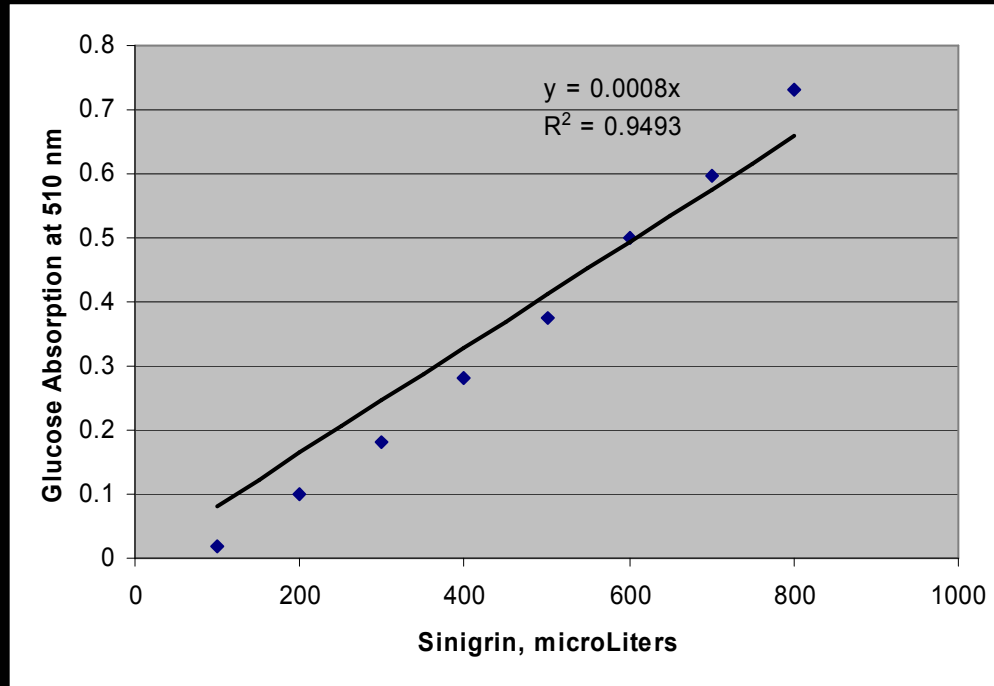
- Separation was accomplished by adsorption on DEAE- Sephadex A-25 (2-[diethylamino] ethyl ether) ion exchange resin of 40-125 μm bead size.
- DEAE was pre-swelled overnight in 2M ammonium bicarbonate.



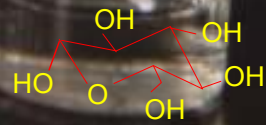
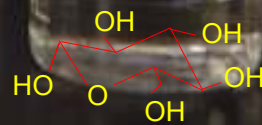
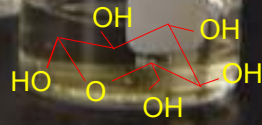
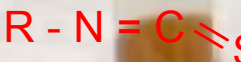
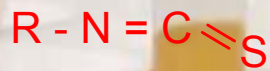
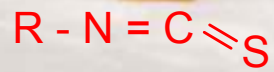
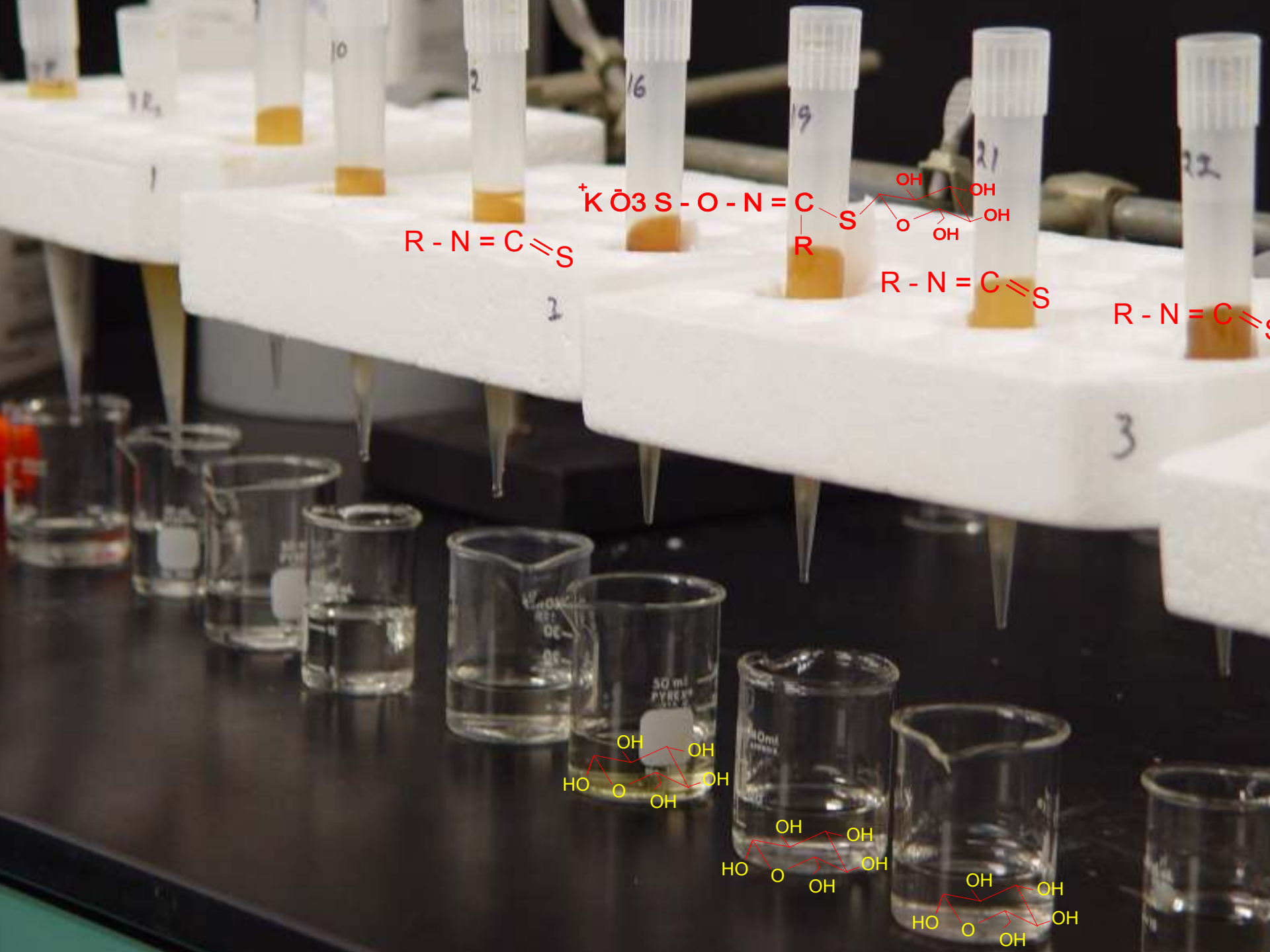
- The column (35 cm x 3 cm) was eluted with aqueous ammonium acetate (0.1M, 0.5M, 1M and 2M), one liter of each.
- Fractions containing GSLs were then combined, evaporated to dryness under reduced pressure and quantified.

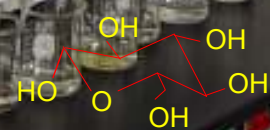
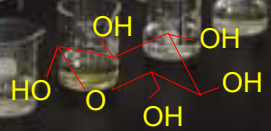
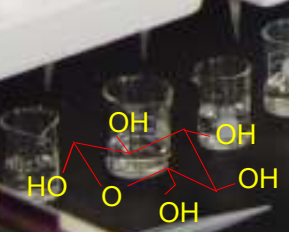
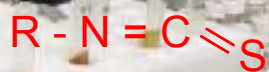
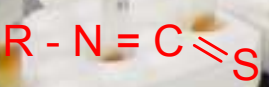
Glucosinolates Separation



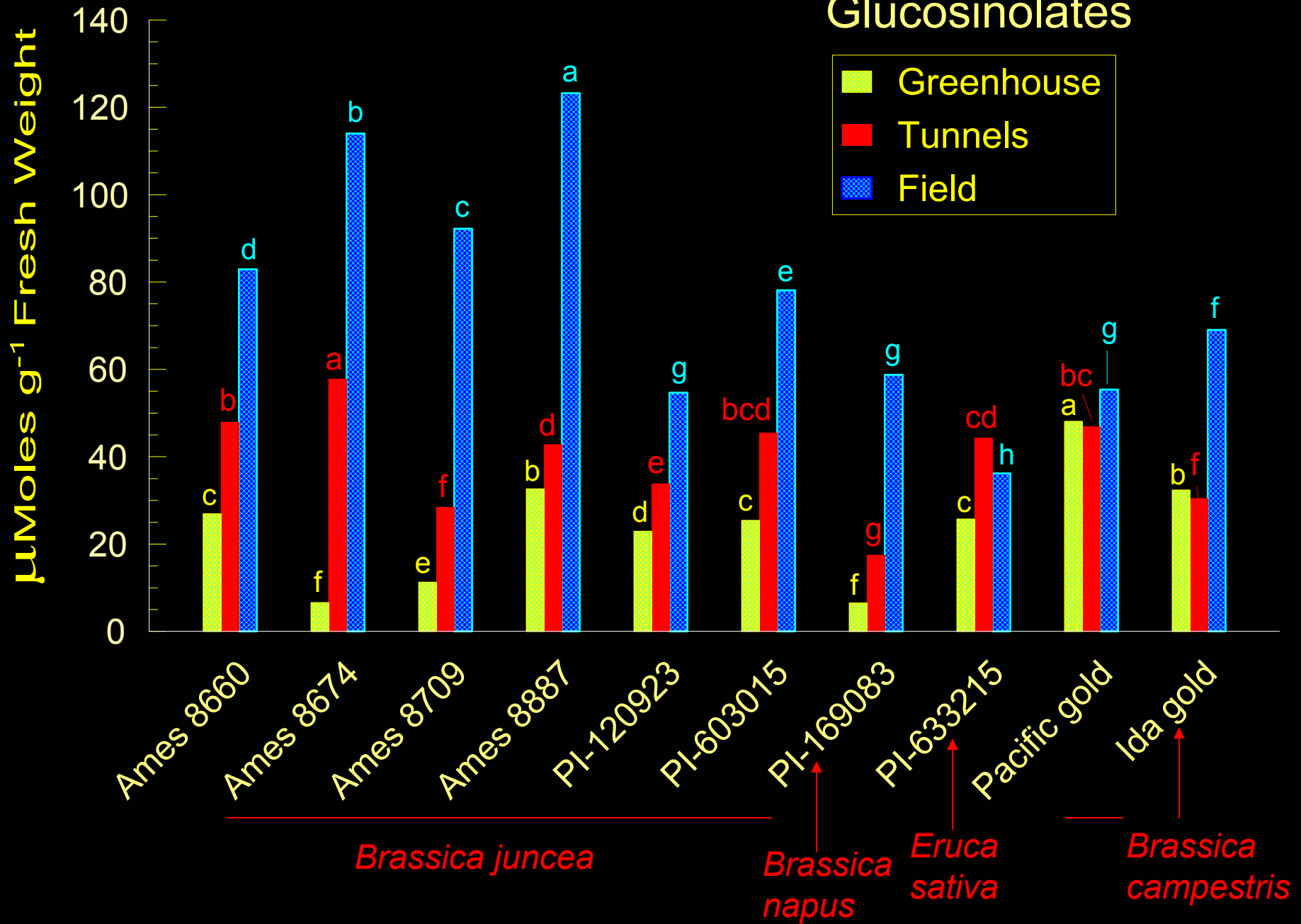


Sinigrin = volatile mustard oil + myrosinase, 5 mg/mL
were incubated at 37 °C for 4 hrs.





Glucosinolates



Conclusion

Environmental stress on growing plants can increase the concentration of GSLs per gm fresh tissue.

Brassica Juncea accession “Pacific gold” & *Brassica napus* accession PI-169083 are the most promising biofumigation cover crops among those tested.

THANK YOU