

TOXINS AS REPRODUCTIVE STRATEGIES IN BITTERSWEET NIGHTSHADE BERRIES

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INTRODUCTION

Background: Bittersweet nightshade, *Solanum dulcamara*, is an invasive vine found in North America that bears small berries. The berries are toxic due to high glycoalkaloid levels. We hypothesized that glycoalkaloid levels are initially high and then decrease as the berries mature, as a reproductive strategy that prevents premature seed dispersal by herbivores. By determining the levels of glycoalkaloids at different stages of development, we may be able to examine the way that *S. dulcamara* allocates its resources towards defense metabolites.

Maturity Classification: As the berries mature, they change colour going from green-green (GG), green (G), orange-green (OG), orange (O), orange-red (OR), red (R), and red-red (RR) when fully ripened (Fig 1, 2).

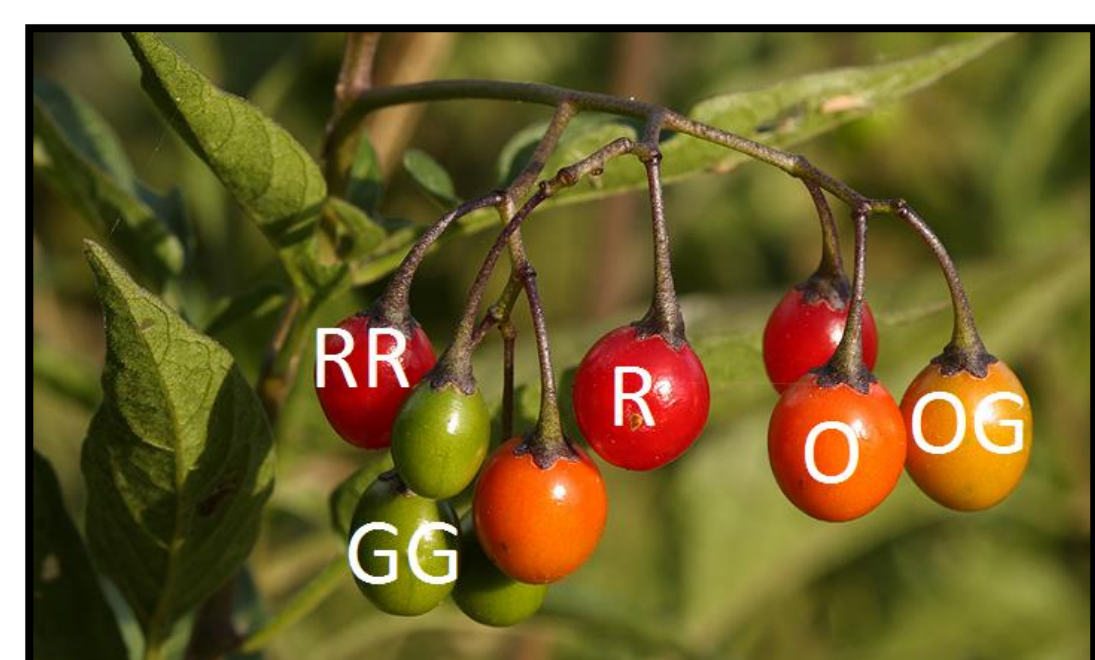


Figure 1. *Solanum Dulcamara* berries, labelled with select maturity classifications. Photo by: Nature Wonders.

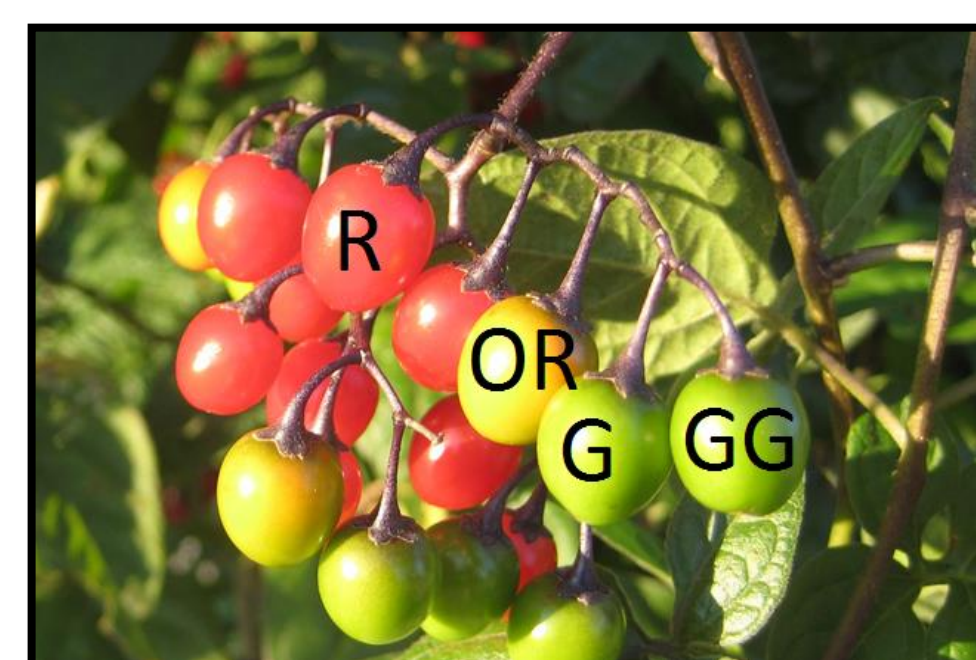


Figure 2. *Solanum Dulcamara* berries, labelled with select maturity classifications. Photo by: Ylem.

OBJECTIVES

To determine the glycoalkaloid levels of *S. dulcamara* berries collected at different stages of maturity based on pigmentation. By comparing the levels of glycoalkaloids at different stages we can determine if the levels decrease as the berries mature, which would be evidence that the toxins act as a deterrent to prevent premature seed dispersal.

METHODOLOGY

Sample Collection: From each of 3 different sites (Fig 3, 4) we collected several berries at each of the 7 maturity levels. Classification by maturity is easy because just like tomatoes, the first fruits (closer to the stem) ripen before the apical fruits (farther from the stem). The GG berries are closer to the stem, and moving towards the distal end they progress towards RR.

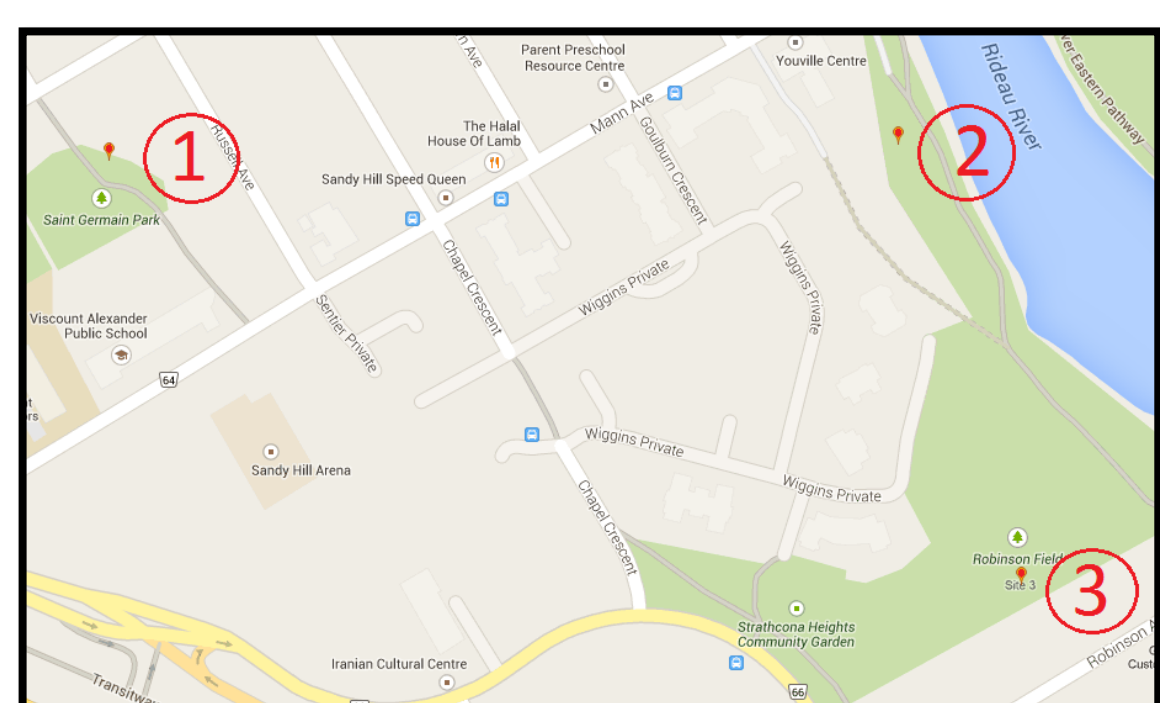


Figure 3. Map of the 3 collection sites in Sandy Hill, Ottawa. Map by: Google Maps.

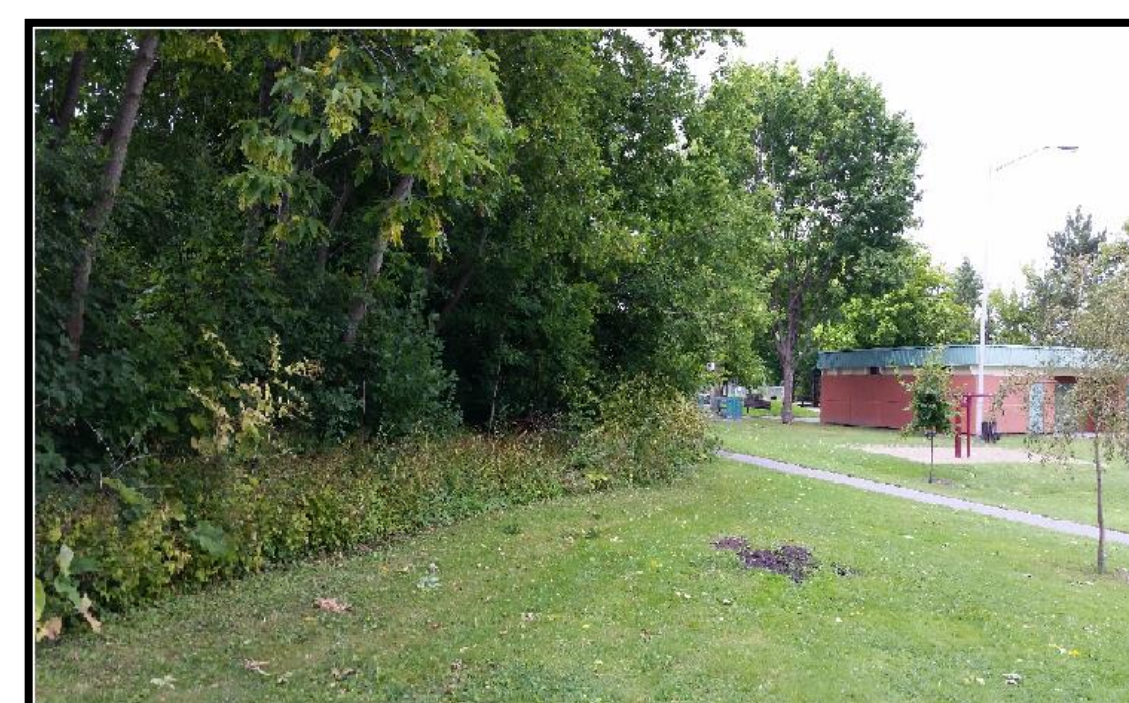


Figure 4. Picture of Site 2 showing typical flora surrounding bittersweet nightshade berries. Photo by: G Sidhu.

Sample Extraction: To make a dry extract, skins were taken off, water was removed by freeze drying, and we then extracted this with 95% ethanol. This was centrifuged and the supernatant was removed (Fig 5). The pellet was freeze dried to remove any traces of water.

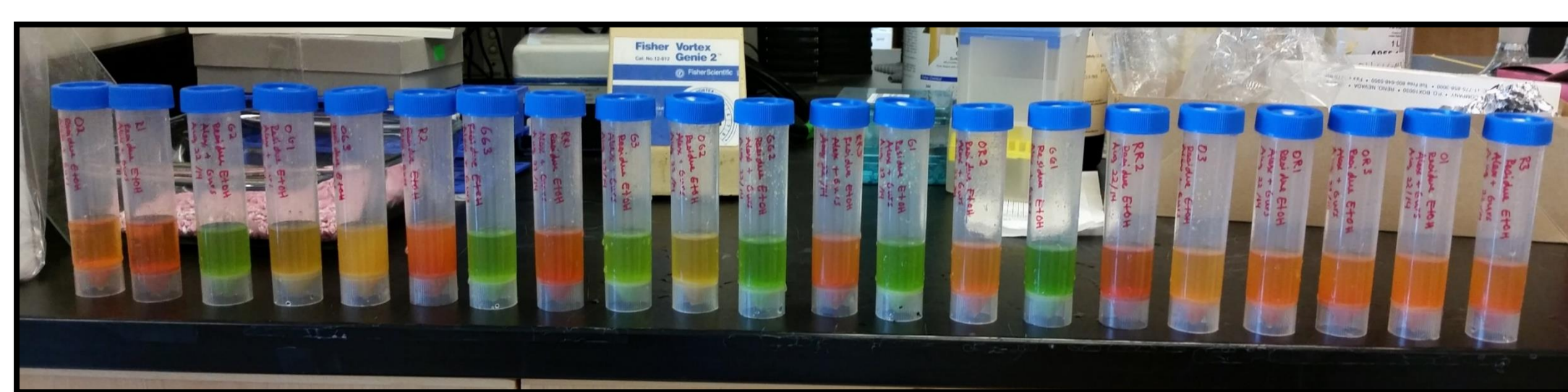


Figure 5. All 21 samples (7 maturity levels at 3 sites) lined up in no particular order after extraction, prior to centrifugation. Photo by: G Sidhu.

Sample Analysis: We analyzed glycoalkaloid content using high pressure liquid chromatography coupled with mass spectrometry (HPLC-MS). Samples of each dry extract were solubilized in 99% ethanol to a concentration of 10 mg/mL, then filtered through a 2.0 um nylon syringe. Relative quantification was achieved by comparing the relative ion intensity of the parent alkaloid, which was determined by MS.

Identification of specific glycoalkaloids was based on literature for MS data of *S. dulcamara* glycoalkaloids (Fig 7-10).

RESULTS

Using mass spectrometry, we positively identified 2 alkaloids with those known in literature (Fig 6), solasonine (884.6 g/mol) and solamargine (868.6 g/mol). 3 other glycoalkaloids were identified, but no matches from literature were available to characterize them. Figure 8 shows the concentration of all 5 identified glycoalkaloids, including the positively identified ones.

We arbitrarily chose units to be concentration of alkaloid per mg of fresh berry. This was the simplest method to compare their concentration.

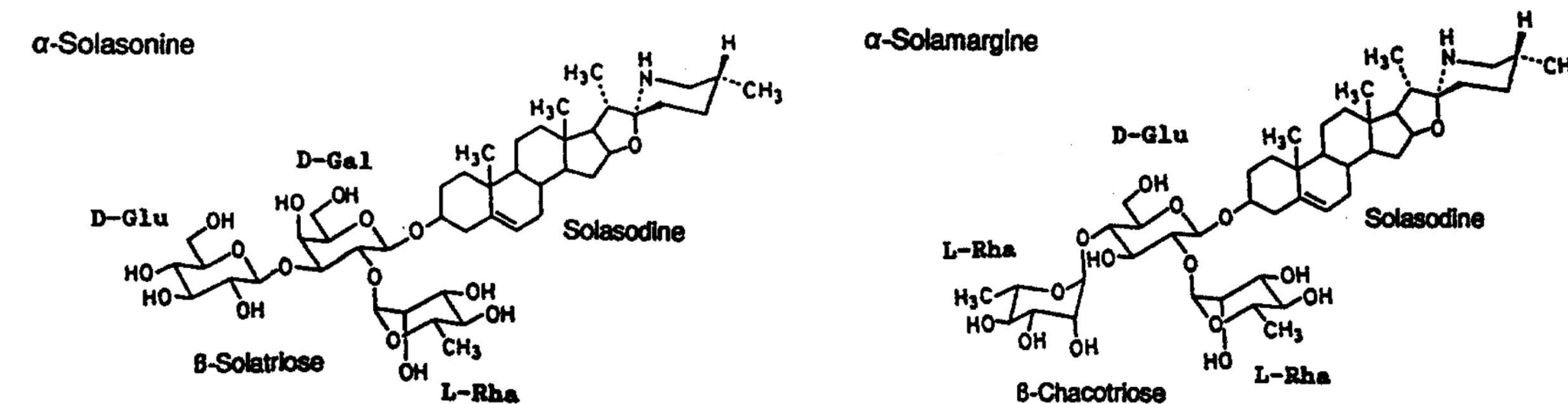


Figure 6. Structures of glycoalkaloids α -solasonine (left) and α -solamargine (right). These were the only ones positively identified from literature. Modified from Cipollini *et al.*

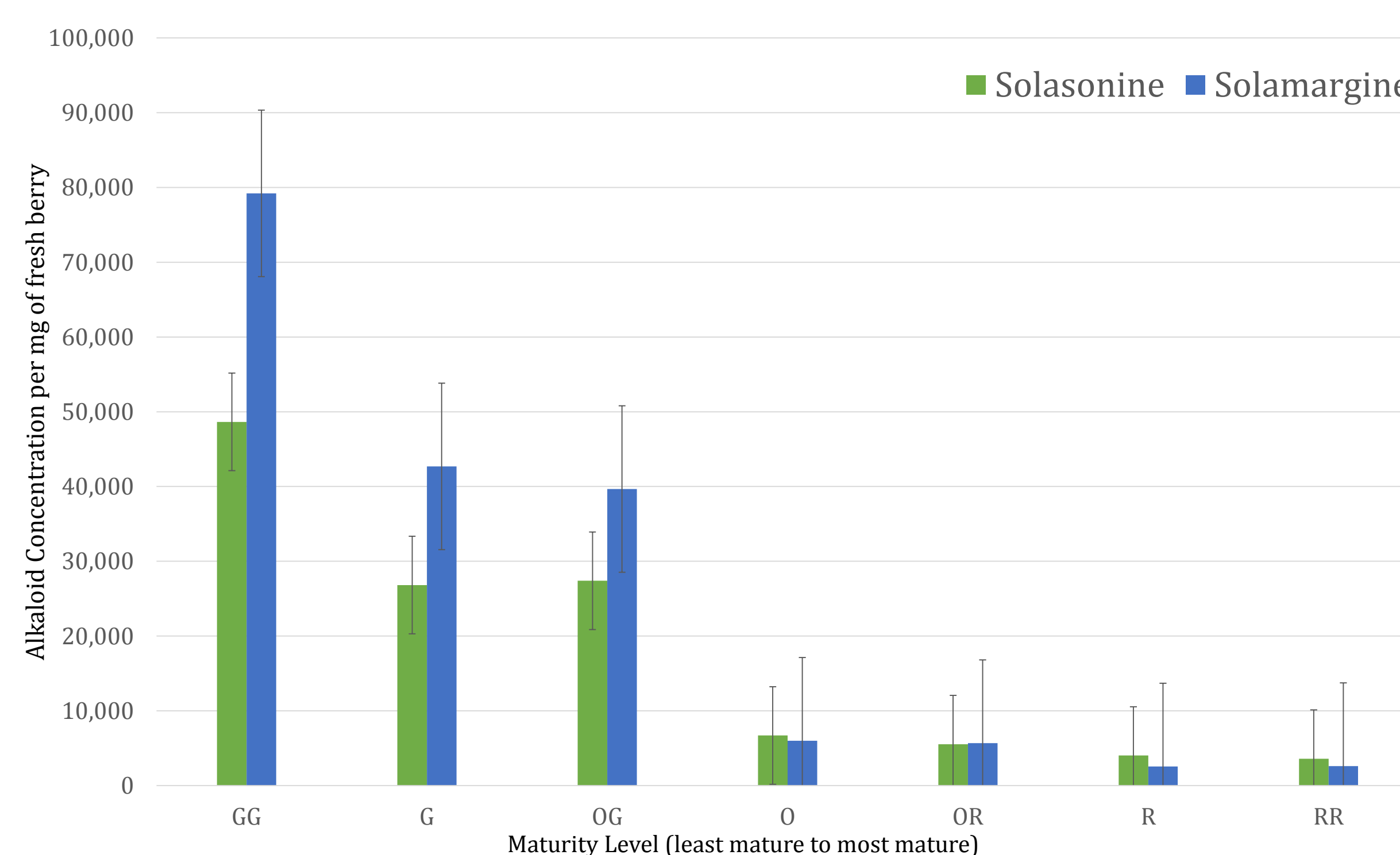


Figure 7. Concentration of major alkaloids (area per mg of fresh berries) against maturity. Solasonine and solamargine shown.

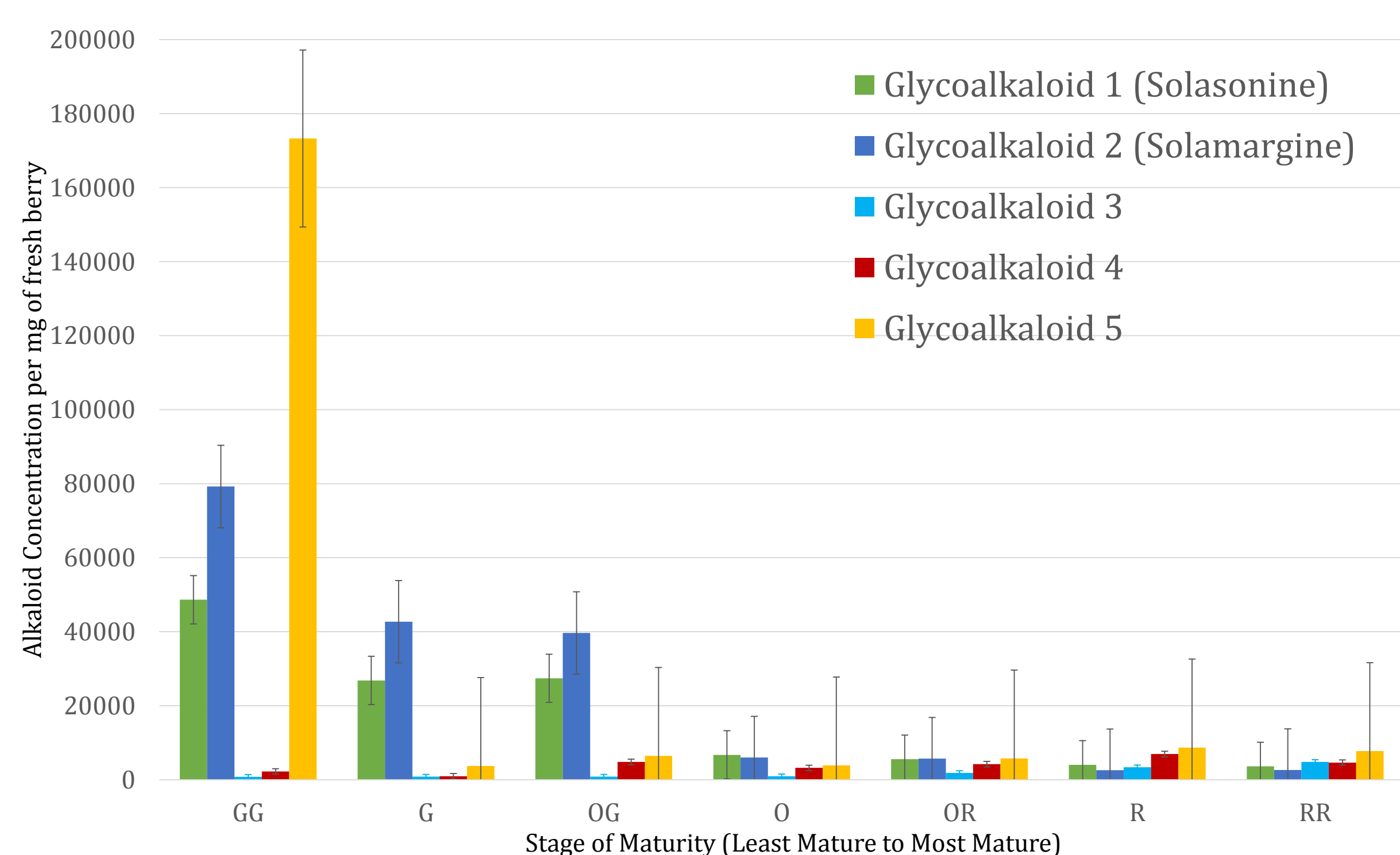


Figure 8. Concentration of minor alkaloids (per mg of fresh berries) against maturity. Minor alkaloids are shown but not identified since no match with literature was made.

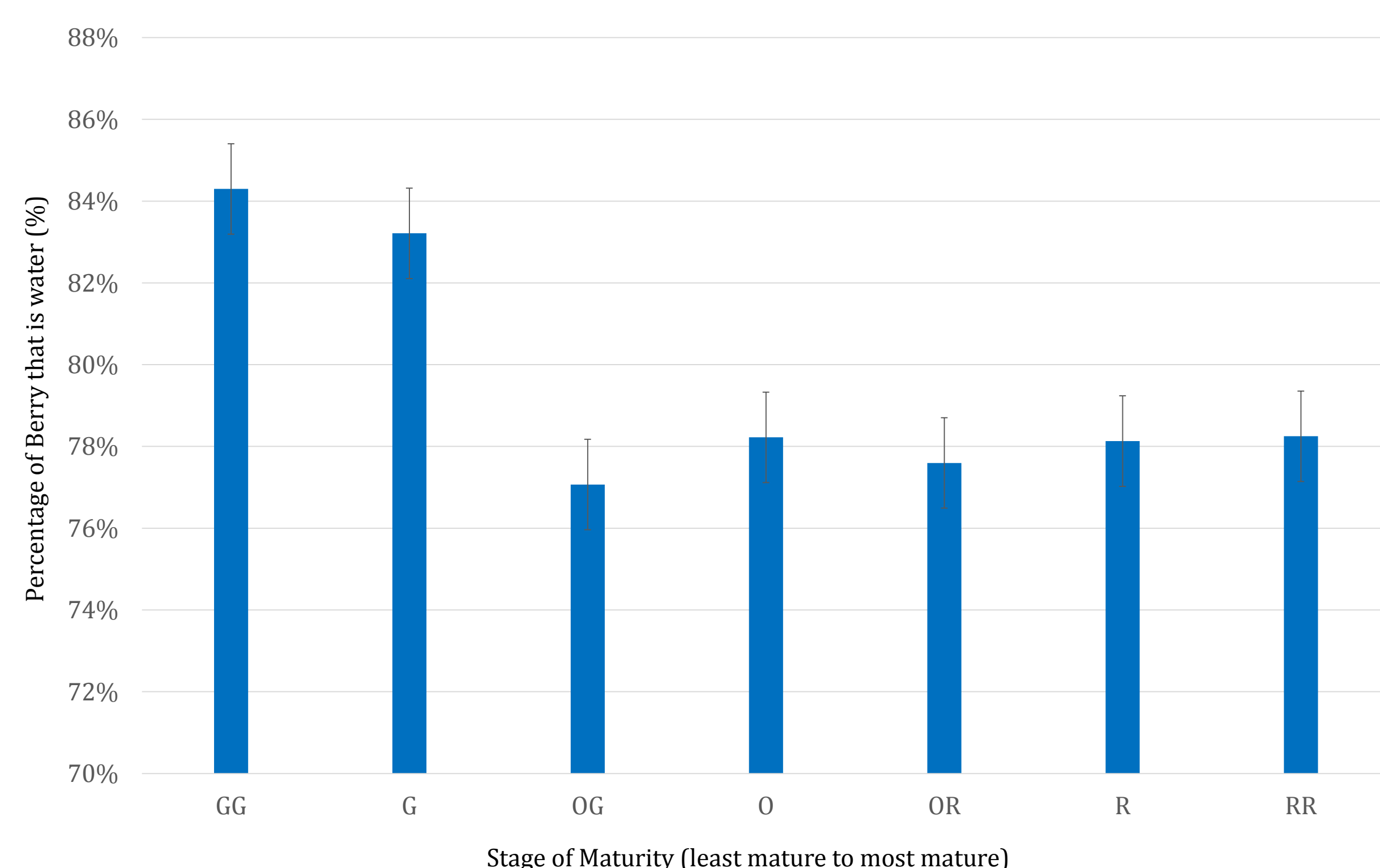


Figure 9. Percentage of fresh berries that is water against maturity.

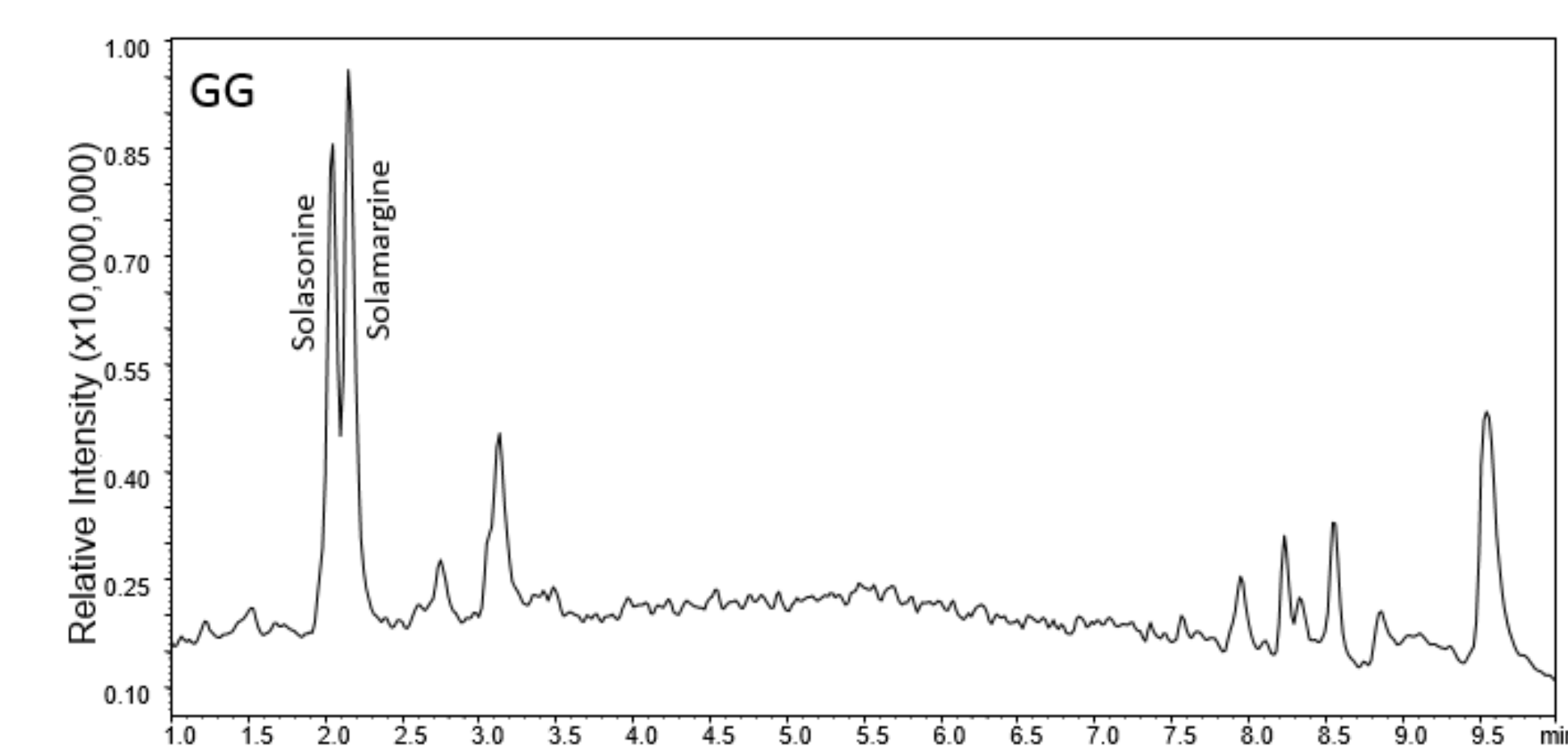


Figure 10. An example of a MS total ion chromatogram, shown for a GG berry extract.

CONCLUSION

We observed that levels of toxic glycoalkaloids solasonine and solamargine do decrease as the berries mature, and so our prediction was correct. [3] The glycoalkaloids most likely act as toxic deterrents to prevent premature seed dispersal, and decrease once the berries are mature. Although herbivores are initially attracted to the colourful pigments, the berries' bitterness combined with the toxicity ultimately deters them. [1]

The sudden decrease in glycoalkaloid levels coincides with a burst of pigmentation from G to OG. This indicates that both colour and glycoalkaloid levels change to display maturity to herbivores. From G to OG, water levels also decrease suddenly from about 83% to 78%, thereafter plateauing. Although other metabolites were not analyzed there may be increased sugar production at this stage, incentivizing seed dispersal along with decreased glycoalkaloid levels.

The levels of some minor alkaloids (Fig 8) also decrease with maturity, but their levels are not usually as high as solasonine and solamargine, so we do not believe that their presence is a major factor in influencing herbivore consumption habits. This indicates that they may be degradation products, forming throughout development. [2]

These results offer insight into herbivores' interactions with these berries, and with further research they could give us a better idea of the defense and reproduction strategies of *S. dulcamara*.

A sugar analysis could be done in the future to corroborate these results and see if primary metabolites are also produced in higher levels as the berries mature. This could show whether primary metabolites are incentives or deterrents to seed dispersal.

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