

# Investigation of Metabolism of Anthocyanidins by *Heliothis virescens*

Lindsay N. Ewert, Greg L. Thompson and James M. Chapman  
Rockhurst University, Kansas City, MO



## ABSTRACT

Punctured flower buds, devoured flowers, and leaves punched full of holes are sure signs that the plants are infested with tobacco budworms (*Heliothis virescens*). They feed on a large variety of bedding or potted plants and are particularly fond of geraniums and petunias. The small green caterpillars grow to about 1½ inches, but they can be difficult to spot; one of their mechanisms of camouflage lets their bodies take on the color of their recent meal. The colors that the caterpillars incorporate into their tissues and excrete in their frass in the case of the petunias and geraniums are based upon anthocyanin structures. The anthocyanins found in the flower petals are glycosylated, but are also frequently acylated with caffeic or coumaric acid moieties. The presence of the glycosylated forms of the anthocyanins in the flower tissues leads to the speculation that the caterpillars may be ingesting and metabolizing these compounds for the attached carbohydrates. The associated anthocyanin core is potentially of no use to the caterpillar as a food source, but may be sequestered in the tissues for the purpose of predator avoidance. It is certainly excreted in the frass as evidenced by the colorful deposits on and around the plant. This investigation has examined the anthocyanidins present in the flower petals and compared these to those found in the frass and sequestered in the tissues of the caterpillar in an attempt to ascertain the metabolic fate of the anthocyanidins.

## INTRODUCTION

Petunia Hybrid Blue Wave is available through most retailers and is a hardy and colorful bedding plant. The characteristically deep purple color of Blue Wave is due to anthocyanins, a large family of glycosylated polyhydroxy and polymethoxy derivatives of flavilium salts. Blue Wave is principally composed of malvidin-based anthocyanins. Anthocyanins, in fact, are responsible for many of the fruit and floral colors in nature. Also present in the petals are flavanols and flavones that are glycosylated and acylated similarly to the anthocyanins. Both anthocyanins and flavanols are easily characterized through the utilization of reversed-phase HPLC with UV-Vis detection coupled to electrospray ionization mass spectrometry (ESI-MS). The ingestion of the flower petals by the budworms is presumably carried out to obtain energy. Since the anthocyanidins, flavanols, and flavones are glycosylated, it could be speculated that the metabolism of these compounds would provide glucose to the budworm. The potential sequestering of the pigments into the tissues of the budworm is of interest as another fate of the anthocyanins since the worms become purple themselves. The frass of the budworm is highly colored, therefore a significant amount of the anthocyanidin is expelled without metabolism. However, it is not known how the attached sugars and acylating groups are handled. The purpose of this study is the characterization and identification of anthocyanins present in Blue Wave petunias and the elucidation of the metabolic fate of these anthocyanins in petunia budworms.

## EXTRACTION AND ISOLATION OF ANTHOCYANINS

Fresh petunia petals, frass, and the skin of the petunia budworm were treated with 50/50 methanol/ water with 0.1% formic acid, ground with a glass stirring rod and placed in sonicator for several minutes to extract the anthocyanins. The mixture was filtered through a cotton plug in a glass transfer pipette to remove large particles. A small aliquot of the aqueous fraction was filtered through a 0.2 µm nylon syringe filter (Whatman Inc., Clifton, NJ) prior to introduction into the LC.

## INSTRUMENTATION – HPLC/DAD/ESI-MS/MS ANALYSES

LC/ESI-MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x .5 mm i.d., Zorbax SB-C18 5 µm (Agilent, Palo Alto, CA). Solvents were (A) 0.1% formic acid/ 99.9% water (v/v) and (B) 0.1% formic acid/ 99.9% acetonitrile (v/v). Solvent gradient was 0-20 min, 10-50% B; 20-31 min, 50% B; and 31-35 min, 50-10% B. Flow rate was 6.000 µL/min, injection volume was 0.5 µL, and column temperature was 25 °C. The ESI parameters were as follows: nebulizer, 13 psi; dry gas (N<sub>2</sub>), 4.00 L/min; dry temperature, 325 °C; trap drive, 76.5; skim 1, 40 V; lens 1, -5.00 V; octopole RF amplitude, 150 Vpp; capillary exit, 158.5 V. The ion trap mass spectrometer was operated in positive ion mode scanning from m/z 100 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 30000 units and maximal accumulation time was 300000 µs. MS-MS was operated at a fragmentation amplitude of 1.2 V, and threshold ABS was 3,000,000 units.

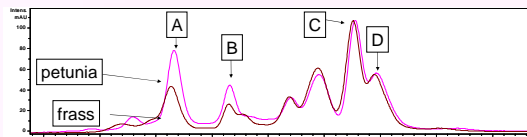


Figure 1. HPLC trace at 530nm normalized to illustrate qualitative reduction in two of the anthocyanin peaks.

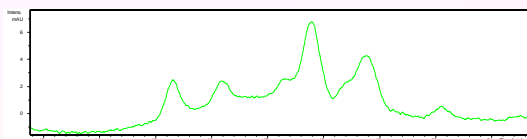


Figure 2. HPLC trace at 530nm of the extract obtained from the skin of the budworm



Figure 3. Budworm which has ingested petunia leaves (left) and one that has ingested petunia blooms (right)



## RESULTS

### MASS SPECTRAL ANALYSIS

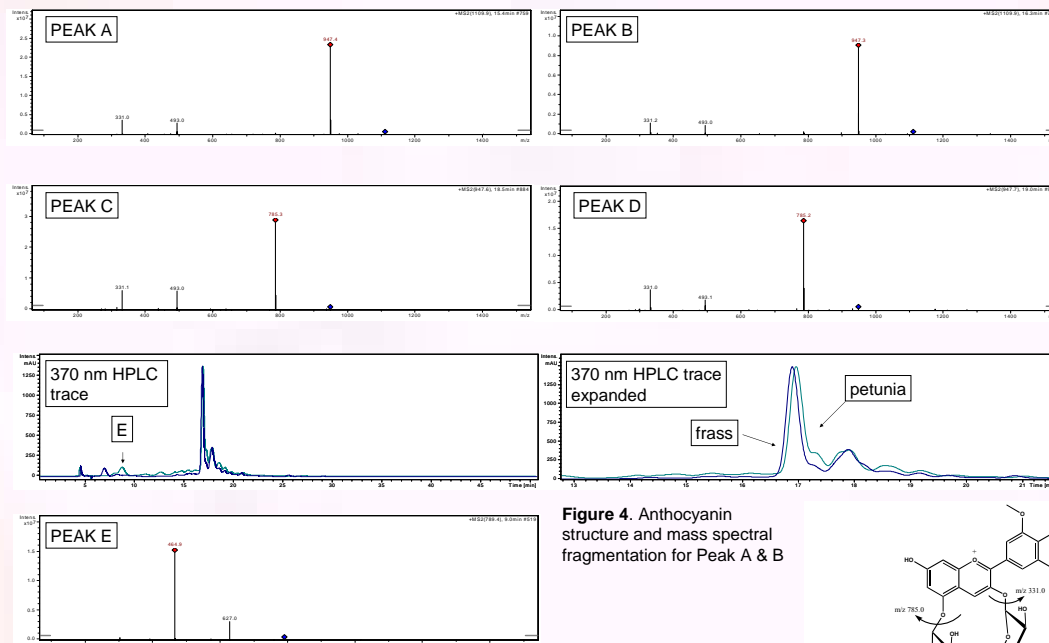
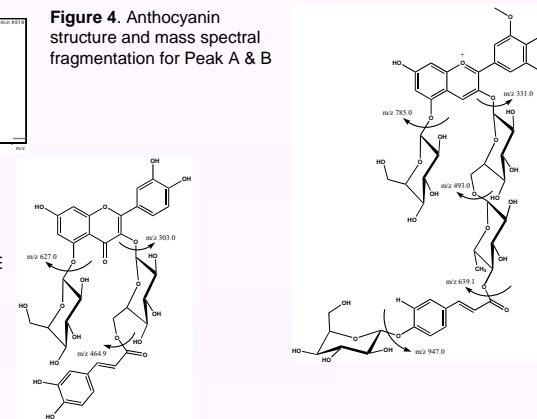


Figure 4. Anthocyanin structure and mass spectral fragmentation for Peak A & B

Figure 5. Flavanol structure and mass spectral fragmentation for Peak E



## CONCLUSIONS

The examination of the anthocyanins and flavanols present in the blooms led to the tentative identification of 6 anthocyanins and 3 flavanols. Of these, two anthocyanins and one flavanol were easily identified as being reduced in relative quantity to the other anthocyanins in the frass. While it was not possible to quantify the amount of flower petals the budworms ingested, it was possible to compare the relative amounts of the anthocyanins within a select sample. The worms cannot synthesize the anthocyanins and as a result peak heights could not increase, therefore the HPLC traces were normalized with respect to the highest remaining concentration in the frass. Figure 1 illustrates the HPLC trace of the anthocyanins in the flower petals and overlaid with that found in the frass. The two anthocyanin Peaks A & B were tentatively identified as the cis/trans isomers of malvidin [3-O-(6-O-(4-O-(4-O-glucopyranosyl) coumaroyl) rhamnosyl) glucopyranoside]-5-O-glucopyranoside, Figure 4. The terminal glucose attached to position 3 or the glucose attached to position 5 could be removed to yield a metabolized peak of mass 947, which was already present in the bloom and identified as Peaks C & D. Peaks C & D did increase with respect to A & B in the frass sample indicating the metabolism of the glucose residues did occur. There did not seem to be any evidence of the further metabolism of C & D and this could possibly be explained by the presence of the coumaryl group in the side chain which would require a different reaction and would not be of use to the budworm as an energy source. Our hypothesis at this point is that the coumaryl group blocks subsequent hydrolysis of additional sugar residues. With regards to the sequestering of the anthocyanins into the tissues of the skin, we were able to demonstrate the presence of several of the anthocyanins in the tissues of the budworm.

## FUTURE WORK

We are currently developing analytical methods of analysis to allow us to further characterize the metabolic fate of the pigment molecules. We also plan to examine the "petunia petal naïve" green budworms to determine if the presence of the pigments in the purple worms are truly unique.