The Influence of Light on Annonaceous Acetogenin Activity In Pawpaw (*Asimina triloba*) Stem and Leaf Tissue

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Introduction

•The pawpaw [*Asimina triloba* (L.) Dunal] tree grows wild in the hardwood forests of 26 states in the eastern United States, including Kentucky, and is usually found in shaded areas of the forest understory (Pomper and Layne, 2005).
•Pawpaw twigs and fruit contain Annonaceous acetogenins compounds which have antitumor and pesticidal properties; these compounds also have antioxidant activity (Pomper et al., 2007; McLaughlin, 2008).
•Annonaceous acetogenin compounds are potent inhibitors of mitochondrial (complex I) as well as cytoplasmic (anaerobic) production of adenosine triphosphate (ATP) and the related nucleotides (McLaughlin, 2008).
•Higher extractable acetogenin levels would be desirable for future product development.
•In beech and oak higher antioxidant enzymatic activities and antioxidant concentrations are induced in leaves at higher light levels than shaded leaves (Hansen et al., 2002).
•Because pawpaw trees prefer the low-light conditions of the forest understory, our working hypothesis was that high light levels stress the pawpaw plant and induce high acetogenin activity in the stem and leaf tissue.
•Brine shrimp mortality is often used to assess the activity of pharmaceutical compounds and can be used to assess acetogenin activity (Ratnayake et al., 1993).

Objective

•To determine if there was a positive correlation between increasing light level and acetogenin activity in the stems and leaves of pawpaw seedlings.

Materials and Methods

•In May of 2008 pawpaw seeds were sown into one-gallon tree pots in ProMix and grown in a greenhouse, until moved outside under 0%, 35%, and 80% shade treatments on August 5, 2008 (Figure 1A).
•A randomized block design was used in the experiment with three replicate seedlings in each treatment in three replicate blocks (3 plants x 3 treatments x 3 blocks) for a total of 27 plants. Plant height (one cm from soil line), stem diameter and number of leaves were recorded at the start of the experiment and six weeks later at destructive harvest (Figure 1B). Stems and leaves were dried at 50ºC.
•Acetogenins were obtained by extracting 2 grams of dried twig tissue with 100% ethanol.
•Following quantification a 2.5 mg/ml solution was made using 95% ethanol, and extract added to 2 dram vials to correspond to 0, 1, 5, 10, and 50 parts per million (ppm).
•After the ethanol was evaporated, ten brine shrimp larvae, taken 48 h after initiation of hatching in artificial sea water, were added to each vial, and the final volume of each vial was adjusted to 5 ml using the artificial sea water.
• After 24 h, survivors were counted and standard deviation was measured from three replicate extracts (Ratnayake et al., 1993) (Figure 1C).
•LC50 (ppm) values were computed using probit regression analysis and log10 transformation for extract concentration using the statistical software SPSS (SPSS Inc, Chicago).

Results

Table 1. Pawpaw Growth Data During Shade Treatments

	Height (cm) 8/5/08	number of leaves 8/5/08
unshaded	15.3	8
35% shade	18.5	8
80% shade	17.6	8
	ns	ns
	Height (cm) 9/22/08	number of leaves 9/22/08
unshaded	20.9	13
35% shade	24.4	12
80% shade	25.0	13
	ns	ns
	Change in Height (cm)	Change in number of leaves
unshaded	+5.6	+5
35% shade	+5.9	+4
80% shade	+7.4	+5
	ns	ns

Figure 1. A) Shade frames containing pawpaw seedlings at 35, 80, and 0% shade. B) Measuring pawpaw seedling height, diameter, and leaf number at destructive harvest. C) Brine shrimp larvae as seen in petri dish under microscope. D) Chemical structure of the acetogenin Asimicin.

Figure 2. Brine Shrimp Mortality for Pawpaw Stem Extracts

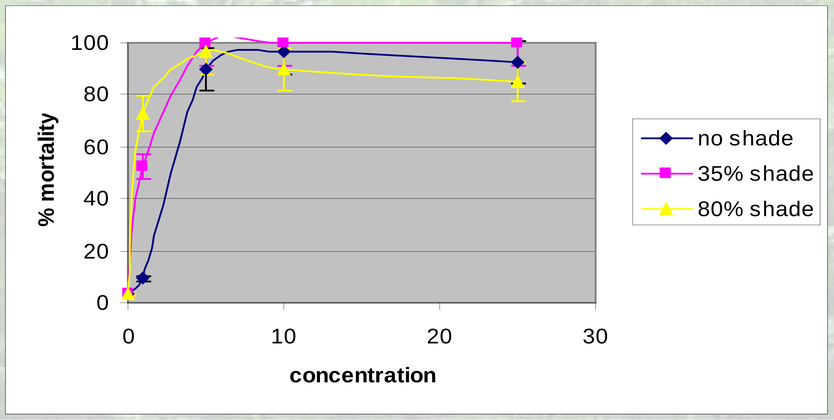
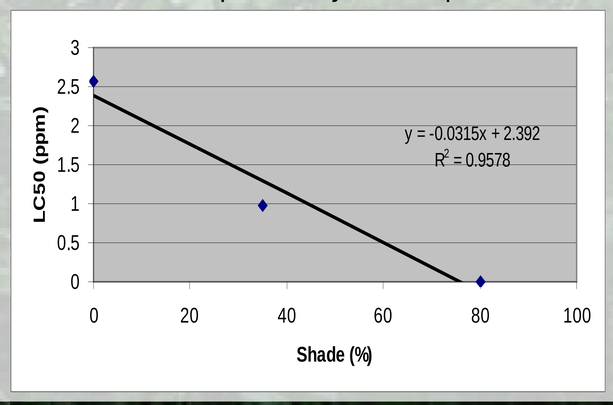


Figure 3. Brine Shrimp Mortality for Pawpaw Stem Extracts



Results

- There were no significant differences in seedling height and leaf number among shade treatments at the beginning or end of the experiment; however, all plants did show an increase in height and leaf number at the end of experiment (Table 1).
- In all shade treatments, brine shrimp mortality increased with higher concentrations of stem extract, with mortality reaching over 90% at 10 ppm or above in each shade treatment (Figure 2).
- With the 1 ppm extract, mortality was highest in the 80% shade treatment, followed by the 35% shade treatment, with the lowest activity in the unshaded control.
- The LC_{50} for each shade treatment was 2.564, 0.982, and 0.002 ppm for the 0%, 35 %, and 80% shade treatments, respectively.
- There was a negative correlation (R^2=0.96) between LC_{50} (ppm) and shade level (%) (Figure 3).

Discussion

- Acetogenin activity, as evaluated using the brine shrimp assay, increased with shading.
- Although acetogenins may be antioxidants, they are apparently not involved in acclimation to high light environments, as has been reported for antioxidants in other plants.
- Other antioxidant compounds could be involved in high light acclimation in pawpaw.
- Acetogenins may serve as anti-feeding compounds under the shaded environment that usually promotes pawpaw growth.
- We reject our hypothesis that high light levels stress the pawpaw plant and induce high acetogenin activity in the stem and leaf tissue.

Conclusion

- There was a negative correlation between LC_{50} (ppm) and shade level (%), indicating shading actually increased acetogenin activity in pawpaw stems.

Literature Cited

- Hansen, U., B. Fielder, and B. Rank. 2002. Variation of pigment composition and antioxidative systems along the canopy light gradient in a mixed beech/oak forest. Trees 16:354-364.
- McLaughlin, J.L. 2008. Paw Paw and Cancer: Annonaceous Acetogenins from Discovery to Commercial Products. Journal of Natural Products, 71:1311-1321.
- Pomper, K.W., J. D. Lowe, S.B. Crabtree, and W. H. Keller. 2007. Assessment of Annonaceous Acetogenin Activity in Pawpaw (*Asimina triloba*) Fruit. HortScience 42:939.
- Pomper, K.W. and D.R. Layne. 2005. The North American pawpaw: botany and horticulture. Hort. Rev. 31:351-384.
- Ratnayake, S., J.K. Rupprecht, W. M. Potter, and J.L. McLaughlin. 1993. Evaluation of the pawpaw tree, *Asimina triloba* (Annonaceae), as a commercial source of the pesticidal annonaceous acetogenins. p. 644-648. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York.