Potential of Ripe Pawpaw Fruit Extract as an Insecticide and Feeding Deterrent for Striped Cucumber Beetle (Coleoptera: Chrysomelidae) on Squash[1]

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Abstract Laboratory experiments were performed to study the effects of pawpaw, *Asimina triloba* (L.) Dunal, fruit extract on mortality and feeding deterrence of striped cucumber beetle, *Acalymma vittatum* (F.). Recently, fruit tissues of pawpaw were found to contain phenolic and antioxidant compounds, as well as annonaceous acetogenin compounds having insecticidal activity. Ripe pawpaw fruit pulp from a range of pawpaw varieties was extracted with 100% ethyl alcohol to obtain acetogenin compounds. Pulp extracts of 0, 10, 100, 1,000, 10,000 and 50,000 ppm were then used to assess feeding deterrence and mortality of beetles. Buttercup squash leaf disks 3.5 cm in diameter were treated individually with each concentration and placed on water moistened filter paper in plastic Petri dishes (9 cm diam). Five striped cucumber beetles were placed on each leaf disk. All Petri dishes were then placed in an environmental growth chamber at 27°C and a 16:8 h light:dark photoperiod. Feeding activity was recorded 1, 4 and 24 h after beetle introduction. After 24 h the beetles were removed. Beetles did not feed on treated squash leaves at either 1 or 4 h of exposure. However, significant feeding occurred between 4 and 24 h after beetle introduction. Feeding was lowest and feeding damage least on 50,000 ppm pawpaw-treated leaf disks compared with leaf disks treated with < 10,000 ppm dilutions. Pawpaw fruit extract reduced feeding by 89% and 97% in the 10,000 and 50,000 ppm treatments, respectively. The calculated LC_{50} value was 50,538 ppm whereas the LCF_{10} (concentration at which only 10% of the leaves were consumed) was 2,033 ppm. At 10,000 ppm 10% of the beetles were killed; however, only 3% of the leaf tissue was consumed. Thus, pawpaw fruit extract may be an effective insect feeding deterrent. The duration of treatment effectiveness and susceptibility of other pest and beneficial insect species to the extracts also needs to be examined.

Key Words pawpaw fruit pulp, feeding deterrence, *Acalymma vittatum, Asimina triloba*

The striped cucumber beetle, *Acalymma vittatum* (F.), is an important pest of pumpkin, squash, watermelon and cucumber. In the adult stage, it is also a pest of sweet corn, beans, peas, and other vegetables (Metcalf and Metcalf 1993). Adult beetles are 5 - 7 mm long and are yellow-green with 3 black stripes. Adults feed on foliage, flowers, pollen and roots and can transmit the bacterium that causes bacterial wilt, *Erwinia tracheiphila* (E.F. Smith) Holland, a severe disease of pumpkins, squash and cucumbers (Hoffman 1998).

The conventional method of controlling the striped cucumber beetle on vegetable crops is to use synthetic chemical insecticides such as imidacloprid, permethrin and

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carbaryl. Organic methods of control include companion planting by planting trap crops and beneficial insect-attracting plants, using aluminum plastic mulch, and/or botanical compounds such as neem, rotenone and pyrethrum (Cline et al. 2008).

Annonaceous acetogenins are a large class of unique structurally homogenous polyketide (C32 or C34 fatty acid) compounds found in the Annonaceae family, which includes both the genera *Annona* and *Asimina* (Mikolajczak et al. 1988, Alali et al. 1999, McLaughlin 2008). These compounds are potent inhibitors of mitochondrial (complex I) as well as cytoplasmic (anaerobic) production of adenosine triphosphate (ATP) and related nucleotides. Acetogenin compounds are powerful cytotoxins and display *in vivo* antitumor, pesticidal, antimalarial, anthelmintic, piscicidal, antiviral, and antimicrobial properties, suggesting many potentially useful applications (McLaughlin 2008).

The North American pawpaw, *Asimina triloba* (L.) Dunal, is a tree fruit in the initial stages of commercial production in the United States (Layne 1996, Pomper and Layne 2005). Over 40 bioactive acetogenins and other compounds have been identified in the crude extracts of twigs, unripe fruits, seeds, root, and bark of the pawpaw (Ratnayake et al. 1992, Zhao et al. 1992, Gu et al. 1999). The month of highest activity in pawpaw twigs is in May, when the trees are actively growing, with the concentrations of asimicin, bullatacin, and trilobacin also peaking concurrently (Gu et al. 1999).

Insecticides based on acetogenins are slow-acting toxins and can be particularly effective against chewing insects (Isman 2006). Acetogenin compounds are toxic to several pest insects including German cockroach nymphs, *Blattella germanica* (L.); Colorado potato beetle, *Leptinotarsa decemlineata* (Say); blowfly larvae, *Calliphora vicina* Robineau-Desvoidy; Mexican bean beetle larvae and adults, *Epilachna varivestis* Mulsant; bean leaf beetle, *Cerotoma trifurcata* (Forster); yellow fever mosquito larvae, *Aedes aegyptii* (L.); two-spotted spider mites, *Tetranychus urticae* Koch; striped cucumber beetles; European corn borers, *Ostrinia nubilalis* (Hübner); melon aphids, *Aphis gossypii* Glover; and the nematode, *Caenorhabditis elegans* (Maupas) (Rupprecht et al. 1986, Alkofahi et al. 1989, Ratnayake et al. 1992, Lewis et al. 1993, He et al. 1997). The role of acetogenin compounds as insect feeding deterrents has not been fully explored. Twig extracts contain acetogenins (e.g., ascimicin) that are effective against several pest species, including the adult striped cucumber beetle, at 5,000 ppm (Mikolajczak et al. 1988). However, twig extracts were never successfully commercialized due to limited availability of twig biomass and high production costs. Ripe pawpaw fruit was recently reported to be rich in acetogenins (Pomper et al. 2009), potentially offering a much larger and more cost effective source of acetogenins. Acetogenin compound composition may vary between pawpaw twig and fruit tissues and additional substances, such as phenolic and antioxidant compounds in the fruit pulp (Kobayashi et al. 2008), may influence crude extract efficacy. Although ripe pawpaw fruit extracts show positive brine shrimp testing activity (Pomper et al. 2009), bioactivity against insects needs to be demonstrated. Therefore, the objective of this study was to determine insecticidal activity and feeding deterrence of ripe pawpaw fruit pulp extract on striped cucumber beetle on buttercup squash, *Cucurbita maxima* Duchesne var. *turbaniformis* (M. Roemer) L.H. Bailey.

Materials and Methods

Plant rearing. Untreated buttercup winter squash seeds were grown in flats using Premier Professional ProMix® potting soil (Premier Horticulture Inc., Quakertown, PA).

Flats were placed in an environmental growth chamber at 27°C and a 16:8 h light:dark photoperiod. Once plants reached the 2 - 3 leaf stage, flats were moved into a solarium, and new plants were started. Plants were used for bioassays when leaves were large enough to have a 3.5 cm disk cut from them.

Insect rearing. Insects used were obtained from a colony maintained in the laboratory. Fifteen untreated Buttercup squash seeds were placed in rows in a 3.8-L clear plastic container containing ProMix potting soil. Striped cucumber beetle eggs were collected from squash plants and filter papers of previously infested 0.45-L Mason jars by gently brushing the eggs with a small camel's hair paint brush moistened with distilled H_2O into a 200 ml beaker containing approx 150 ml distilled H_2O. Eggs on the sides and bottoms of jars were rinsed with distilled H_2O and brushed into the same beaker. Eggs were then removed from the beaker with a 5 ml pipette and placed on top of the soil in which squash seeds were planted. Approximately 200 eggs were placed in each container, and each container was then covered with nylon organdy, secured with a rubber band and then placed in an environmental growth chamber set at 27°C and a 16:8 h light:dark photoperiod. Fresh cotyledons were replaced as necessary in containers where larvae were feeding. Containers were checked for adult emergence every other day. Fresh squash stems, cotyledons and leaves were replaced as needed in the containers when adult beetles were emerging.

Twenty to 25 adult beetles were collected from containers and placed into ventilated 0.45-L Mason jars containing a 7-cm diam filter paper disk, squash stems, cotyledons and leaves. Adults from these jars were transferred to fresh jars every other day, and the entire process repeated to insure colony continuance. Adults used in bioassays were 1 - 2 wks old.

Plant material and pulp extraction. Ripe pawpaw fruit were collected from 6 cultivars in September 2006 and the fruit mixed prior to processing. Pulp was separated from skin and seeds, homogenized in a food processor, placed in zip-lock bags, and stored in a freezer at -15°C. Ten grams of frozen pawpaw pulp was weighed into a beaker, and the pulp allowed to barely soften. Twenty-five ml of 100% ethanol was added to the pulp in 5-ml increments while thoroughly stirring. The beaker was covered with parafilm and placed in a dark cabinet for 48 h. The homogenate was filtered through coarse filter paper to remove pulp solids. This procedure was repeated 3 times to insure that there would be enough stock solution. Once the homogenate was filtered, the 3 separate solutions were combined into a 250-ml Wheaton bottle. One ml of extract was then placed into a preweighed microcentrifuge tube and incubated at 50°C for 48 h to allow the ethanol to evaporate. The tube was reweighed, and the difference between weights allowed for calculation of the extract concentration in mg/ml. Dilutions of 10, 100, 1,000, 10,000 and 50,000 ppm were then prepared from this stock solution along with 50% ethanol and 50% distilled H_2O. A control (0 ppm) was made using 50% ethanol and 50% distilled H_2O.

Bioassays. A filter paper disk was placed into each 9-cm plastic Petri dish and moistened with 2 ml distilled H_2O. Buttercup squash leaf disks 3.5 cm diam were treated individually by dipping each disk into their respective concentrations for 5 s, shaking excess moisture from the disk and placing them on the moistened filter paper. Five striped cucumber beetle adults were placed on each treated or control leaf disk. Feeding activity was recorded 1 h after introduction. Petri dishes were then placed in an environmental growth chamber set at 27°C with a 16:8 h light:dark photoperiod. Feeding activity was checked again at 4 and 24 h. After 24 h, the number of dead beetles was recorded, the beetles were removed, and photographs were taken of

each leaf disk. The amount of leaf tissue consumed was quantified with a personal computer using the public domain NIH Image J program developed at the U.S. National Institutes of Health (NIH Image 2008). Percentage consumed was calculated by subtracting the area remaining after feeding from the uneaten area before striped cucumber beetle introduction, dividing this by the unconsumed area before introduction and multiplying by 100. For runs 1 and 2, photographs of each leaf disk were taken after removing the striped cucumber beetles at 24 h. Each disk was then "painted" using Adobe Photoshop 6.0 to create the unconsumed area before introduction (Adobe Photoshop). For runs 3 and 4, photographs of each leaf disk were taken before introducing the striped cucumber beetles and again after removing the beetles at 24 h.

Statistical analyses. Leaf consumption and mortality data were analyzed using ANOVA and a protected Fisher's LSD test using CoStat Statistical Software (CoHort Software 2006). The LC_{50} and leaf consumption (LFC_{10}) values were computed using probit regression analysis and a log10 transformation for extract concentration using the statistical software SPSS (SPSS 2005). Mean and standard error were calculated using Excel software (Microsoft Office Excel 2003).

Results and Discussion

Little feeding was observed on the squash leaf disks at 1 and 4 h, but feeding occurred between 4 and 24 h after beetle introduction (Fig. 1). About 20 - 40% of the leaf disks were consumed by the striped cucumber beetles for disks treated with 0 - 1,000 ppm pawpaw pulp extract (Table 1). However, feeding by beetles was greatly deterred by application of higher concentrations of extract to the leaf disks. Feeding was reduced 89% in the 10,000 ppm and 97% in the 50,000 ppm extract treatments. A LFC_{10}

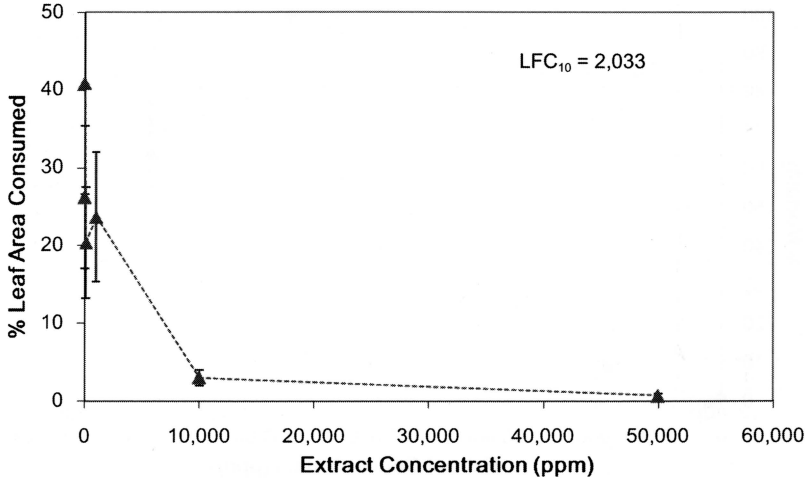

Fig. 1. Average percentage of squash leaf area consumed (± SE for 4 replicates) after 1, 4 and 24 h by striped cucumber beetle under increasing concentrations of ripe pawpaw fruit extract applied to squash leaves.

Table 1. Laboratory assay of striped cucumber beetle mortality and foliage consumption in 24 h on squash treated with ripe pawpaw fruit extract.

Concentration (ppm)	%Consumed*	%Mortality*
0	26.2 ± 8.3ab	1.7 ± 1.7a
10	40.9 ± 14.6a	1.7 ± 1.7a
100	20.4 ± 8.7bc	1.7 ± 1.7a
1,000	23.7 ± 8.4ab	0.0 ± 0.0a
10,000	3.0 ± 0.8cd	10.0 ± 7.9a
50,000	0.7 ± 0.2d	64.9 ± 13.7b
Df	5,15	5,15
F value	6.40	19.04
P value	0.0023	0.00001

* ± SE for 4 replicates.

value of 2,033 ppm was calculated for the extract concentration required to limit feeding to 10% of the leaf. Beetle mortality was greatest between 4 and 24 h after feeding (Fig. 2). The LC_{50} value of 50,538 ppm was calculated for the extract concentration required to kill 50% of the beetles. At 10,000 ppm, leaf consumption was limited to 3% and only 10% of the beetles had been killed. This indicates that the reduction in feeding was due to feeding deterrence of beetles and not just fewer beetles consuming the leaf disks.

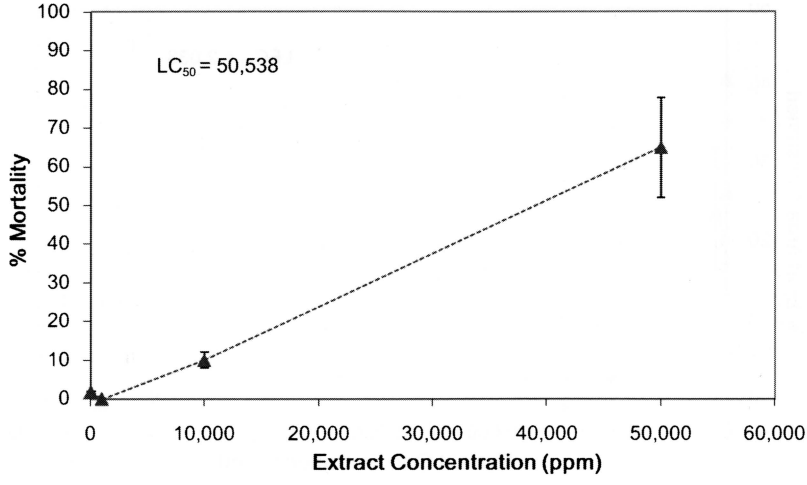

Fig. 2. Average beetle mortality (± SE for 4 replicates) after 1, 4 and 24 h by striped cucumber beetle under increasing concentrations of ripe pawpaw fruit extract applied to squash leaves.

A number of synthetic chemicals are used currently to control many pests of horticultural crops, including striped cucumber beetle, in Kentucky (Bessin et al. 2009). Botanical insecticides are attractive alternatives to synthetic chemical insecticides for pest management of crops because they pose little threat to the environment or to human health; however, few botanicals are currently used and even fewer are allowed for use on organic farms (Isman 2006). Crude extracts containing natural mixtures (acetogenin compounds) as conventional and organic pest control agents have certain advantages, e.g., mixtures may act synergistically, or additively, or slow the development of insect resistance. The short residual activities of alternative botanical insecticides might be less harmful than conventional insecticides to nontarget organisms and the environment. Development of a new botanical pesticide from pawpaw fruit could provide both conventional and organic vegetable and small fruit growers economically viable, environmentally sound and socially responsible production practices. The identification of a larger biomass source of acetogenin compounds would be helpful in developing commercial products based on acetogenin compounds. Mature pawpaw trees produce more fruit tissue (15.9 kg fruit fresh weight/6.4 kg dry weight) than twig tissue (2.3 kg fresh weight/0.8 kg dry weight). Pawpaw fruit represents a larger and more cost effective source of acetogenins than twigs. At high concentrations (i.e., 2,033 ppm and above) pawpaw fruit pulp extract could serve as a feeding deterrent for striped cucumber beetle on cucurbit species.

Additional experiments need to be conducted to determine the optimal concentration of ripe pawpaw fruit extract for striped cucumber beetle feeding deterrence. The persistence of treatment effectiveness and susceptibility of other pest and beneficial insect species to the extracts need to be determined and different pawpaw varieties may have different activity and acetogenin profiles. Finally, ultra violet light stability and field testing should be undertaken with all practical expedience.

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