# Effect of Striped Cucumber Beetle (Coleoptera: Chrysomelidae) Foliar Feeding on Pumpkin Yield<sup>1</sup>

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**Abstract** Field and laboratory studies were conducted to examine the relationship between foliar feeding damage caused by striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), and yield of pumpkin, *Cucurbita pepo* L. (var. Spookie). In field trials in 1996 and 1997, plants were artificially infested with varying numbers of beetles at the cotyledon-, first-, second-, or third-leaf stages. At harvest, fruit number, fruit mass, and fruit maturity were evaluated. Foliar feeding damage had little effect on yield other than a slight increase in number of fruit per plant for plants with 1 to 20% damage. The stage of plant development at infestation had no effect on yield, nor did the interaction of foliar feeding damage and growth stage. In a laboratory trial, varying numbers of *A. vittatum* were allowed to feed for 1, 2, 3, or 4 d on plants at cotyledon-, first-, second-, or third-leaf stages. For all stages, significant and differing positive linear relationships were observed when the percentage of foliar feeding damage was regressed against beetle-days. The results indicated that small fruited, vining pumpkins can tolerate relatively high levels of striped cucumber beetle feeding injury. Foliar feeding damage thresholds are conservatively estimated to be ~60% during cotyledon through third leaf stage of growth.

**Key Words** Acalymma vittatum, Cucurbita pepo, pumpkin, threshold, foliar feeding damage, yield

The striped cucumber beetle, *Acalymma vittatum* (F.), is an important pest of cucurbits. Adults feed on foliage, stems, flowers and fruits (Latin and Reed 1985). Heavy infestations of these beetles can destroy stems and cotyledons of young plants (Davidson and Lyon 1987), occasionally resulting in the loss of entire plantings. Larvae burrow into the roots and stems where they feed for 2 to 6 wk, complete a minimum of three stadia, and then pupate in the soil (Jewett 1927). A survey of fresh market vegetable producers in New York, New Jersey, and Pennsylvania showed that cucumber beetles were considered to be the most important insect pest on pumpkins and all other cucurbits (Hoffmann et al. 1997). In addition, cucumber beetles can vector or predispose plants to several diseases (Rand 1916, Gargerich et al. 1986). Bacterial wilt caused by *Erwinia tracheiphila* (Smith) is a particularly important disease vectored by cucumber beetles in melons, *Cucumis melo* L., and cucumbers, *C. sativus* L. The disease has historically been less important in pumpkins *Cucurbita pepo* L. and squash *C. maxima* Duch, but now there is a more virulent strain affecting these cucurbits as well (Zitter 1999).

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A notable characteristic of the striped cucumber beetle is the rapid colonization of young cucurbit seedlings following transplanting or just as they emerge. The rapid appearance of large numbers of beetles and their damage to young plants often results in an insecticide application by the producer with little regard for the relationship between damage to the crop and yield loss.

An understanding of the relationship between foliar feeding damage and yield is fundamental to the development of an appropriate action threshold for striped cucumber beetle damage to pumpkin. To date, there has been little research on the importance of cucumber beetle leaf feeding injury on pumpkin yield. However, research on other cucurbits suggests that tolerance can be quite high. Brewer et al. (1987) reported that summer squash infested with striped and spotted cucumber beetles at second and third leaf stages were typically able to compensate for growth delays before fruit production. Burkness and Hutchison (1998) reported that young cucumbers withstood high levels of simulated beetle foliar feeding damage before yield loss occurred. Ayyappath et al. (2002) demonstrated that winter squash was also relatively tolerant of beetle foliar damage. Hoffmann et al. (2000) found that pumpkins could tolerate relatively high levels of simulated foliar feeding damage, but the damage in that study was artificial (leaf area was cut away) and was a single event, in contrast to continuous feeding that would occur with natural infestations. To address that shortcoming, we examined the relationship between foliar feeding damage caused by striped cucumber beetles and yield of pumpkin under field conditions. We also conducted a laboratory experiment to help understand the relationship between number of beetles per plant and leaf feeding damage over time.

#### Materials and Methods

Field trials. Trials were conducted in 1996 and 1997 at the Cornell Univ. Depart. of Entomology Research Farm, Freeville, NY, to determine how yield of pumpkin 'Spookie' (Harris Seeds, Rochester, NY) would be affected by cucumber beetle feeding injury to cotyledon-, first-, second-, and third-leaf plants. 'Spookie' is a small fruited, vining cultivar with medium-sized leaves. Transplants were grown in 36-cell flats (2 plants/cell) containing general purpose growing medium (Premium Horticultural Inc., Redhills, PA) in a greenhouse maintained at ~28°C under normal daylight conditions. Cotyledon-stage seedlings were transplanted (21 June 1996, 18 June 1997) into rows 1.5 m apart, and spaced 1.2 m apart within rows, similar to commercial plantings. Once established, alternate hills within rows were thinned to a single plant leaving 256 and 320 individual plants of similar size for the planned experiments in 1996 and 1997, respectively. The unthinned hills were considered buffers and were retained so that overall plant density approximated that of a commercial planting. Immediately after thinning, all experimental plants were covered with cages (50 cm height, 25 cm diam) made of No-See-Um nylon organdy mesh (Balson-Hercules, NY) to prevent feeding injury by natural populations of striped cucumber beetles. The cages were held in position using bamboo sticks. In both years, plots received banded applications of 60 kg N ha<sup>-1</sup> (ammonium nitrate) before transplanting and 120 kg N ha<sup>-1</sup> (ammonium nitrate) as top dressing when vines began to run.

To create a range of foliar injury to plants at the cotyledon-, and first-, second- and third-true leaf stages of plant development, cages were infested with varying numbers of beetles soon after thinning of hills. A leaf was considered to be a true leaf once it was completely unfolded. The beetles used had been collected from nearby com-

mercial fields of cucurbits. The number of beetles introduced to each cage when plants were at the cotyledon-, and first-, second-, and third- true leaf stages of plant development were: 0, 5, 10, and 15; 0, 10, 15, and 20; 0, 15, 20, and 25; and 0, 20, 25, and 30 beetles, respectively. The reason for increasing the number of beetles as stage of plant development increased was to help ensure that we had approximately the same range of foliar damage (percentage leaf area) at all stages of plant development. The beetles were typically in cages for 2 to 4 d and the plants generally remained in their age class during that time period. After cages were infested with beetles, plants were observed each day and leaf feeding damage was visually estimated.

Because the cotyledons were similar in size across plants we used them as our standard to estimate leaf area damage for the entire plant. Each cotyledon was visually divided into 10 equal "cotyledon units". The area of true leaves were subsequently also estimated in cotyledon units. For example, a true leaf that was twice the size of a cotyledon would have 20 cotyledon units. A true leaf one-half the size of a cotyledon units to estimate the total area damaged by beetle feeding we estimated the number of cotyledon units damaged divided by the total number of cotyledon units for the entire plant. The result was a reasonably accurate estimate of percent leaf area damage for the entire plant despite the constraints of not being able to manipulate or directly measure plant leaf area or damage. Feeding on the leaf surface (scouring) was considered to be the equivalent of feeding that extended through the leaf creating holes. To minimize variation, all estimates were made by the same person.

Individual plants from each growth stage were categorized using a scale that reflected percentage of total leaf area of the plant with feeding damage; 0 = 0%, 1 =1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, or 5 = >80%. At the end of the infestation period, plants were protected from further damage by removing the beetles and by spraying the plants with 0.5% carbaryl (Sevin XLR Plus, Rhone-Poulenc Ag. Company, Research Triangle Park, NC) to kill any beetles that may have been missed. Possible confounding associated with the duration of time plants were in cages (e.g., cage effect) was minimized because all plants were caged for the same length of time. When cages were removed, the soil was drenched with 0.5% diazinon (Diazinon 4E, Ciba-Geigy, Greensboro, NC) to minimize confounding the experiment with root damage, caused by larval progeny of the caged beetles. Our focus was limited to leaf feeding damage and any plant damage or stress related to root feeding could potentially have affected yield. Following cage removal and as the crop matured, the fungicides triadimeton (Bayleton 50% DF, Bayer, Kansas City, MO), benomyl (Benlate, Dupont, Wilmington, DE), and chlorothalonil (Bravo 720, ISK Bioscience, Mentor, OH) were applied at the label rates as needed to control powdery mildew.

On 8 October 1996 and 6 October 1997 pumpkins were harvested and the number, fresh weight, and maturity of fruit per plant were recorded. Pumpkin maturity was determined using a scale based on the percentage of the individual fruit surface with an orange-yellow color: 1 = 1 to 15%, 2 = 16 to 50%, 3 = 51 to 85%, and 4 = >86%. A few pumpkins were <1 kg, completely lacked yellow color or were malformed, rotten, or diseased. These were discarded and excluded from analyses.

The experiments were conducted using a completely randomized design with individual plants considered a replicate. Yield data were analyzed using a mixed model analysis of variance (PROC MIXED). Plant growth stage and foliar feeding damage were considered fixed effects, and year as a random effect. Mean separations were accomplished by *t*-tests of differences between least squares marginal means using Tukey's adjustment. Because we used different numbers of beetles to generate a range of damage levels at each stage of plant growth we did not attempt to relate beetle numbers to ultimate yield and maturity. Our goal was to relate various levels of damage to yield and maturity.

Laboratory trials. The objective of this laboratory study was to determine the rate at which beetles damaged pumpkin foliage (e.g., beetle days). Striped cucumber beetles used in this experiment were collected from commercial fields of cucurbits during mid-June 1996, held at ~28 C and provided pumpkin foliage ('Spookie') for nutrition.

Trials were conducted beginning mid-July 1996, on pumpkin 'Spookie' seedlings at cotyledon-, first-, second-, and third-leaf stages. Pumpkin plants were grown individually in 15 cm diam pots containing general purpose growing medium (Premium Horticultural Inc., Redhills, PA), in a greenhouse maintained at ~28 C. Once plants reached the appropriate growth stage they were transferred to a growth chamber maintained at 16:8 L:D,  $28 \pm 2$  C, and 60% RH. Plants were covered with cages (50 cm height, 25 cm diam) made of white No-See-Um netting (Balson-Hercules, NY) and held in position using bamboo stakes. Cotyledon and first-leaf stage plants received 2, 4, 8, or 16 beetles. Second and third leaf stage plants received 4, 8, or 16, beetles. Thus, there were 14 combinations of stage x beetle number. Insects in each cage were counted daily, and dead beetles replaced. Beetles were allowed to feed for 1, 2, 3, or 4 d to determine the rate of foliar feeding damage caused by a known number of beetles over a known period of time. Plants typically remained at their respective stages of growth throughout the experimental period.

Experimental treatments were arranged in 6 randomized complete blocks with each of the 14 combinations (plant growth stage x beetle number) represented 4 times in each block for a total of 336 possible observations. On each of 4 d, one plant of each growth stage x beetle number combination was removed from each block. Upon removal, leaf surface area measured with a leaf area meter (Model LI-3050A, LI-COR, Lincoln, NE) using the following methods. To calculate total leaf area we traced onto paper what we presumed to be the outline of an undamaged leaf. If the leaf edge was damaged we estimated (presumed) the leaf outline as if no damage was present. This was then cut out and the area measured. A second tracing of the leaf was made, but this time including areas fed upon by beetles and areas scoured. This new area was also cut from the paper and area measured. The difference between the first and second tracing provided an estimate of leaf area damaged by beetles. To provide a simple measure of rate of damage by beetles, we calculated 'beetle-days' at each stage of plant development. Beetle-days were calculated as the number of beetles per plant x number of days the beetles were allowed to feed on plants.

Least squares linear regression analyses were performed with percentage foliar feeding damage regressed on beetle-days. Multiple comparisons of regression slopes were conducted using an ANCOVA model for the raw data rewritten as a weighted ANOVA model (SAS Institute 2000).

#### Results

**Field trials.** Over the 2 yrs, 32 (5.55%) out of 576 infested plants died, apparently due to complete consumption by beetles, injury to the hypocotyl or stem, or for

reasons unknown. However, the level of plant mortality within main effects was never significantly different (P > 0.05) from the mortality in the respective controls thus could not be attributed to treatment affect.

In 1996 and 1997 our goals were 16 and 20 replications, respectively, of each combination of growth stage and foliar feeding damage. However, attaining damage levels >60%, especially at the second- and third-leaf stages, was not always accomplished, thus the number of replicates per treatment combination varied (Fig. 1). We were rarely able to obtain >80% leaf damage except for cotyledon stage plants, and damage per increment beetle tended to decrease for third-leaf plants (Fig. 2). Because these particular data were sparse they were not included in subsequent analyses.

The overall effect of foliar feeding damage on yield and maturity was minimal. The mean number of fruit per plant was affected by level of foliar feeding damage (F = 2.52; df = 4,469; P = 0.041) but not by plant growth stage nor by an interaction of these factors. Comparisons of least squares means indicated that the difference was largely attributable to a slight increase in the number of fruit in plants experiencing 1 to 20% leaf damage (Table 1). Fruit mass per plant and maturity rating were not affected (P > 0.05) by stage of plant growth, foliar feeding damage, or by their interaction.

Laboratory trials. The procedures used resulted in a range of leaf damage levels. Up to 40% leaf damage was attained for most growth stages but relatively few plants at the first leaf stage or older incurred damage levels >60% (Fig. 3). Significant positive linear relationships were observed between percentage foliar feeding damage and beetle-days for all growth stages (Table 2). Further, the percentage of damage caused by a given level of beetle-days depended on growth stage at time of infestation with cotyledon stage plants showing the largest change in foliar feeding damage with changes in beetle-days (Fig. 4). Feeding damage to second- and third-leaf stage plants was similar and was less sensitive to an increase in beetle-days than were cotyledon-stage plants. Feeding damage to plants at the first leaf stage was not

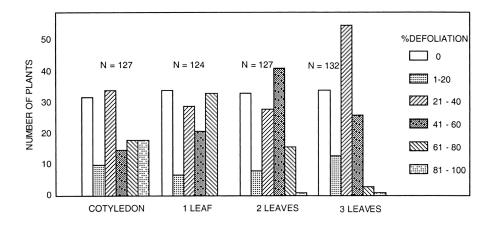


Fig. 1. Number of 'Spookie' pumpkin plants in each growth stage and foliar feeding damage category in field trials conducted in 1996 and 1997.

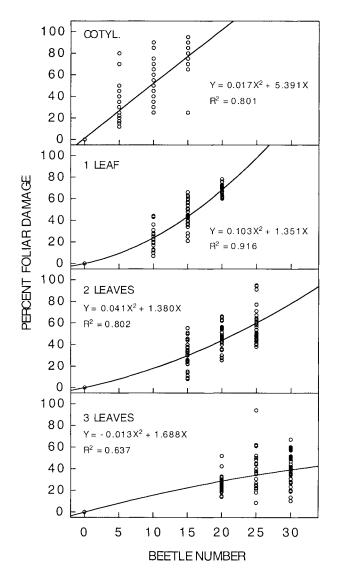


Fig. 2. Influence of striped cucumber beetle number on percent foliar damage to 'Spookie' pumpkin plants for cotyledon through third-leaf plants. Combined data from 1996 and 1997 are presented.

statistically different from either cotyledon-, second-, or third-leaf stage plants, suggesting an intermediate response to beetle pressure (Table 2).

## Discussion

Our studies indicate that a small vining variety of pumpkin, such as "Spookie" can tolerate considerable foliar feeding damage by striped cucumber beetle without a

Feeding damage (%)	Fruit mass/plant Fruit/plant (Kg) Maturity rating/pla				
0	2.34 ± 0.36 ab*	4.43 ± 1.12 a	2.11 ± 0.70 a		
1-20	2.84 ± 0.39 a	5.11 ± 1.16 a	2.15 ± 0.71 a		
21-40	2.26 ± 0.36 b	4.47 ± 1.12 a	2.23 ± 0.70 a		
41-60	2.25 ± 0.37 b	4.54 ± 1.12 a	2.22 ± 0.70 a		
60-80	2.17 ± 0.40 b	4.19 ± 1.16 a	2.28 ± 0.71 a		

Table 1. Least squares means (±SEM) estimates of harvest variables after incurring various levels of foliar damage to pumpkin 'Spookie'

\* Values followed by same letter are not significantly different (P < 0.05, Tukey's Studentized Range (HSD) Test [SAS Institute 1998]).

#### Table 2. Linear regression statistics (± std. err. of parameter) for percentage foliar feeding damage regressed onto beetle-days, for pumpkin 'Spookie'

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Growth stage	Slope	Intercept	ŕ	F	Р	
cotyledon	1.17 ± 0.21 a*	22.18 ± 6.26	0.76	29.64	0.0006	
1st leaf	0.78 ± 0.05 ab	12.40 ± 1.56	0.96	209.53	0.0001	
2nd leaf	0.49 ± 0.06 b	$9.82 \pm 2.04$	0.89	60.21	0.0002	
3rd leaf	0.48 ± 0.06 b	6.13 ± 1.84	0.91	71.97	0.0001	

\* Slope values followed by same letter are not significantly different (*P* < 0.05, Tukey's Studentized Range (HSD) Test [SAS Institute 2000]). Intercepts were not tested.

negative impact on yield or maturity. We observed similar results in trials that simulated beetle leaf damage to pumpkin (Hoffmann et al. 2000). Those trials showed that neither the level of leaf area removed nor plant stage at the time of simulated leaf damage had a significant effect on pumpkin yield. Related studies in other cucurbits (Brewer et al. 1987, Burkness and Hutchinson 1998, Ayyappath et al. 2002) also demonstrated a general tolerance for foliar feeding damage by striped cucumber beetle.

In the field experiments that we conducted, foliar feeding damage >60% was difficult to achieve for plants with two or more leaves despite increasing the numbers of beetles placed on such plants (see Fig. 2). Partial regression coefficients for the second order terms declined with progression from first to second to third leaf stages, indicating a diminished response with incremental increases in beetle number. By the third leaf stage, an injury plateau was predicted at approximately 55% and 50 to 60 beetles per plant. However, although beetle number may exceed 50 to 60 beetles per plant, typically relatively few plants in a field are heavily infested, thereby diluting overall field damage. A combination of rapid growth by the pumpkin plants, compensating for leaf damage, and interference in feeding behavior because of crowded conditions may limit the rate and extent of damage.

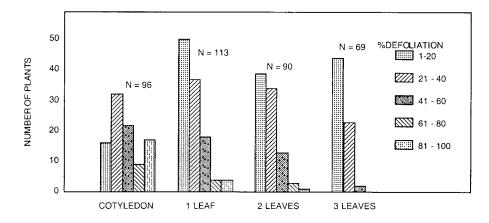


Fig. 3. Number of 'Spookie' pumpkin plants in each growth stage and foliar feeding damage category in laboratory trials conducted in 1996.

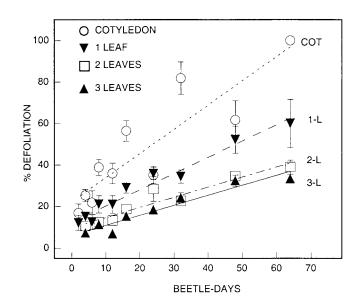


Fig. 4. Least squares linear regressions for growth stages of 'Spookie' pumpkin subjected to varying numbers of striped cucumber beetles and varying durations.

In our laboratory trial, >60% foliar feeding damage was most commonly observed on cotyledon stage plants and percentage leaf damage accrued more rapidly in cotyledon-stage plants, than in second- or third-leaf plants. Plants at the first-leaf stage of growth exhibited an intermediate response. The laboratory trial was conducted at a constant 28 C, and conditions were probably more optimal for beetle feeding than they would be in natural settings. Because the foliar feeding damage may have been greater than typical in field settings, our results should be interpreted with caution as our studies may represent worst case scenarios.

A risk-averse approach to beetle management would be to protect cotyledon and first leaf plants at risk of suffering leaf damage in excess of 60%, or if substantial stem feeding is observed. However, we observed relatively little mortality due to stem feeding in our field trials. Because beetles are highly mobile, estimating beetle number and/or their duration of feeding is difficult under field conditions. Leaf damage, however, is apparent and is relatively easy to estimate.

Plants with >20% foliar feeding damage had reduced fruit number when compared to plants experiencing 1 to 20% leaf damage. Although fruit number declined with increasing foliar feeding damage, total fruit mass was unchanged. This suggests compensation by the plant. The economic effect of this relationship would depend on whether pumpkins are sold by weight or by size. Both forms of marketing are common in the northeastern U.S.

Although we could document no yield reduction in our studies, we propose a conservative action threshold of ~60% leaf area damage for the entire field. In general, monitoring at least bi-weekly during the early stages of crop development is necessary because beetle damage can occur rapidly, especially at the cotyledon stage. Once the true leaves are established, cucumber beetle damage should be monitored at least weekly until the beetle population declines. We issue the caveat that these conclusions pertain only to foliar feeding damage and does not take into account subterranean feeding by striped cucumber beetle juveniles or late-season feeding on fruit. At present, there is a dearth of knowledge regarding the effect of striped cucumber beetle root feeding on plant growth and yield. However, unpublished research conducted by this group has identified no correlation between number of beetles and number of eggs or larvae in the soil. Hence, adjusting thresholds by using beetle number or foliar damage as a proxy for egg or larval density is deemed unlikely.

The results reported here, along with results from the simulated feeding injury studies on pumpkin (Hoffmann et al. 2000), should improve the management of cucumber beetles in pumpkins and reduce pesticide applications. This new information will facilitate the development of improved action thresholds for these important pests of cucurbits in regions where the new virulent strain of bacterial will does not occur. Where the virulent strain exists, even more conservative thresholds will likely be necessary.

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