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## Confirmation of bean leaf beetle, *Cerotoma trifurcata*, feeding on cucurbits

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### Abstract

The objective of these studies was to assess the degree to which bean leaf beetle, *Cerotoma trifurcata* (Forster), will feed on cucurbits. In 2003, we documented an infestation of *C. trifurcata* in a commercial pumpkin field near Rosemount, MN, USA. To evaluate *C. trifurcata* feeding on cucurbits, we conducted laboratory no-choice and choice test feeding studies. In the laboratory, *C. trifurcata* fed most heavily on cotyledon-stage cucumber plants, followed by pumpkin and squash. With soybean plants present, *C. trifurcata* still fed on cucumber plants. However, *C. trifurcata* appeared to prefer soybeans until the quality of the soybean plants was diminished through feeding damage. This is the first known report of *C. trifurcata* feeding on cucurbits. The pest potential of *C. trifurcata* in cucurbit cropping systems should be further evaluated.

**Keywords:** *Cerotoma trifurcata*, host plant, squash, pumpkin, cucumber

### Introduction

The bean leaf beetle, *Cerotoma trifurcata* (Forster), is a pest of leguminous crops throughout the eastern United States (Kogan *et al.* 1980). Adults feed on above ground plant tissue, such as leaves, stems, and pods (Kogan *et al.* 1980). *C. trifurcata* can also vector various pathogens, such as bean pod mottle virus, cowpea mosaic virus, and southern bean mosaic virus (Walters 1969). In Minnesota, *C. trifurcata* completes one generation per year, with peaks in adult abundance occurring in late-May to early-June and late-August (Loughran and Ragsdale 1986). In the Midwestern United States, the abundance of *C. trifurcata* has increased over the past several years (Bradshaw and Rice 2003). Concurrently, the severity and importance of the bean leaf beetle as a pest in leguminous crops has increased (e.g., Hutchison *et al.* 2002; Hutchison *et al.* 2003).

Despite being known to feed primarily on legumes, *C. trifurcata* will also feed on non-legumes. Feeding on non-legume hosts appears to be an early season phenomenon. Helm *et al.* (1983) documented feeding on wild non-legume plants, such as *Urtica dioica*, *Laportea canadensis*, and *Euonymus atropurpurea*. Metcalf and Metcalf (1993) reported that they may feed on corn, *Zea mays*. However, Zeiss and Pedigo (1996), under laboratory conditions, observed no feeding on grasses, such as *Z. mays*, oats, *Avena sativa*, and wheat, *Triticum aestivum*. While *C. trifurcata* may possibly feed on non-leguminous crops (e.g., Metcalf and Metcalf 1993), Tallamy *et al.* (1991) have shown that cucurbitacins from cucurbits serve as a feeding deterrent.

While monitoring for striped cucumber beetle, *Acalymma vittatum* (Fabricius), in pumpkin fields near Rosemount, MN, we

observed a high infestation of *C. trifurcata* and feeding damage on cotyledon-stage pumpkin plants. Cucurbits are grown on ca. 1,900 ha in Minnesota, for a total value of \$8,477,000 (Hutchison and O'Rourke 2002). Historically, *A. vittatum* has been the most important pest in Minnesota cucurbits (Ives and Walters 1985; Subramanyam *et al.* 1993), with the early growth stages of the plants being most vulnerable to attack (Brewer *et al.* 1987; Burkness and Hutchison 1998). The objective of the studies presented in this paper was to assess the degree to which *C. trifurcata* feeds on cucurbits.

### Materials and Methods

#### Field Observations

The pumpkin variety, 'Magic Lantern', was planted on 25 May 2003 into a 4-ha field near Rosemount, MN. The field was planted with soybeans the year before. Pumpkin plants began emerging on 6 June. Cotyledon stage volunteer soybean plants were also present in the field at this time. On 13 June, we began using whole-plant visual inspections to monitor the field for early season pests. Subsequent whole-plant visual inspections were conducted on 16, 17, 18, 20, 23, and 24 June. On each sample date, 60 to 140 plants were examined, and counts of *C. trifurcata* were recorded.

#### No-Choice Feeding Study

To determine if *C. trifurcata* will feed on cucurbits, we conducted a no-choice feeding study and a choice test feeding study in the laboratory. For the no-choice feeding study, seed from pumpkins ('Magic Lantern'), slicing cucumbers ('Marketmore 76'),

and squash ('Turks Turbin') were planted separately into 3.8 liter pots containing universal soil mix. Only one seed was planted in each pot. The pots were held in a growth chamber at 27° C with a 16:8 (L:D) cycle. When the plants reached the cotyledon stage, a small cage was placed over each potted plant. The cages were made from two-liter clear plastic bottles with the bottoms cut off. The large opening at the bottom of the bottles was buried about 2 cm into the soil. Many pinholes were punched into the bottles to allow ventilation. Each cage was infested with ten *C. trifurcata* adults, which were collected from a soybean field at the Rosemount Research and Outreach Center, University of Minnesota, Rosemount, MN. Until infestation, *C. trifurcata* were held in a growth chamber at 27° C with a 16:8 (L:D) cycle, and fed soybean foliage. The cucumbers, pumpkins, and squash were each replicated in six pots. At 72 h post-infestation, the cages were removed, and the plants were assessed for damage. Damage was recorded as follows: percentage of cotyledon area with holes going completely through the cotyledons; percentage of the upper surface of the cotyledons with surface scarring; percentage of the lower surface of the cotyledons with surface scarring; presence or absence of damage to the stem; and presence or absence of damage to the small, folded first true leaf. Data for each injury type were arc-sine square root transformed, and analyzed with analysis of variance and the Ryan-Einot-Gabriel-Welsch multiple range test (SAS, 1995).

#### Choice Test Feeding Study

For the choice test feeding study, a single seed of a slicing cucumber ('Marketmore 76') and a soybean ('M96-133 151') were planted into 3.8 liter pots containing universal soil mix. Plants were grown under the conditions described for the no-choice feeding study. When the plants reached the cotyledon stage, a small cage, as in the no-choice feeding study, was placed over the pair of plants within each pot. Each cage was then infested with 10 *C. trifurcata* adults that were collected from the same location as the beetles used in the no-choice feeding study. Beetles were maintained under the same conditions until infestation as in the no-choice feeding study. This feeding trial was replicated in nine pots. Plants were visually inspected at 4, 8, 18, 24, and 72 h, and the number of beetles per plant and presence or absence of damage to the plants was recorded. At 24 and 72 h post-infestation, plants were more thoroughly assessed for damage. Damage to the cucumber and soybean plants was recorded as in the no-choice feeding study. Data on the abundance of *C. trifurcata* on plants within the cages were analyzed by time period using a two-sample t-test on the difference between the number of beetles on soybean and cucumber plants. The mean difference was compared to zero, which would indicate no preference. Data on the occurrence of damage on the caged plants was analyzed by time period using a two-sample t-test on the mean difference of arc-sine square root transformed data on the percent of plants with damage for soybean versus cucumber. Statistical analyses were not necessary at 4, 8, and 72 h post-infestation, because there was no variability for the percentage of damaged plants for cucumber and soybean. Data on the damage ratings conducted at 24 and 72 h post-infestation were analyzed using a two-sample t-test on the mean difference of arc-sine square root transformed data on the percent damage to soybean and cucumber plants for each injury type. The mean difference was

compared to zero, which would indicate no difference in the amount of damage.

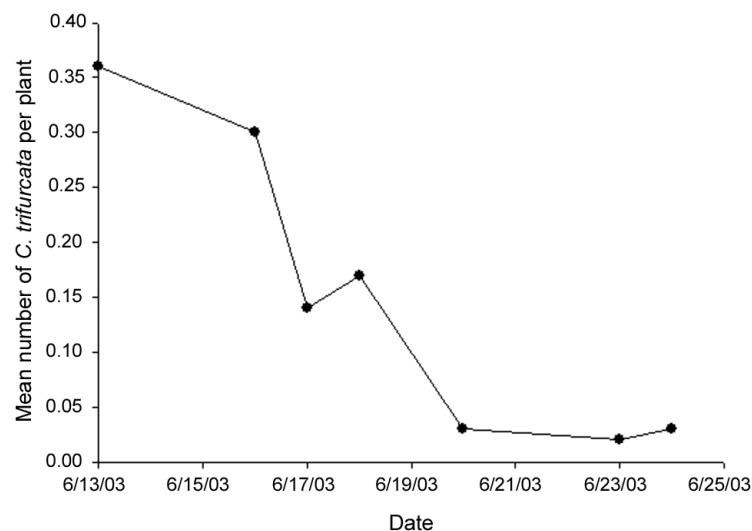
## Results

### Field Observations

*C. trifurcata* adults were present on the pumpkin plants with associated damage at each sample date (Fig. 1, 2, 3). From 13 June to 24 June 2003, the abundance of *C. trifurcata* decreased from 0.36 to 0.03 individuals per plant (Fig. 1). In addition, *A. vittatum* was present on the plants at each sample (RLK unpublished data).

### No-Choice Feeding Study

Cucumber plants were generally more heavily damaged than



**Figure 1.** Abundance of *Cerotoma trifurcata* on pumpkin ('Magic Lantern') plants near Rosemount, MN, 2003.



**Figure 2.** Photograph of an adult *Cerotoma trifurcata* on a pumpkin ('Magic Lantern') cotyledon with associated damage. Image was taken in a commercial pumpkin field near Rosemount, MN, 13 June 2003.





**Figure 3.** Photograph of the lower surface of a heavily damaged pumpkin ('Magic Lantern') cotyledon. Image was taken in a commercial pumpkin field near Rosemount, MN, 13 June 2003



**Figure 5.** Photograph of a heavily damaged cucumber ('Marketmore 76') plant from the laboratory no-choice feeding study. Note the surface scarring and distinct holes in the cotyledon. Also, feeding damage to the stem resulted in the upper portion of the plant hanging downward. Damage to the plant was caused by ten *Cerotoma trifurcata* adults over 72 h.



**Figure 4.** Photograph of squash ('Turks Turbin') (top left), cucumber ('Marketmore 76') (bottom center), and pumpkin ('Magic Lantern') (top right) plants from the laboratory no-choice feeding study. Note the decrease in cotyledon size and increase in intensity of *Cerotoma trifurcata* feeding damage from squash to pumpkin to cucumber. Damage to each plant was caused by ten *C. trifurcata* adults over 72 h.

squash plants, with pumpkin plants having an intermediate amount of damage (Table 1; Fig 4; 5). The percentage of plants with stem damage was significantly greater for cucumber and pumpkin plants compared to squash plants ( $F = 5.00$ ,  $df = 2, 15$ ,  $P = 0.022$ ), while the percentage of plants with damage to the first true leaf did not differ significantly ( $F = 0.83$ ,  $df = 2, 15$ ,  $P = 0.45$ ) (Table 1). Significantly more cotyledon area had distinct holes on cucumber plants than pumpkin or squash plants ( $F = 11.88$ ,  $df = 2, 15$ ,  $P = 0.0008$ ) (Table 1). The percentage of the lower ( $F = 16.86$ ,  $df = 2, 15$ ,  $P = 0.0001$ ) and upper ( $F = 9.21$ ,  $df = 2, 15$ ,  $P = 0.0025$ ) surface of the cotyledons scarred for cucumber and pumpkin plants was significantly greater than that of squash plants (Table 1).

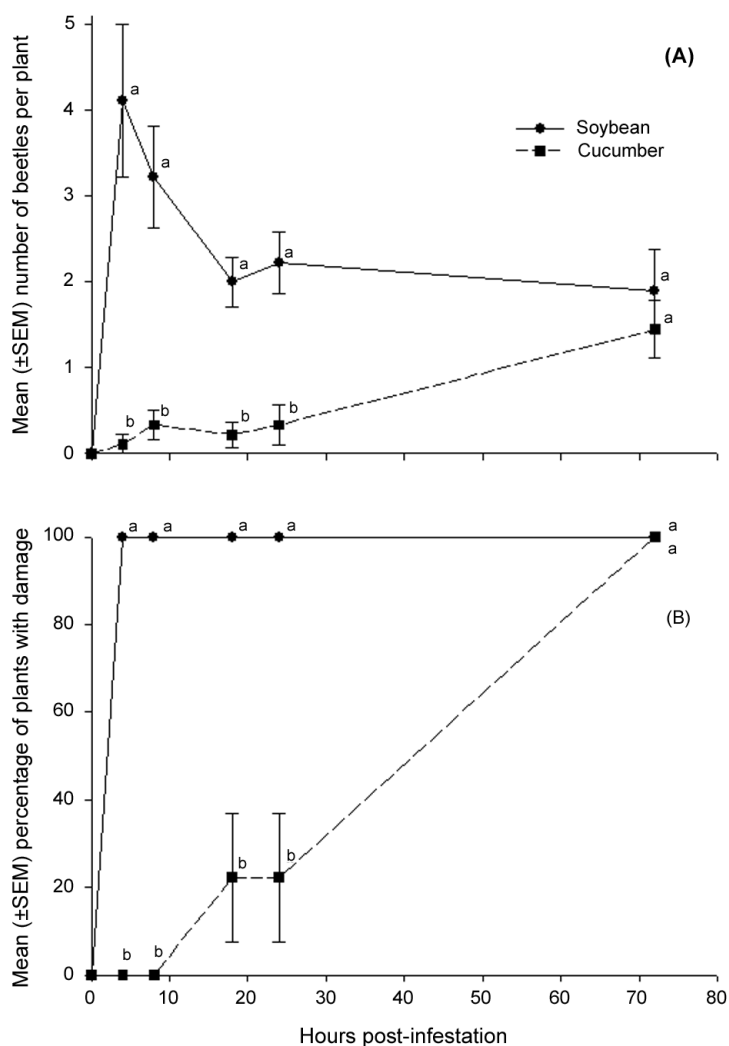
#### Choice Test Feeding Study

More *C. trifurcata* were found on soybean than on cucumber (Fig. 6A). The difference in abundance on soybean

**Table 1.** *Cerotoma trifurcata* feeding damage on cucumber, pumpkin, and squash from laboratory no-choice feeding study.

Injury type	Mean (SEM)		
	Cucumber	Pumpkin	Squash
% of plants with stem damage	66.67 (21.08) a	66.67 (21.08) a	0 b
% cotyledon area with distinct holes	30.00 (9.40) a	1.17 (0.60) b	0 b
% upper surface area of cotyledon scarred	4.83 (1.42) a	6.33 (2.82) a	0.13 (0.08) b
% lower surface area of cotyledon scarred	8.25 (2.68) a	11.67 (2.47) a	0.07 (0.02) b
% of plants with damage to 1st true leaf	33.33 (21.08) a	33.33 (21.08) a	66.67 (21.08) a

Means within a row followed by different letters are significantly different ( $P < 0.05$ ), analysis of variance and the Ryan-Einot-Gargiel-Welsch multiple range test on arc-sine square root transformed data.



**Figure 6.** Results from laboratory choice test feeding study. (A) Abundance of *Cerotoma trifurcata* on cucumber and soybean plants. Means within an observation date are significantly different ( $P < 0.05$ ), two-sample  $t$ -test on mean differences in abundance of beetles on soybean and cucumber plants. (B) Occurrence of *C. trifurcata* feeding damage on cucumber and soybean plants. Means within an observation date are significantly different ( $P < 0.05$ ), two-sample  $t$ -test on the mean difference of arc-sine square root transformed data on percent of damaged soybean versus cucumber plants.

compared to cucumber plants was significant at all observations, except for 72 h post-infestation (4 h:  $t = 4.63$ ,  $df = 8$ ,  $P = 0.0017$ ; 8 h:  $t = 5.12$ ,  $df = 8$ ,  $P = 0.0009$ ; 18 h:  $t = 6.4$ ,  $df = 8$ ,  $P = 0.0002$ ; 24 h:  $t = 5.38$ ,  $df = 8$ ,  $P = 0.0007$ ; 72 h:  $t = 0.61$ ,  $df = 8$ ,  $P = 0.56$ ) (Fig. 6A). The abundance of *C. trifurcata* on soybeans decreased from  $4.11 \pm 0.89$  (mean  $\pm$  SEM) individuals per plant at 4 h post-infestation to  $1.89 \pm 0.48$  individuals per plant at 72 h post-infestation (Fig. 6A). Abundance on cucumber ranged from  $0.11 \pm 0.11$  to  $0.33 \pm 0.24$  individuals per plant from 4 to 24 h post-infestation, respectively (Fig. 6A). At 72 h post-infestation, abundance on cucumber reached a maximum of  $1.44 \pm 0.34$  individuals per plant. *C. trifurcata* will feed on cucumber plants when soybean plants are present (Table 2; Fig. 6B). However, they fed on soybean plants nearly immediately, with 100 % of soybean plants being damaged

at 4 h post-infestation (Fig. 6B). Cucumber plants were not damaged so rapidly. Only  $22.22 \pm 14.70$  % of plants were damaged at 18 and 24 h post infestation (Fig. 6B). However, at 72 h post infestation, 100 % of the cucumber plants were damaged (Fig. 6B). The percentage of damaged plants was significantly greater for soybeans than cucumber at all observations, except 72 h post infestation (8 h:  $t = 5.29$ ,  $df = 8$ ,  $P = 0.0007$ ; 18 h:  $t = 5.29$ ,  $df = 8$ ,  $P = 0.0007$ ) (Fig. 6B).

All types of injury, due to *C. trifurcata* feeding, were more intense on soybean than on cucumber plants (Table 2). At 24 h post-infestation, significantly more soybean than cucumber plants had incurred stem damage (Table 2). The percentage of cotyledon area with distinct holes ( $t = 3.34$ ,  $df = 8$ ,  $P = 0.01$ ) and percentage of the lower surface area of the cotyledon scarred ( $t = 13.62$ ,  $df = 8$ ,  $P < 0.0001$ ) was greater on soybean compared to cucumber plants (Table 2). At 72 h post-infestation, significantly more soybean than cucumber plants had stem damage and damage to the first true leaf (Table 2). The percentage of cotyledon area with distinct holes on soybean and cucumber plants did not differ significantly ( $t = 1.82$ ,  $df = 8$ ,  $P = 0.11$ ) (Table 2). The percentage of surface area scarred on the upper ( $t = 10.10$ ,  $df = 8$ ,  $P < 0.0001$ ) and lower surfaces ( $t = 21.76$ ,  $df = 8$ ,  $P < 0.0001$ ) of the cotyledons was significantly greater on soybean than on cucumber plants (Table 2).

## Discussion

In mid to late-June, *C. trifurcata* was observed on pumpkin plants at the first true-leaf growth stage with associated damage to the plants in the field (Fig. 2, 3). Adults rarely migrate from one field to another within a growing season (Waldbauer and Kogan

Table 2. *Cerotoma trifurcata* feeding damage on cucumber and soybean plants in laboratory choice-test feeding study.

Injury type	Mean (SEM)	
	Cucumber	Soybean
<i>24 hours post-infestation</i>		
% of plants with stem damage	0 a	100 b
% cotyledon area with distinct holes	0.11 (0.11) a	2.89 (1.09) b
% upper surface area of cotyledon scarred	0.33 (0.24)	NA*
% lower surface area of cotyledon scarred	0 a	32.22 (4.01) b
% of plants with damage to 1st true leaf	NA**	NA*
<i>72 hours post-infestation</i>		
% of plants with stem damage	0 a	100 b
% cotyledon area with distinct holes	3.13 (2.19) b	4.61 (1.15) b
% upper surface area of cotyledon scarred	0.74 (0.32) a	61.89 (7.73) b
% lower surface area of cotyledon scarred	1.16 (0.73) a	52.78 (4.72) b
% of plants with damage to 1st true leaf	0 a	100 b

Means within a row followed by different letters are significantly different ( $P < 0.05$ ), two-sample  $t$ -test on the mean difference of arc-sine square root transformed data on percent damage for soybean versus cucumber.

\* Cotyledons had not opened, so the upper surface of the cotyledon and first true leaf could not be evaluated.

\*\* First true leaf was not apparent.



1976), and over winter under stubble in the field or in wooded areas adjacent to soybean fields (Lam and Pedigo 2000). The pumpkin field that we sampled was likely at high risk to being infested by *C. trifurcata*, because it was planted to soybeans the year before, contained soybean stubble and volunteer soybeans, and was surrounded by wooded habitat on three sides. The observed decline in the abundance of *C. trifurcata* (Fig. 1) temporally matched the decline of overwintered adults observed by Loughran and Ragsdale (1986), suggesting that the decline was due to natural population dynamics rather than repellency from the pumpkin field.

Our laboratory studies confirmed that *C. trifurcata* will feed on cucurbits. Damage to cucurbits caused by *C. trifurcata* appeared similar to damage caused by *A. vittatum*, with feeding occurring between the veins of the leaves, consuming either a layer of tissue from the top or bottom the leaf; eventually feeding may result in a hole through the leaf (Burkness 1996). Cucumber plants appeared to be more susceptible to *C. trifurcata* than either pumpkin or squash (Table 1; Fig. 4, 5). Differential susceptibility of the various cucurbits to feeding may have been due to differences in cucurbitacin concentration (e.g., Tallamy *et al.* 1991) or differences in size and thickness of the leaf tissue (Fig. 4). *C. trifurcata* fed on cucumber plants, albeit a small amount, when soybean plants were present, which corroborates our observation of their presence on pumpkin plants in the field when volunteer soybean plants were present.

To our knowledge, this is the first report of *C. trifurcata* feeding on cucumber, squash, or pumpkin. However, their pest potential for cucurbits remains uncertain and needs further investigation. Burkness and Hutchison (1998) found that for cotyledon to first true leaf stage cucumbers, only 10-15 % defoliation was necessary to cause a significant yield loss. In our no-choice feeding study, densities of 10 *C. trifurcata* per cucumber plant resulted in about 19 % of the leaf area having distinct holes. With maximum densities of 0.36 *C. trifurcata* per plant observed in the field (Fig. 1), it seems unlikely that feeding will result in economic losses. However, densities of ten *C. trifurcata* per plant did result in severe damage to some of the cucumber plants, which would likely lead to plant death (Fig. 5). *C. trifurcata* may be even less of a threat to pumpkin and squash plants, due to the ability of pumpkin and squash plants to tolerate higher levels of defoliation (Hoffmann *et al.* 2000), and the relatively low levels of feeding on pumpkin and squash plants (Table 1; Fig. 4). Therefore, additional feeding studies, including the relationship between cucurbitacin concentration and feeding damage should be examined.

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