

## THE INFLATED CALYX OF *PHYSALIS ANGULATA*: A REFUGE FROM PARASITISM FOR *HELIOTHIS SUBFLEXA*

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**Abstract.** *Heliothis subflexa* and *H. virescens* are the only known hosts of the parasitoid *Cardiochiles nigriceps*. *H. subflexa*, a specialist on plants in the genus *Physalis*, appears to have a lower parasitism rate compared to the polyphagous *H. virescens*. *Physalis* is characterized by producing fruits that are concealed within a paper-like lantern, within which *H. subflexa* larvae feed. We conducted an experiment to determine whether *H. subflexa* is protected from parasitism by feeding within the lanterns of *Physalis*. We compared parasitism rates of wild *H. subflexa* larvae on normal *P. angulata* plants to parasitism rates of larvae on plants that had the tips of all the lanterns removed to allow parasitoids access to the larvae. A higher percentage of larvae were parasitized on plants with cut lanterns (48.25%) than uncut lanterns (6.74%). A second experiment was conducted to determine whether volatiles released from cut lanterns influenced the searching of *C. nigriceps*. This experiment consisted of the two treatments from the previous experiment and a third in which the tips of lanterns were removed and the resulting hole was stapled shut. Cutting plants did not make the plants more attractive to searching parasitoids. We conclude that *H. subflexa* gains protection from parasitism by feeding within the inflated calyx of *Physalis*.

**Key words:** *Cardiochiles nigriceps*; *Heliothis subflexa*; *monophagy*; *parasitism*; *Physalis angulata*; *refuge*; *specialist*.

### INTRODUCTION

Predators may play an important role in the evolution of a narrow host range in phytophagous insects because they impose selection pressure on herbivores to utilize enemy-free space (Bernays and Graham 1988). Jeffries and Lawton (1984) define enemy-free space as “ways of living that reduce or eliminate a species vulnerability to one or more species of natural enemies.” The focus of this paper is the potential for monophagy to result in avoidance of a natural enemy.

The insect examined in this paper is *Heliothis subflexa* (Gn.) (Lepidoptera: Noctuidae), a close relative of the tobacco budworm, *H. virescens*. The two are so closely related that they can be hybridized, producing fertile females and sterile males (Laster 1972). *H. virescens* is highly polyphagous, feeding on 37 species of plants in 14 different plant families (Sheck and Gould 1993). In contrast, *H. subflexa* is reported to be a specialist feeding only on plants within the genus *Physalis* (groundcherry) (Laster 1972). *Physalis* is

characterized by producing fruit that are enclosed within the inflated calyx of the flower, referred to here as a lantern (Fig. 1). The larvae of *H. subflexa* feed on the fruit within the lantern by boring a small hole in the lantern to gain access to the fruit. Generally, only one larva is found per fruit. To complete development, larvae need to feed on a number of fruit and often do not eat all of any single fruit (M. Sisterson, *personal observation*). Damaged and infested lanterns are often abscised by the plant and heavily infested plants usually have a large number of lanterns on the ground.

This study was motivated by survey data on parasitism rates of *H. virescens* and *H. subflexa* on their respective host plants by the braconid parasitoid *Cardiochiles nigriceps* (Lewis et al. 1967). *C. nigriceps* is only known to parasitize *H. subflexa* and *H. virescens* and is attracted to the salivary secretions of both (Lewis et al. 1967, Lewis and Vinson 1971). In field collections of larvae, parasitism rates of *H. virescens* by *C. nigriceps* can attain 96% on cotton (Lewis et al. 1972) and 72% on tobacco (Neunzig 1969). Due to the economic importance of *H. virescens*, there are far more studies that report parasitism rates for it than for *H. subflexa*. Some collections of *H. subflexa* have found no parasitism (Roach 1975; M. Sisterson, *unpublished data*). Lewis et al. (1967) reported five parasitized larvae out of a col-

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FIG. 1. Photo of a group of lanterns of *Physalis angulata*. Half of the lantern on the left has been removed to allow us to view the fruit and a *Heliothis subflexa* larva. The other lanterns have been left intact.

lection of 240 (2.1%) at one site and 12 parasitized larvae out of 138 at another (8.7%). Combined parasitism rates of *H. subflexa* by *C. nigriceps* and another *Heliothis* parasitoid, *Campoletis sonorensis*, have been reported at 12% (Smith et al. 1976). These survey data indicate that the specialist *H. subflexa* may have a lower parasitism rate than the generalist *H. virescens*. We tested the hypothesis that feeding within the lanterns of *P. angulata* confers an advantage to *H. subflexa* by providing protection from parasitism.

#### MATERIALS AND METHODS

*Experiment 1.*—This study was conducted from 8–29 September 1996 in a 2.5-ha soybean field in Orangeburg County, South Carolina, USA. The field contained ~1000 reproductive phase *P. angulata* plants which were naturally infested with wild *H. subflexa*.

Sixty pairs of *P. angulata* plants that were similar in size and in close proximity to one another were paired and marked with flagging tape. Lanterns that had fallen off plants were removed. In each pair, one plant had the tips of all lanterns removed (referred to as cut) by cutting diagonally with a pair of scissors, ~0.5 cm from the tip of the lantern. Cutting the lanterns created a hole large enough for a parasitoid to enter (~1.0 cm diameter), potentially making any residing larvae more vulnerable to attack. Also, any lanterns that were too small to cut were removed by hand (this was done only for the cut treatment). This was necessary because the lanterns develop quickly and by the

end of the experiment the small lanterns would be fully formed. Removing the newly developing lanterns insured that only cut lanterns were present on the plant. The lanterns on the other plant in the pair were left intact (referred to as uncut), allowing larvae to feed within the completely enclosed lantern. The naturally existing infestation was used for this test. After a week, lanterns from all cut and uncut plants were collected. For each plant all lanterns that had fallen to the ground beneath the plant during the week were placed into a sealable plastic bag. We then collected all of the lanterns on the plant and placed them into a separate sealable plastic bag. This protocol was followed because, often, when removing lanterns from a plant many fall to the ground. This protocol enabled us to distinguish lanterns that had fallen on the ground from those remaining on the plant. The experiment was repeated on three separate dates for a total of 60 pairs of plants (8–14 September, 12 pairs; 14–21 September, 23 pairs; 22–29 September, 25 pairs).

In the laboratory, we determined for each plant the total number of lanterns on the plant and on the ground. For each bag we also recorded the total number of larvae and the identity of each larva as *H. subflexa* was verified (McElvare 1941). To rear out any parasitoids, the larvae were fed fruits placed in 30-mL plastic cups that were held at ~24°C and 14L:10D. Undamaged lanterns were saved and stored in the refrigerator to feed to the larvae. Each larva was monitored for pupation, death, or parasitoid emergence. If larvae died in the lab before pupation or parasitoid emergence they were preserved in alcohol. They were later dissected as described in Lewis and Brazzel (1966) to determine if they were parasitized.

*Experiment 2.*—Volatiles released from damaged plants are important host-finding cues for *C. nigriceps* (Vinson 1976). Cutting the plant with scissors may have released plant volatiles, making the plant more attractive to searching *C. nigriceps*. Therefore, another experiment was conducted with laboratory reared insects and *P. angulata* grown in pots to determine whether the difference in parasitism rates between cut and uncut lanterns was a result of providing parasitoids access to the feeding larvae in cut lanterns, or if cutting the lanterns released volatiles that made the plants more attractive to searching parasitoids.

The *H. subflexa* used in this study originated from field collections in Orangeburg, South Carolina, during August and September 1996. For regular colony maintenance, larvae were reared individually on a corn-milk-soy diet modified from Burton (1970) in 30-mL cups at ~24°C and 14L:10D. Upon pupation they were individually placed into new cups half filled with moistened vermiculite to prevent desiccation. Pupae were checked daily for adult emergence. Adults were

mated in groups of ~25 moths in one-gallon buckets that contained a piece of tissue paper saturated with 5% sucrose solution for nourishment and a cutting of host material (*P. angulata*) to enhance oviposition. Cheesecloth was placed over the top of the bucket to act as an oviposition substrate. The buckets were stored at ~27°C. Eggs were collected every other day and washed in a 1% solution of Chlorox bleach and water. Eggs were held at ~18.5°C and neonates were placed onto the corn–milk–soy diet using a camel hair brush. The three day old larvae that were to be used for the experiment were reared three to five per cup.

Field collected seed of *P. angulata* was germinated outdoors in 15-cm pots. To enhance germination, seeds were placed outdoors in an empty pot for ~2 mo prior to germination. Individual seedlings were transferred to new 15-cm pots. Plants were fertilized with one teaspoon of Osmocote (NPK 14:14:14) per month. Plants were allowed to grow until they developed at least 13 lanterns. They were transported to the study site in a truck with a covered bed.

The study was conducted at the Oxford Tobacco Research Station in Oxford, North Carolina, USA. Potted *P. angulata* plants were placed along the field margin of a 0.1-ha tobacco field. There were two more tobacco fields of larger size in the vicinity. This site was selected as a location where *C. nigriceps* adults were likely to be searching for hosts.

A randomized complete block design was used for this test. Each block consisted of a group of three plants. Each plant was infested with 10 *H. subflexa* larvae that had been reared on the corn–milk–soy diet for 3 d. The larvae were left on plants overnight to allow feeding to begin. The next day, one plant in each block received one of the following treatments: (1) lanterns were left uncut, as in the previous experiment; (2) tips of all the lanterns were cut off, as in the previous experiment (cut); and (3) tips of all lanterns were cut off and then the resulting hole was stapled shut using a small stapler (stapled). The stapled treatment allowed us to examine the effect of releasing volatiles that may be attractive to *C. nigriceps*, while still providing a barrier to the parasitoids. On all plants in all treatments, developing lanterns that were too small to cut and staple were removed. Four days later all of the lanterns were collected and data recorded in the same manner as in the previous experiment. Larvae were reared in the laboratory on corn–milk–soy diet and monitored for parasitoid emergence. Replicates of this experiment were begun on nine dates for a total of 35 blocks. Replication was not equal on all dates (26 June–1 July, 2 blocks; 1–6 July, 3 blocks; 2–7 July, 8 blocks; 3–8 July, 3 blocks; 6–11 July, 4 blocks; 7–12 July, 2 blocks; 12–17 July, 1 block; 14–19 July, 5 blocks; 15–20 July, 7 blocks).

## ANALYSIS

For each plant, the number of parasitized larvae found on that plant was tallied. The number of parasitized larvae was divided by the total number of larvae on that plant to determine the percentage of larvae parasitized on each plant. Because the data in Experiment 1 and Experiment 2 were not normally distributed, nonparametric tests were used. These tests allowed us to use the untransformed data. The percentage of larvae parasitized per plant was compared between treatments in Experiment 1 by Wilcoxon's signed-rank test. Experiment 2 was analyzed as a randomized complete block using Friedman's two-way ANOVA (Factor 1, treatment; Factor 2, block) followed by a multiple comparison test (Neter et al. 1996).

In Experiment 1, pairs of plants were dropped from the analysis for the following reasons: (1) if one or both plants in a pair yielded no larvae (19 pairs) or (2) if both plants in a pair yielded no parasitized larvae (16 pairs). Lehmann (1975) reviews the treatment of zeroes in the Wilcoxon test and suggests that pairs without a treatment effect are uninformative and should be dropped. Therefore, 25 pairs of plants were used for the Wilcoxon signed-rank analyses.

The same criteria were used to determine if any blocks should be dropped from the analysis for Experiment 2. All plants set up before 12 July 1997 yielded at least one larvae, and at least one parasitized larvae per block. All plants set up on or after 12 July 1997 yielded no parasitized larvae. Therefore, only the 22 blocks set up before 12 July 1997 were used for the Friedman's two-way ANOVA.

To insure that any observed effect was a result of the treatment and not due to inherent differences in plant size, tests were conducted using the number of lanterns on each plant as an indicator of its size. Pairs or blocks of plants excluded from the previous analysis were not used for these tests. In Experiment 1, the number of cut and uncut lanterns per plant were compared using a paired *t* test ( $\alpha = 0.05$ ). In Experiment 2, the number of cut, uncut, and stapled lanterns per plant was compared using a two-way ANOVA (Factor 1, treatment; Factor 2, block) followed by the least-squared difference test to compare means ( $\alpha = 0.05$ ).

We were also interested in determining if the treatments had an effect on larval survival. All pairs or blocks of plants were used for these analyses. The number of larvae collected per plant in each treatment in Experiment 1 was compared using a paired *t* test ( $\alpha = 0.05$ ). In Experiment 2 the number of larvae collected per plant in each treatment was compared using a two-way ANOVA (Factor 1, treatment; Factor 2, block) followed by the least-squared difference test to compare means ( $\alpha = 0.05$ ).

TABLE 1. Mean  $\pm$  1 SE for percentage of parasitized larvae per plant, number of lanterns per plant, and number of larvae per plant in Experiment 1 and Experiment 2. Within a row, means followed by the same letter are not significantly different ( $\alpha = 0.05$ ).

Measure	Treatment		
	Uncut	Cut	Stapled
Experiment 1			
Percentage of parasitized larvae per plant	6.74 $\pm$ 4.17 <sup>a</sup>	48.25 $\pm$ 7.25 <sup>b</sup>	
Number of lanterns per plant	53.88 $\pm$ 7.55 <sup>a</sup>	55.04 $\pm$ 11.57 <sup>a</sup>	
Number of larvae per plant	3.58 $\pm$ 0.51 <sup>a</sup>	2.60 $\pm$ 0.33 <sup>b</sup>	
Experiment 2			
Percentage of parasitized larvae per plant	14.54 $\pm$ 2.34 <sup>a</sup>	61.40 $\pm$ 6.34 <sup>b</sup>	10.03 $\pm$ 2.79 <sup>a</sup>
Number of lanterns per plant	25.64 $\pm$ 3.14 <sup>a</sup>	20.23 $\pm$ 1.44 <sup>a,b</sup>	18.73 $\pm$ 1.86 <sup>b</sup>
Number of larvae per plant	7.14 $\pm$ 0.31 <sup>a</sup>	4.40 $\pm$ 0.34 <sup>b</sup>	5.49 $\pm$ 0.44 <sup>c</sup>

### RESULTS AND DISCUSSION

In Experiment 1, the percentage of parasitized larvae per plant was significantly higher in the cut lantern treatment than in the uncut treatment ( $P < 0.0001$ ; Table 1). In Experiment 2, there was a significant treatment effect on the percentage of parasitized larvae per plant ( $df = 2$ ,  $\chi^2 = 22.89$ ,  $P < 0.001$ ; Table 1). The cut treatment had a significantly higher percentage of parasitized larvae per plant than the uncut treatment ( $P < 0.0001$ ) and the stapled treatment ( $P < 0.0001$ ). The uncut and stapled treatments did not have a significantly different percentage of parasitized larvae per plant ( $P < 0.4404$ ; Fig. 2). Therefore, we conclude that cutting lanterns with a pair of scissors does not make the plants more attractive to searching parasitoids and that the results observed in Experiment 1 are explained by increased physical access to parasitoids.

In Experiment 1, the number of lanterns per plant in the two treatments was not statistically different ( $df = 24$ ,  $t = 0.091$ ,  $P < 0.928$ ; Table 1). In Experiment 2, there was nearly a significant treatment effect on the

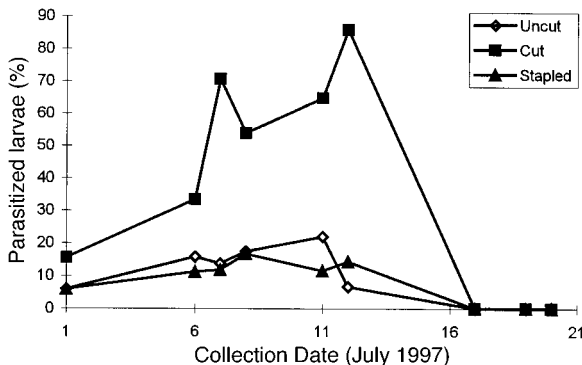


FIG. 2. The percentage of parasitized larvae in each treatment on each collection date (Experiment 2). *C. nigriceps* adults were frequently observed in the study area between 1 and 10 July. *C. nigriceps* was not observed again in the study area after 10 July.

number of lanterns per plant ( $df = 2$ ,  $42$ ,  $F = 2.98$ ,  $P < 0.0614$ ; Table 1) and the block effect was insignificant ( $df = 21$ ,  $42$ ,  $F = 1.47$ ,  $P < 0.1425$ ). The number of lanterns per plant in the cut treatment did not differ significantly from the number of lanterns per plant in the stapled treatment ( $P < 0.62$ ). The number of lanterns per plant in the uncut treatment was not significantly greater than the number of lanterns per plant in the cut treatment ( $P < 0.07$ ), but was significantly greater than the stapled treatment ( $P < 0.02$ ). Thus, the greater parasitism rates in the cut treatment in both experiments did not result from differences in plant size. The results of Experiment 1 and the comparison of stapled vs. cut lanterns offer strong support for the hypothesis that lanterns protect larvae from parasitism.

In Experiment 1, fewer larvae were collected from cut lanterns than uncut lanterns ( $df = 59$ ,  $t = 2.01$ ,  $P < 0.049$ ; Table 1). This difference was unexpected as we sampled an equal number of plants. Likewise, in Experiment 2, the ANOVA showed that there was a significant treatment effect on the number of larvae recovered ( $df = 2$ ,  $68$ ,  $F = 15.35$ ,  $P < 0.0001$ ; Table 1) and the block effect was insignificant ( $df = 34$ ,  $68$ ,  $F = 1.26$ ,  $P < 0.2107$ ). The number of larvae collected from plants differed significantly for each pairwise comparison. These results suggest that experimental manipulation of the plants in the cut and stapled treatments did have some effect on resident larvae. Disturbance caused by cutting and/or stapling lanterns may have resulted in larvae leaving the plant. A greater number of larvae were collected from plants with stapled lanterns compared to plants with cut lanterns suggesting that some additional factors, besides disturbance, affected the number of larvae collected in the cut treatment. Larvae residing in cut lanterns have been demonstrated to be more vulnerable to *C. nigriceps* than larvae residing in uncut or stapled lanterns. It seems reasonable to hypothesize that they may also be more vulnerable to generalist predators, and that this additional source of mortality may account for the sig-

nificant difference between the number of larvae collected in the stapled and cut treatments.

This paper demonstrates that the inflated calyx of *P. angulata* provides *H. subflexa* with a refuge from parasitism. Structural barriers, such as this one, have been implicated as a means of defense for other species of insects (Price et al. 1980). For example, the gall making insects *Euura lasiolepis* and *Eurosta solidaginis* avoid parasitism when they reside in galls that have a diameter that is larger than the attacking parasitoid's ovipositor (Weis and Abrahamson 1985, Price and Clancy 1986). It has been hypothesized in both cases that the mortality caused by the parasitoids may cause selection on both the host plant and the herbivore. Gomez and Zamora (1994) demonstrated the impact a herbivore may have on plant fitness and its relationship to natural enemies. Weevils in the genus *Ceutorhynchus* feed on the seed of their host *Hormathophylla spinosa* (a woody crucifer). Plants attacked by weevils have a significant decrease in the size and abundance of their seeds. The weevil larvae are attacked by a guild of parasitoids and parasitized larvae have a decreased impact on plant fitness. Therefore, parasitoid activity is thought to enhance plant fitness. Similar selection forces may be acting within our system.

Geitzenauer and Bernays (1996) demonstrated that *H. virescens* has greater survival when exposed to the generalist predator, *Polistes arizonensis*, when feeding on *Physalis pubescens* (a non-host) than on sunflower (*Helianthus annuus*, a regular host). They tested the influence of plant structural complexity, host odors, and larval host plant on attack rate. All three parameters had a negative impact on attack rate by the generalist natural enemy. The results of our study combined with those of Geitzenauer and Bernays (1996) indicate a number of possible benefits of feeding on *Physalis* spp. This supports our hypothesis that the host plant of a specialist insect may confer an advantage to that insect.

Jeffries and Lawton's (1984) definition of enemy-free space, as ways of living that reduce or eliminate a species vulnerability to one or more species of natural enemy, applies to our system. The close relationship of *H. subflexa* to *H. virescens*, their extremely different host range, and different vulnerability to parasitoids suggest that natural enemies may have played a key role in the evolution of monophagy in *H. subflexa*.

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