ABSTRACT

PETZOLD, JENNIFER LEE. Interactions Between a Specialist and Generalist Moth and Their Host Plants. (Under the direction of Fred Gould and Wendy Boss).

Among the most significant issues regarding plant-herbivore interactions today are 1) elucidating the genetic architecture of host plant use and determining what factors are involved in locating and ovipositing on host plants, 2) understanding chemical interactions between insects and their host plants, and 3) understanding how plants evolve in response to the ecological pressures imposed on them by their insect pests, and how these insects in turn respond to these evolved mechanisms of host plant defense. The purpose of this research was to address these questions using a model system composed of two Heliothine moths, Heliothis subflexa and H. virescens.

Heliothis subflexa (Hs) is a specialist that feeds only on plants in the genus Physalis, while H. virescens (Hv) is a broad generalist. To determine what genetic factors are involved in changes in host range, these two species were hybridized, and backcrosses to both parent species were produced. Larval feeding preference of Hs, Hv, and backcross lines was assessed using choice and no choice feeding tests on Physalis and tobacco (a host of Hv), and oviposition behavior was assessed by observing moths in a large outdoor cage containing Physalis and tobacco. We found that backcrosses in the Hs direction always resembled Hs in feeding and oviposition preference. Backcrosses in the Hv direction resembled Hv in oviposition behavior, but had intermediate feeding behavior and fed on both Physalis and tobacco. Hybrids showed strong preference for tobacco in oviposition behavior. These results show that at least one major locus is likely involved in feeding preference between the two host plants and that preference for Physalis is a dominant trait; however, genetic control of oviposition behavior on the two host species is more complicated to understand, possibly involving multiple genetic loci and a threshold effect, or few genes and a heterozygous disadvantage. Field experiments were also conducted to determine how Hs locates its host plants. Results showed that vision is an important host location cue, and confirmed prior studies that showed that Hs lays approximately 20% of its eggs on nearby non-hosts. A possible reason for this could be to avoid host plant defenses—Physalis plants were observed to respond to eggs of Hs; they do so by forming undifferentiated cells or a hypersensitive
response directly under some Hs eggs. Laboratory and field studies demonstrated that eggs that elicited a response had a 25% lower probability of hatching, and a 28% lower probability of remaining on the plant. This resulted in a fitness cost of 18% for Hs, and it was concluded that response to eggs could be a factor that selected for non-host oviposition by Hs. Plants in this genus have also evolved to abscise fruits in response to frugivory by Hs, but the degree to which chemical interactions are involved in this defense response was unknown. By using a combination of mechanical damage, natural damage, and Hs saliva and regurgitant treatments applied to fruit, it was determined that mechanical damage was sufficient to cause fruit abscission and insect oral secretions were not important in this response.
Interactions Between a Specialist and Generalist Moth and Their Host Plants

by

Jennifer Lee Petzold

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APPROVED BY:

Co-Chair of Advisory Committee
Fred Gould

Co-Chair of Advisory Committee
Wendy Boss

Thomas Wentworth

Edward Vargo
DEDICATION

I would like to dedicate this dissertation to my parents, Bob and Jackie Petzold, who have provided me with their unwavering support throughout my life. Never missing one athletic game, recital, or important event for as far back as I can remember, it has been their constant love, encouragement, and support that has enabled me to get to where I am today. I simply could not have done it without them, and I am grateful for their presence in my life.
Jen Petzold was born in Manchester, NH, where she grew up with her little sister, Heidi, and was raised by her parents Bob and Jackie. Summers filled with outdoor adventures during family camping trips in southern NH instilled a fascination with biology early on in Jen’s life, and she quickly decided to major in Biology when she went to Trinity College in Hartford, CT. While in college, a study abroad trip to the rainforests of Australia, and an internship at Harvard Forest confirmed her love of plants and ecology, and she took a one-year internship studying endangered plants in Philadelphia, PA. In graduate school, a fortuitous rotation in Fred Gould’s lab introduced her to the world of plant-insect interactions, where her interests have remained ever since. Jen will relocate to Iowa for a postdoctoral position at Iowa State continuing her studies with plants and insects.
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INTRODUCTION

Why study ecological specialization of insects?

Many organisms exhibit specialized patterns of resource use, drawing on only a subset of all resources available (see review Jaenike 1990). Specialized interactions dominate many ecosystems, and these highly specialized relationships have been attributed to be a major factor in producing much of the diversity that exists on earth. With estimates ranging as high as 30 million species (Erwin 1982), insects are the most diverse group of organisms. Plants are also extremely speciose: seed plants and the insects that feed on them make up half of all described species on the planet. Both the rate and the magnitude at which diversification of these two groups has occurred is unmatched by most other taxa (Janz et al. 2006). Therefore, these two groups provide ideal systems for answering many important ecological questions regarding how biodiversity is generated. Many insects feed primarily on one or several closely related plant species (specialists), while others use many different plant species as hosts (generalists). The vast majority of all phytophagous insect species are specialists on one group of plants (Janz et al. 2001, and references therein), and the majority of these insects have formed highly specialized interactions with their plant hosts. Ehrlich and Raven stated in their seminal paper on coevolution that specialized interactions between insects and their plant hosts have led to rapid diversification of both insect and plant species (1964). Therefore, studying the interface of plant/herbivore relationships by examining the specialized relationships that occur between specialist insects and their host plants provides information essential to our overall understanding of broad ecological concepts, such as speciation, evolution, and diversification.
**The Heliothis subflexa/Physalis interaction**

_H. subflexa_ (hereafter referred to as Hs) is a monophagous insect, feeding only on plants in the genus _Physalis_ (Solanaceae) (Laster et al. 1972). Larvae feed on the fruit of _Physalis_ plants, where they are enclosed in the inflated calyx that surrounds the fruit, a characteristic shared by all plants in the genus _Physalis_. Many characteristics of the interactions between _H. subflexa_ and plants in the genus _Physalis_ seem to have resulted from a common evolutionary history. Although strict pairwise coevolution cannot be assumed to have occurred, interactions between these two taxa very likely influenced each other’s evolution. For example, De Moraes & Mescher (2004) found that _P. angulata_ fruit do not contain linolenic acid, a compound essential to almost all insect taxa for growth and development. The authors suggest that _H. subflexa_ has adapted to circumvent the need for linolenic acid in development, and by doing so has gained access to _Physalis_ fruit as a food source not sufficient for the development of other insects (De Moraes & Mescher 2004). Furthermore, Sisterson and Gould showed that _H. subflexa_ gains protection from the parasitoid _Cardiochiles nigriceps_ by feeding inside the enclosed calyx of _Physalis angulata_, since parasitism rates were decreased by about 40% due to protection from the calyx (1999). Other experiments have shown that _H. subflexa_ feeding on _Physalis_ fruit induces fruit abscission, which acts as an induced defense against these insects (Benda 2007). Also, most _H. subflexa_ larvae feeding on fruit exit the fruit several hours before abscission occurs, indicating that larvae may be able to sense a cue from the plant indicating when the fruit will drop (Petzold, unpublished data). Although many aspects of the Hs/Physalis interactions seem highly specialized, phylogenetic studies have shown that the specialist Hs and a closely related generalist, _H. virescens_, only recently diverged from a common polyphagous ancestral species (Mitter et al. 1993, Fang et al. 1997). Because much of our knowledge of plant/herbivore interactions has come from studies of highly specialized plant/insect systems that share a long evolutionary history of relationships, more information is needed from systems that have had more recent evolutionary associations, like that of Hs and _Physalis_ species.
The study of plant-herbivore interactions can also be used as a tool to gain a better understanding of the genetics of host use. Although understanding what genetic factors are necessary for a change in an insect's host range has important implications for both agriculture and for our understanding of speciation, there is little known about the genetic architecture of host use in insects. This is mainly due to lack of appropriate system for studying the genetics of host range. Because line crosses of insects with differences in host ranges are ideal for uncovering the genetic factors associated with host use, systems with closely related insects that differ in host ranges are necessary. *H. virescens* (hereafter referred to as Hv) is a broad generalist that is closely related to Hs, and it feeds on plants in over 14 families. Despite the drastic difference in host ranges of Hs and Hv, these two species can be hybridized in the laboratory (Laster 1972; Sheck & Gould, 1993). The ability to hybridize these two species and the distinct differences in their host ranges provide a unique opportunity to examine the genetic basis of differences in host use between generalists and specialists. Thus, the Hs/Hv model is highly suitable for advancing our understanding of the genetic architecture of host use, and the *Hs/Physalis* system provides a useful model to study the intricacies of a newly specialized system, both in terms evolution of plant defenses, and the evolution of host range.

**MECHANISMS OF PLANT DEFENSE**

The evolution of plant defenses against insect species is one factor that has been attributed to providing much of the diversity on earth (Ehrlich & Raven 1964). Damage to plant tissue by insect herbivores can cause a significant reduction in plant fitness, and plants have evolved both constitutive and herbivore-induced defenses to limit this damage (Simms & Rausher 1987, Nunez-Farfan & Dirzo 1994, Karban & Baldwin 1997). Induced defenses are often generated by defense- and wound-response pathways (Walling 2000) that are secondarily activated by chemical and physiological changes in the plant caused by herbivory. These defenses against herbivores include increased amounts of toxic or repellent allelochemicals.
produced at the feeding site or throughout the whole plant (Karban & Baldwin 1997), a change in plant nutrient content (Haukioja & Niemela 1997), physical changes in plant tissue such as induction of trichome growth (Traw & Bergelson 2003), and emission of enemy-attracting volatiles (see reviews by Dudareva et al. 2006, Dicke & van Loon 2000). Another important inducible defense mechanism for some plant species is the abscission of the plant part on which the herbivore is feeding (Karban & Baldwin 1997), and the formation of necrotic or specialized tissue of leaves following oviposition. Both of these mechanisms of plant defenses are discussed below.

I. Abscission

Plants are faced with a variety of environmental stresses throughout their life, and they are equipped with a number of mechanisms for defense against these stresses. A number of plants respond to herbivory of reproductive structures by abscising the damaged organ; however, the defense-induced pathway that initiates the induction of fruit abscission in many systems is unknown. Understanding abscission in a generic sense is also of great importance economically (for farmers, growers, and horticulturalists), and in its potential for providing a better understanding of the physiology of hormone action and the resulting coordination of cellular events. Abscission has been studied for well over a century, and is used as an important tool for the understanding of hormonal control and regulation (Taylor & Whitelaw 2001).

Abscission zones in plants

The process of abscission has been studied for many years, as early botanists recognized the predictable places at which abscission occurred in plants and sought to examine and describe the tissues where separation took place. Von Mohl was the first botanist to call the point of separation the ‘abscission zone’ at a time when it was believed that leaf drop resulted from the plant cutting off resources to the organ (Sexton & Roberts 1982). Inman (1848, see
Sexton & Roberts (1982) disproved this theory, and described a cellular disintegration at the abscission zone. He observed cells “becoming rounder and separating their walls from each other as to destroy their cohesion” (Sexton & Roberts 1982). The structure of the abscission zone in relation to its function became better understood with future investigation. In 1860, Von Mohl recognized that cells at the proximal end of separation acquired a protective layer, while wall breakdown occurred in the cells at the distal end (Sexton & Roberts 1982). Later work showed that there was indeed a corky, suberized protective layer on the side of separation closest to the plant and a layer of expanding cells distal to this protective layer (nearest to the abscising organ) (Addicott 1982). This early work paved the way for later botanists to describe the features of cells in the abscission zone.

The abscission zone consists of several rows of specialized cells arranged in a parallel layers. These specialized cells respond to abscission inducing signals, allowing cell separation to take place at a genetically predetermined site (Brown, 1997). They are usually smaller and less vacuolated, have a denser cytoplasm than surrounding cells, and have highly branched plasmodesmata (Sexton & Roberts 1982). Cells in the abscission zone vary in thickness between species; in tomato the abscission zone consists of eight to ten cell layers and separation occurs between two (Tabuchi & Arai 2000). Conversely, in Sambucus nigra, the abscission zone contains approximately 50 cell layers and separation takes place between many layers (Taylor & Whitelaw 2001).

It is widely accepted that cells in the abscission zone are functionally different from surrounding cells. These cells are composed of subclasses of different cell types, including the separation layer and the protective layer. Cells in the abscission zone are considered Type 2 cells—cells characterized by elevated growth in response to ethylene and suppressed growth by IAA (Gonzalez-Carranza et al. 1998). Cells that comprise the body of the plant are classified as Type 1 cells—longitudinal elongation is enhanced by IAA but not ethylene (Taylor & Whitelaw 2001). The abscission zone is formed very early in development; in newly germinated Phaseolus vulgaris seedlings, Osborne recorded abscission after
application of ethylene of unexpanded new leaves (1989). Studies using periclinal chimeras of the *jointless* tomato mutant, which lacks abscission zones, have shown that the genotypic origin of the L3 layer in the apical meristem (the layer that gives rise to vascular tissue and internal tissues) is responsible for the formation and differentiation of the pedicel abscission zone (Szymkowiak & Irish 1999). Only the chimeras with a wild-type L3 layer had functional pedicel abscission zones; chimeras with L1 and L2 from the *jointless* mutant formed normal abscission zones. It is not known, however, if the cell layers receive the signals for differentiation in the meristem or during development of the pedicel, and it is proposed that cells destined to become abscission zone cells may receive a series of developmental signals during differentiation that “confer morphological, functional, and positional information” (Roberts *et al.* 2002).

*Secondary Abscission Zones*

Abscission occurs at highly predictable, genetically predetermined places. These regions on a plant include leaves, flowers, sepals, petals, filaments, seeds and pods, fruits, and spines (Roberts *et al.* 2002). The abscission layer for these organs forms as organ development takes place. In addition to the formation of abscission layers in these predictable positions, it has been well documented that mature cells are able to change into cells that have the ability to differentiate into abscission zone cells, resulting in secondary or adventitious abscission zone formation (Webster & Leopold 1972, McManus *et al.* 1998). These secondary abscission zones are formed where abscission would not normally occur. Lloyd (1914, see Addicott 1982) first documented the formation of an abscission zone in a shoot following decapitation of the shoot between nodes, with the abscission layer forming just above the first internode, leading to subsequent shedding of the stem above the node. It has since been shown that the formation of secondary abscission zones can, in some cases, be stimulated by hormonal application (Pierik 1980), but this is not a universal trait shared by all plants (Roberts *et al.* 2002). Using secondary abscission zones as a tool for studying the formation of abscission zones cells, as well as hormonal and enzymatic control in abscission, will be
important in understanding how cells in the abscission zone respond differently from surrounding cells to cues preceding abscission.

**Cellular Adhesion in Plants**

To understand cell separation processes, the initial state of cells in plants must be understood. Plant cells, unlike animal cells, do not move once formed (with very few exceptions), and remain attached to adjacent cells throughout their lives (Jarvis et al. 2003). Adhesion between plant cells takes place during cytokinesis. As the cell plate is laid down between two daughter cells, primary cell walls are formed on either side of the cell plate. The cell plate eventually becomes the middle lamella and the site of intercellular attachment (Jarvis et al. 2003). Composed of proteins and cross-linked pectins, which are chains of linked D-galacturonic acid units, the middle lamella is described as a three dimensional gel-like network that adheres together the walls of adjacent cells (Jarvis 1984). Plant cell walls consist of cellulose microfibrils embedded in a matrix containing pectins and hemicelluloses (McNeil & Darvill 1984), and it is thought that the microfibrils of primary cell walls are covalently linked to the pectic network in the middle lamella (Jarvis et al. 2003). The cell wall is rigid and retains cell shape, and its covalent polymer network can be disrupted either by the cleaving of the chains between crosslinks, or by the breaking of the cross-links themselves (Jarvis et al. 2003). This process dissociates adjacent cells and allows cell separation to occur.

**Abscission due to herbivory – the possible role of insect oral secretions**

**Insect oral secretions and plant defenses.**

Although mechanical damage from feeding is an important factor in the induction of some plant defenses, chemical stimuli in insect oral secretions are now known to play a crucial role in the induction of many important plant defenses. However, the role of insect oral secretions in abscission of reproductive structures is largely unknown. Insect oral secretions, or regurgitant, are defined as fluids collected from the insect following
disturbances such as squeezing or pinching and contain components from the alimentary canal as well as saliva (Felton & Eichenseer 1999). Elicitors of plant responses have been found in both regurgitant and in pure saliva (Turlings et al. 1990, Matiacci et al. 1995, Musser et al. 2005).

There are a number of plant defenses that are not associated with wounding or artificial damage alone, but can be induced by the application of elicitors from herbivore secretions to artificial plant wounds (Mattiacci et al. 1995, Alborn et al. 1997, McCloud and Baldwin 1997, Felton & Korth 2000, Musser et al. 2005). In addition to inducing direct plant defenses, application of elicitors in oral secretions of insects has been shown to cause the release of volatile compounds that attract natural enemies of the herbivore (Turlings et al. 1990, Mattiacci et al. 1995, Alborn et al. 1997). There are some cases in which defenses are elicited without any mechanical damage. For example, Turlings et al. (1993) showed that corn leaves incubated in a beet armyworm regurgitant solution resulted in terpene emission that attracted parasitic wasps without any damage to the leaf blade. More often, however, both wounding by herbivores as well as components of oral secretions play important roles in the elicitation of plant defenses (Mattiacci et al. 1995, Turlings et al. 1995, McCloud & Baldwin 1997, DeMoraes et al. 1998, vonDahl et al. 2006).

**Oral secretions and abscission**

Though many studies have demonstrated that induced defenses in leaves can be initiated by insect oral secretions or wounding, the literature lacks information about the effects of oral secretions on reproductive structures. Many plants are known to abscise fruit or flowers upon damage by herbivores (Marr & Pellmyr 2003, Kostenyuk & Burns 2004, Ruperti et al. 2002; reviewed by Sallabanks & Courtney 1992). However, the possible role of oral secretions versus mechanical damage in abscission of reproductive parts is very rarely addressed. Two exceptions include studies that show varying results. Shackel et al. (2005) injected small quantities of *Lygus* bug salivary enzymes into cotton and alfalfa flowers to determine whether damage by *Lygus* bugs on these crops was caused by stylet damage, or
biochemical responses to components in saliva. Browning of flowers and an arrest in growth occurred not only at the injection site, but also in surrounding areas. It was concluded that components in the saliva caused the withering and abscission of both cotton and alfalfa flowers (Shackel et al. 2005). Levine and Hall (1978) injected protein extracted from boll weevil larvae into cotton flowers and found that the resulting flower bud abscission was most likely not from boll weevil salivary extracts, but rather from molting fluid in larvae (Levine & Hall 1978, Coakley et al. 1969). Other authors attribute bud abscission to mechanical damage by boll weevils (Santos et al. 2003).

Using Heliothis subflexa and Physalis species as a model for studying herbivore-induced abscission

Herbivory by Heliothis subflexa larvae is known to cause fruit abscission in Physalis species (Benda 2007). Larvae feed on the Physalis fruit, which are enclosed in the inflated calyx that surrounds the fruit. This inflated calyx provides a structural refuge to H. subflexa (Sisterson & Gould 1999), and has been shown to be one of the causal factors for uncharacteristically low parasitism rates by Cardiochiles nigriceps (Oppenheim & Gould 2002). H. subflexa larvae bore a hole into the calyx to reach the enclosed fruit, and must consume several fruit in order to complete development. A mechanism employed by members of the Physalis genus to avoid further fruit herbivory is abscission of the fruit on which H. subflexa feed. This prevents subsequent movement to other fruit on the plant, unless the larva can relocated and reestablish itself onto the plant following fruit abscission. Plants in the genus Physalis have been shown to vary substantially in both the probability and rate of abscission of herbivore-damaged fruit (Benda 2007), however, the specific mechanism (mechanical damage or elicitors in oral secretions) that causes fruit abscission in Physalis species had not been studied.
Ethylene has long been known to be important for fruit abscission. Herbivory by insects can cause the induction of the jasmonic acid pathway, and it has been shown that jasmonic acid and ethylene can act synergistically in producing chemical defenses against herbivory (O’Donnell et al., 1996). In several cases, an increase in levels of jasmonic acid has been shown to induce increased ethylene production, which causes fruit abscission. This is true for citrus fruits (Hartmond et al., 2000), tomato and apple (Saniewski et al., 1987a, Saniewski et al., 1987b), and cherry tomatoes (Beno-Moualem et al., 2004). Martin et al. (1988) injected excised cotton squares with Pseudatomoscelis seriatus salivary extracts to measure the ethylene-inducing effects of these extracts after previous studies reported a difference in cotton square abscission caused by feeding versus artificial damage. They found that pectinases in the salivary extracts of these insects caused substantial ethylene production. In sum, these findings indicated that it is feasible that damage caused by herbivory could initiate the jasmonic acid pathway, leading to increased ethylene production and ultimately abscission of fruit in *Physalis* species.

In *Physalis* plants, it has been shown that parasitoid-attracting volatiles, albeit modest amounts, are emitted by plants whose fruit are damaged by *H. subflexa* (De Moraes & Mescher 2004). In addition, visits by the parasitoid *C. nigriceps* were substantially higher for *Physalis* plants infested with *H. subflexa* compared to those infested with the closely related *H. virescens* (Oppenheim & Gould 2002), which inflict similar damage to fruit. This suggests a likely role in parasitoid attraction to *Physalis* plants by the oral secretions of *H. subflexa*, which may be initiated by the jasmonic acid signaling pathway. Because jasmonic acid is often an important signal for fruit abscission, and components in *H. subflexa* oral secretions seem to be important in calling parasitoids, it is possible that elicitors in *H. subflexa* oral secretions are an important cue in initiating the jasmonic acid pathway and subsequent fruit abscission in *Physalis* species.

To our knowledge, there has been no single study that tested for the independent and combined effects of herbivore oral secretions and mechanical damage on fruit abscission.
Because frugivores can cause substantial loss of plant fitness in nature and yield in agriculture, a better understanding of the specific mechanisms by which herbivory causes fruit abscission would be useful.

II. Plant response to insect eggs

The ability of plants to react in a defensive way to insect eggs is a recently discovered phenomenon that, in all cases identified so far, is initiated by a chemical cue from the insect. This is an important finding, because it adds another dimension of complexity in the evolutionary ‘arms race’ between plants and insects. Chemical cues from insect eggs elicit specific plant defenses against the insect before it hatches, effectively reducing the amount of potential herbivory by defending against the least destructive stage of the herbivore. Details on the mechanisms by which plants react to insect eggs are quickly emerging, but very few systems have been studied. Whether oviposition-induced defenses against insect eggs have evolved in many plant species remains to be seen, although it has been hypothesized that this may be a common phenomenon existing in many plants (Agrawal 2000). To date, only six systems have been studied with regard to oviposition-induced defenses. Details on this phenomenon in other systems are essential for a better understanding of the selective impact that insects have on plants. Theories of host-plant specificity, coevolution between plants and their herbivores, and distribution of secondary compounds among plant parts and plant species are all influenced by our understanding of selection by insects for plant defenses (Fritz & Simms 1992).

Plants that respond to insect eggs have been shown to do so in two ways: 1) directly, by physically reacting to the egg at the site of deposition and 2) indirectly, by emitting volatile chemicals that attract egg parasitoids.
Direct defenses

Physical reactions to insect eggs can be characterized by either a neoplasmic (tumor like) growth that forms directly under the egg, or browning and death of tissue under the egg that resembles a hypersensitive response (HR). Neoplasmic growths, or neoplasms, occur when cell division is stimulated in non-meristematic areas. Pea plants of a specific genotype react by forming neoplasms in response to pea weevil eggs (Doss et al. 1995). These neoplasms elevate the egg above the surface of the pea pod, resulting in disorientation of the pea weevil upon hatching and eventual desiccation (Doss et al. 2000). The only other plants known to form neoplasms directly under an insect egg are those in the genus Physalis (discussed below). A hypersensitive response, first described in the context of defense against microbes (Ward & Ward 1902; Klement & Goodman 1967), also occurs as a defense against insect eggs and is characterized as the formation of necrotic tissue at the site of egg placement. There are two described cases in which plants respond to eggs with an HR: mustard (Brassica nigra) in response to Pieris brassicae and Pieris napi eggs, and a potato hybrid in response to Colorado potato beetle eggs. In the mustard system, tissue directly under eggs undergoes an HR within 24 hours of egg laying; this response was shown to kill eggs by desiccation within three days (Shapiro & Devay 1987). Colorado potato beetle eggs induce the formation of necrotic tissue in leaves of certain potato hybrids in a zone under egg masses, causing detachment from the leaf and subsequent larval death after hatching (Balbyshev & Lorenzen 1997). Thus, both types of physical reactions to eggs, neoplasms and hypersensitive responses, result increased mortality of eggs and larvae.

Indirect defenses

The term indirect defense refers to adaptations that result in the recruitment of organisms that protect plants against herbivorous attackers (Cardé & Millar 2004). Many plant species are known to emit volatiles in response to insects feeding on them. The release of these volatiles is induced by elicitors in insect oral secretions and/or by damage from feeding, and attracts predators or parasitoids of the insects causing the damage (Dicke et al. 1997; Paré &
Plants have recently been shown to respond indirectly to insect eggs by releasing volatiles (=synomones) that attract egg parasitoids (insects that lay their eggs in the eggs of other insects); this phenomenon has been shown in three systems to date. The egg parasitoid *Oomyzus gellerucae* uses volatiles released in response to oviposition by elm trees to orient to and parasitize eggs of the elm leaf beetle, *Xanthogaleruca luteola* (Meiners & Hilker 1997). Similarly, pine tree (*Pinus sylvestris*) needles emit volatiles attractive to the specialist egg parasitoid *Chrysonotomyia ruforum* in response to oviposition by pine sawfly (*Diprion pini*) females. The bean/Nezara system also illustrates the importance of indirect defenses. Two species of bean, *Vicia faba* and *Phaseolis vulgaris*, emit volatiles that attract the egg parasitoid *Trissolcus basalis*, in response to both feeding and egg deposition by the bug *Nezara viridula*.

**Specificity of response**

Plant response to egg deposition, whether directly or indirectly, can occur with a high amount of specificity both in the type of response and the ability to respond. Only lines of peas with a specific genotype form neoplasms in response to bruchins present in several bruchid species (Doss *et al.* 2000). These same lines of pea species are not elicited to form neoplasms by bruchins if they are applied to non-ovipositional sites, such as stems or leaves (Oliver *et al.* 2002). There also seems to be a high amount of specificity for HRs; in both systems studied (*Solanum* and *Brassica*), only certain lines of *Solanum* (Balbyshev & Lorenzen 1997) and only certain individuals in a *Brassica* population (Shapiro & Devay 1987) respond to egg deposition by the herbivore. In the elm/elm leaf beetle system (*Ulmus minor* and *Xanthogaleruca luteola*), only eggs of *X. luteola* elicit a response from *U. minor*; eggs of a closely related species, *Galeruca tanaceti*, do not (Meiners *et al.* 2000). Additionally, elm leaf beetle eggs elicit emission of attractive volatiles only for *Ulmus minor*; volatiles emitted by *Ulmus glabra*, a closely related but less preferred elm species, are not attractive to egg parasitoids following egg deposition (Meiners *et al.* 2000). Thus, the response of the
parasitoid is specific for the plant species most favorable for the elm leaf beetle.

In the pine/pine sawfly system (*Pinus sylvestris* and *Diprion pini*), the response of the egg parasitoid *C. ruforum* was shown to be specific for the plant species, but not as specific for the herbivore species. *Chrysonotomyia ruforum* did not respond to volatiles from a closely related pine species, *P. nigra*, after egg deposition by *D. pini*, even though this parasitoid has been observed to parasitize *D. pini* eggs on *P. nigra* (Mumm et al. 2005). Two diprionid species closely related to *D. pini* (*Gilpinia pallida* and *Neodiprion sertifer*) that also feed on *P. sylvestris* had different effects on the ability to elicit attractive volatile emission in this plant. Eggs of *G. pallida* did not elicit volatiles that were attractive to the parasitoid, while eggs of *N. sertifer* did (Mumm et al. 2005). *Neodiprion sertifer* and *D. pini* are known to cause significantly more defoliation compared to *G. pallida* (Pschorn-Walcher & Eichhorn 1976); the authors suggest that the plant ‘recognizes’ eggs of different species that cause varying amounts of damage by detecting elicitors on the egg surfaces to which it responds differently (Mumm et al. 2005; Stout & Bostock 1999). Although there seems to be a significant level of specificity in the elm/elm leaf beetle and pine/pine sawfly systems, the bean/*N. viridula* system does not seem to be governed by such specificity. In contrast to the elm leaf beetle and the pine sawfly, which have restricted host ranges, *N. viridula* is a generalist that feeds on over 150 plants in more than 30 families (Schaefer & Panizzi 2000). Oviposition by this insect was able to induce very similar volatile blends attractive to its egg parasitoid in both of the bean species tested, and the authors predict that, because of this insect’s broad host range, it most likely induces similar volatile profiles attractive to the egg parasitoid in a variety of annual and perennial host plants (Colazza et al. 2004). Thus, plants that have close associations with their specialist insects respond with a high degree of specificity to eggs of that insect.
Physalis *species and* Heliothis subflexa

In response to oviposition by Hs, at least two species in this genus, *P. angulata* and *P. pubescens*, react to eggs. The tissue directly under the singly laid eggs reacts in one of three ways, either with 1) formation of a neoplasm, 2) an HR, or 3) in both ways. Not all eggs cause a reaction; the reason for this is unknown. Preliminary studies on this response have shown that a reaction to an egg decreases the probability that an egg will hatch by about 20% for *P. angulata*, and about 24% for *P. pubescens*. Reactions to eggs usually occur within three days after oviposition; some eggs that elicit a reaction shrivel up and desiccate on or before day six; the mechanism by which eggs are killed is unknown. The three different responses to eggs vary in the degree to which they cause egg mortality; a neoplasm and HR together cause highest egg mortality (60%), while separately, an HR and neoplasm cause lower mortality (41% and 37%, respectively, not significantly different). The proportions in which these reactions occur differ between the two plant species. The oviposition-induced elicitors that cause reactions are unknown in this system. However, small glass beads the size of Hs eggs placed on the leaf surface of *Physalis* plants do not induce a response, indicating that reactions on leaves are not elicited by the physical presence of an egg. Thus, it is likely that the elicitor is associated with the oviposition secretion, or is present on the surface of the egg.

One puzzling aspect of this system is that *Hs* females do not oviposit exclusively on *Physalis* plants, despite the fact that larvae can only develop on *Physalis* fruit. Instead, females lay approximately 20% of eggs on non-hosts growing in the immediate vicinity of their *Physalis* hosts (Benda 2007). Oviposition on non-hosts could simply be non-optimal behavior, or it could be that there would be disadvantages to *Hs* females that lay 100% of their eggs on *Physalis*. A better understanding of the effects of responses of *Physalis* leaves to *Hs* eggs would allow us to determine if the ability of *Physalis* plants to physically respond to *Hs* eggs may be one factor that could have selected for non-host oviposition.
THE GENETIC ARCHITECTURE OF HOST USE

Understanding the evolution of host range is one of the foremost goals in the field of plant-herbivore interactions, and although this issue has been increasingly addressed in the past several decades, many questions still remain. Despite the fact that 90% of insects are specialists, phylogenetic evidence indicates that specialization is not an evolutionary endpoint and that host plant range can continue to expand and contract over time (Nosil 2002). However, how much of a genetic change is needed for an insect to modify its host range is unknown. Is a change in host use caused by an accumulation of many small genetic changes over evolutionary time, or several large changes? Elucidating the genetic basis of host range evolution will allow for a better understanding of how speciation can occur and how the diversity of plant-feeding (phytophagous) insects has arisen, and will also provide insight on methods for controlling agricultural pest species (Futuyma & Peterson, 1985; Tang et al., 2006).

Because insects in the Lepidoptera comprise the second largest insect order (Arnett 1985), they serve as an important model system for understanding the genetic basis of host use. There is very limited information on the genetics of host use in the Lepidoptera (Nylin et al. 2005, and references therein), despite the fact that insects in the Lepidoptera have considerable economic importance and have served as models of evolution of plant-insect interactions since the publication of the seminal paper by Ehrlich and Raven (1964).

There have been a number of studies examining the genetics of host use by hybridizing subspecies with different host ranges (e.g. Huettel & Bush 1972; van Drogelen & van Loon 1980; Scriber 1983, Tauber & Tauber 1987, Thompson 1988). These studies provide an insight into the genetics of host range, but crossing species with wide differences in host range provides a better opportunity to understanding the genetic basis of differences in host use between generalists and specialists (Hora et al. 2005). However, relatively few studies like this exist, since pairs of species with such extreme differences in host range that can be
hybridized are rare. The few studies that use line crosses of separate species with vast differences in host ranges to study the genetic architecture of host use are discussed below.

*Feeding behavior*

To gain a better understanding of the genetics of host use, Scheck and Gould (1993) performed a line cross analysis of the closely related species Hs and Hv to assess feeding behavior of hybrids and backcrosses. Hs and Hv were crossed to produce F₁ hybrids and a backcross to Hs. Feeding on soybean, tobacco, cotton (hosts of Hv, the generalist), and *Physalis pubescens* (a host of Hs, the specialist) was used to assess larval performance and mortality of hybrid and backcrossed larvae relative to parental performance. Genes from Hs were found to be overdominant for larval survival and dominant for weight gain on *P. pubescens*, although epistatic or gene-environment interactions were also likely involved. Genes for Hv were partially dominant for survival and weight gain on cotton and tobacco, and additive for these traits on soybean (Scheck & Gould 1993). In addition, weight gain and survival were concluded to be controlled by autosomal, and not sex-linked, genes. Later experiments sought to determine if loci from Hv conferring utilization of soybean also controlled utilization of other hosts (Scheck & Gould 1996). This was accomplished by moving these loci from Hv into the genetic background of Hs. Although performance on soybean and cotton was partially correlated in individuals with this genetic makeup, larvae did not perform well on cotton or tobacco, and it was concluded that performance on these plants had an independent genetic basis (Scheck & Gould 1996). This finding is important, because it suggests that a single locus is not responsible for the extreme differences in host range between the specialist Hs and the generalist Hv. Tang *et al.* (2006) also investigated the genetic basis of host use using the generalist *Helicoverpa armigera* and the closely related specialist *H. assulta*. In this study, the feeding preference of progeny from interspecific crosses (F₁, F₂ and backcrosses) of a specialist (*Helicoverpa assulta*) and a generalist (*H. armigera*) was examined. Feeding preferences of hybrids resembled those of the generalist. Backcrosses to the generalist exhibited feeding behavior nearly identical to
that of the generalist, while backcrosses to the specialist had more variable feeding behavior and were grouped into two classes: those that fed primarily on pepper, the specialist’s host plant, and those that fed primarily on cotton, a host plant of the generalist. The findings in this study suggested that (1) larval feeding preference was determined by autosomal genes, (2) \textit{H. armigera}-derived alleles were partially dominant to \textit{H. assulta} alleles, and (3) at least one major autosomal gene was involved in larval feeding on cotton (Tang \textit{et al.} 2006). These studies offer a glimpse into the genetic architecture of host range with respect to feeding behavior, however, more experiments studying the feeding behavior of insects with different host ranges are necessary for a general understanding of what genetic factors are involved with a change in host range.

\textit{Oviposition behavior}

Because the neonate larvae of many phytophagous insects are small and largely immobile, female oviposition behavior is another important component of evolutionary modification of host range. As is the case for feeding behavior, detailed information on the genetic architecture of host use and oviposition behavior is scarce (Fox \textit{et al.} 2004). Several studies have utilized line crosses to examine the genetics of species or population differences in oviposition behavior. However, there are varying results regarding the number of genes controlling host use, the degree to which dominance and epistasis are involved, and whether genes are additive or non-additive. Some studies have detected some dominance and epistasis of genes controlling oviposition behavior (Guldemond 1990, Lu and Logan 1995, Sheck & Gould 1995, Craig \textit{et al.} 2001), while other studies have shown evidence for an additive genetic basis governing oviposition preference (Sezer & Butlin 1998). Fox \textit{et al.} (2004) showed that differences in oviposition preference of different populations of the seed beetle \textit{Callosobruchus maculatus} were explained by complete additivity, however dominance and epistasis were responsible for differences in egg dispersion. In some systems, oviposition genes are thought to be sex-linked (Thompson 1988, Scriber \textit{et al.} 1991, Janz 1998), whereas other systems show no evidence of sex-linkage (Nylin \textit{et al.} 2005, Hora \textit{et al.}
Finally, the number of genes controlling oviposition behavior is also variable, with some systems showing monogenic control (Guldermond 1990, Lu & Logan 1995, Sezer & Butlin 1998), and others showing polygenic control (Sheck & Gould 1995). Sheck & Gould (1995) examined oviposition preference of Hs, Hv, and reciprocal F₁ hybrids in oviposition cages in a greenhouse. Four hosts were supplied as oviposition substrates: cotton, tobacco, soybean (hosts of Hv), and Physalis angulata (host of Hs), and these hosts were ranked for preference. Hs females preferred Physalis, while Hv and both reciprocal hybrids preferred tobacco. There was no evidence of sex-linkage, since both reciprocal hybrids had similar patterns of preference across hosts (Sheck & Gould 1995). Feeding studies using this system have also shown that host utilization traits are not sex-linked (Sheck & Gould 1993).

**Future work**

Further genetic studies employing the use of molecular markers are necessary to determine if there is one or few major loci that govern oviposition behavior, or if oviposition behavior is controlled my many loci. Studies using line crosses aimed at obtaining phenotypic data on oviposition behavior and feeding behavior from a large sample of backcross moths and larvae would allow for the use of the Hs/Hv system to gain a better understanding of the genetic basis of host use. A quantitative trait locus analysis would further allow us to pinpoint specific chromosomes that are necessary for a change in host use.

A broader understanding of the details of newly specialized interactions of insects and their host plants should be a major focus for future work. Understanding the degree of specialization of mechanisms of plant defense and insect response will lead to a better understanding of broad ecological concepts, such as speciation and mechanisms of diversification.


Szymkowiak E.J., Irish E.E. 1999. Interactions between jointless and wild-type tomato tissues during development of the pedicel abscission zone and the inflorescence meristem. The Plant Cell 11: 159-175.


HOST FINDING IN *HELIOTHIS SUBFLEXA*: AN ANALYSIS OF VISUAL AND Olfactory Cues

Jennifer Petzold\textsuperscript{1}, Coby Schal\textsuperscript{2}, Fred Gould\textsuperscript{2}

\textsuperscript{1}Department of Plant Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

\textsuperscript{2}Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA
Host finding in phytophagous insects involves the processing of a number of signals, including visual and olfactory cues. Most studies examining the cues mediating host finding and oviposition take place in the laboratory because of the difficulty in obtaining data in the field. In this study, we examined the relative role of visual and olfactory cues in host finding for *Heliothis subflexa* in a field setting. First, oviposition behavior in a large field tent was assessed to determine if behavior was similar to behavior observed under natural field conditions. We found that moths observed in the field tent laid similar proportions of eggs on 1) hosts and non-hosts, and on 2) various plant parts of its host *Physalis angulata*, and showed the same general oviposition behaviors compared to those observed under natural field conditions. Using cages to cover host plants that differed in visual obstruction of the plants, we compared the number of approaches, lands, and oviposition events on these two types of cages containing *Physalis* plants. Moths laid a higher number of eggs, and had significantly more approaches, approaches resulting in lands, and lands resulting in oviposition events, on cages that did not obstruct the view of the host plant. Our results demonstrate an accurate way to observe host finding in *H. subflexa* behavior in a field setting using a large outdoor tent, and show that vision is an important cue in host finding for *H. subflexa*. 
INTRODUCTION

Understanding the behavioral and sensory mechanisms important in the location and acceptance of host plants for oviposition by herbivorous insects has many significant implications, both in terms of evolutionary processes and for pest management purposes. Oviposition behavior of insects is one of the most important determinants of host range, because neonates are often immobile and must feed on the plant species chosen by the ovipositing female (Honda 1995). However, for most herbivorous insect species, little is known about the natural behavioral events leading up to oviposition.

Host finding can be categorized into three processes: host-habitat location, host location, and host acceptance (defined by oviposition on a plant); these categories can overlap in the sensory mechanisms involved, and are not independent of each other (Ramaswamy 1988). The steps involved in locating and ovipositing on a host plant require the processing of a number of cues by ovipositing females. These cues often involve olfaction, mechanoreception, contact chemoreception, and vision (reviewed by Miller and Strickler 1984, Ramaswamy 1988, Renwick and Chew 1994). Chemical cues, such as volatiles, have been shown to be important in all steps of host finding (e.g. Visser 1986, Ramaswamy 1988, Tingle et al. 1990, Tingle and Mitchell 1991, Bernays and Chapman 1994, Jallow et al. 1999, Witzgall et al. 2005, Derksen et al. 2007). Substrate surface texture, which is sensed most often by mechanoreceptors on the ovipositor, has been shown to be a significant cue for host acceptance in some systems (Ampofo 1985, Kumar and Saxena 1985, Ramaswamy 1988). Contact chemoreception is also important in host acceptance, allowing insects to accept or reject an oviposition substrate based on the presence or absence of specific chemicals (Tabashnik 1985, Qui et al. 1998, Maher et al. 2006, Calatayud et al. 2008, Newland and Yates 2008). Finally, visual cues such as color, leaf shape, and leaf size are imperative cues used in host finding for some species (Rausher 1978, Aker and Udovic 1981, Tabashnik
1985, Bernays and Chapman 1994), but have shown to be unimportant in host finding for other species (Riggin-Bucci et al. 1998, Couty 2006).

*Heliothis subflexa* G. (Lepidoptera: Noctuidae) is a specialist, feeding only on plants in the genus *Physalis* (Laster et al. 1982). Studies examining the role of volatiles and surface chemicals in host finding by *H. subflexa* (hereafter referred to as Hs) have shown that both of these cues are important: methanol extracts of homogenized *Physalis angulata* leaves increased oviposition 8.5-fold on treated non-hosts over untreated controls (Mitchell and Heath 1987), and gravid females showed oriented flight towards volatiles from *Physalis angulata*, but not towards non-host volatiles, in a dual-choice flight tunnel (Tingle et al. 1990).

A large number of studies aimed at understanding cues involved in oviposition take place in the lab, and are focused on the host acceptance process: whether an insect oviposits on a plant once it lands on the plant (Couty et al. 2006). However, fewer studies examine the host location process (involving how insects locate and land on plants when searching for hosts), or host location and host acceptance, in a field setting (but see Prokopy et al. 1983 and Prokopy et al. 1994). In this study, we examine the relative importance of visual and olfactory cues in host location and host acceptance for Hs using a large tent placed in the field.

**MATERIALS AND METHODS**

*Plants*

Observations occurred in 2006 and 2008. Plants used in the 2006 observations were grown in a greenhouse kept at approximately 30/25°C day/night. Seeds were from a lineage of *Physalis angulata* plants originally collected from wild populations in Orangeburg County, SC, USA (33°33′N, 81°04′W) in 1998. Seeds were sown in large flats containing potting soil.
(2 Mix Professional Formula, Fafard), and two teaspoons of fertilizer (Osmocote 14-14-14, N-P-K). Seedlings were transferred to 0.5-l plastic pots three weeks after germination, and were fertilized with one teaspoon of fertilizer, after which they were fertilized every four weeks. Ten-week old plants were transplanted into 7.5-l plastic pots, and were moved to an outdoor field site where they stayed until they were used in the experiment; plants had mature fruit and were 12-15 weeks old at their time of use.

Plants used in the 2008 observations were grown in NCSU’s phytotron, a facility that houses controlled-environment chambers. Seeds of Physalis angulata were started in flats containing potting soil, and were subsequently transplanted singly to small Styrofoam cups one to two weeks after germination. All plants were grown in a chamber kept at 26/22°C day/night, with lights on from 8:00 – 17:00 (fluorescent and incandescent lighting) and a night interruption from 23:00 – 2:00 (incandescent lighting). Light intensity ranged from 550 – 600 μmoles/m²/sec, and humidity ranged from 40 – 65%. Plants were watered twice a day with a nutrient solution (see Saravitz et al. 2008 for nutrient composition). Two weeks later, plants were transplanted into 5-l plastic pots. Four weeks later, Physalis plants were moved to an outside field plot to acclimate to field conditions. Here, plants were transplanted to 7.5-l plastic pots and watered daily. Plants remained outside for 1-3 weeks, and were then placed in the outdoor field cage, where they remained for one week.

Insects

Insects used in the 2006 observations were field collected. Second to fifth instar larvae were collected from Physalis fruits in a field (Central Crops Research Station, Johnston County, NC, U.S.A.) containing both planted and natural populations of P. angulata and P. pubescens plants. Larvae were reared in the laboratory on P. angulata fruit in small cups; fruit were replaced every 2 days. Upon pupation, female and male pupae were separated. Once adult female moths emerged, they were mated singly with male Hs obtained from the field or from our laboratory colony (rearing procedures for colony are described below). Couples were supplied with sugar water (5% sucrose solution), and after three nights males were dissected
to confirm that mating occurred (Groot et al. 2006). Fertile females were marked on one or both wings using a Sharpie marker for identification in the cage.

In order to supplement the number of females available for release in the cage, we also collected adult females from the field. Adult females observed to be ovipositing on *Physalis* plants were collected at dusk (when oviposition occurs) at two field sites where *Physalis* plants were abundant (Central Crops Research Station, Johnston County, NC, U.S.A.; NCSU Lake Wheeler field site, Wake County, NC, U.S.A.). Moths were immediately transferred to buckets containing sugar water, and placed in the greenhouse where they remained until they were used for observations in the field cage the following nights. Moths were used until they were lost in the cage or died.

Because oviposition behavior of laboratory-raised and field collected Hs did not seem to differ in observations in a separate study in 2006 and 2007 (J. Petzold, pers. obs.), insects used in the 2008 observations were from our laboratory colonies. The Hs colony originated from larvae collected from *Physalis angulata* fruits in Florence and Barnwell Counties, SC, USA, in 1996 (Sheck et al. 2006), and has been maintained continuously in the laboratory using the methods outlined by Sheck & Gould (1993). Briefly, moths are mated in groups of 20-30 in large buckets, with cheesecloth used as an oviposition substrate, and neonates are reared on a corn/soymeal diet (Burton 1970). Pupae were taken from the laboratory colony, and separated by sex. Upon emergence, females were mated singly with males and fertile females were marked with Sharpies (described above) and used for observations in the field cage.

**Observations**

All observations took place in a large (7.6 x 15.2 x 2 m) outdoor screen tent (Fig 1a) placed in a tilled field in Wake County on NC State University campus (Raleigh, NC, U.S.A). The ground in the tent’s interior was covered with black woven plastic ground cover (Lumite, 183 cm-wide) to hinder weed growth. Moths were released into the tent for observation at sunset,
when moths typically begin to oviposit (Benda 2007), and were observed until activity ceased (usually 1.5-2 h after sunset).

_Oviposition on hosts and non-hosts_

In 2006, our goal was to determine if moth oviposition behavior in the large field tent accurately represented oviposition behavior in the field. Three treatments were set up to determine how many eggs would be laid on weeds at three distances from host plants (15, 30, and 100 cm), and also to determine the overall percent of eggs laid on weeds. Similar data were collected by Benda (2007) under natural field conditions. We used crabgrass (_Digitaria sp._) taken from a field site containing _Physalis_ plants for our weed species; this was the most common species on which moths laid eggs in previous years’ observations (Benda 2007). A total of 9 _Physalis_ plants were placed in two rows inside the tent. Three plants each were randomly assigned one of the three distance treatments. For plants assigned the 15 cm distance treatment, 6 weeds in 0.5-l plastic pots were placed in a circle around the plant, with approximately 15 cm separating each of the weeds. This was repeated for the plants assigned the 30 and 100 cm distance treatments; 12 and 42 weeds, respectively, were placed in a circle around the _Physalis_ plant for the 30 and 100 cm treatments, in order to have all weeds spaced with approximately 15 cm between each plant. After four nights of observations, the treatment with weeds at 100 cm from _Physalis_ plants was eliminated because no eggs were laid on these weeds. This allowed for 12 _Physalis_ plants to be used in the cage (6 per row), with the 15 and 30 cm distance treatments alternating between plants. Each night, we released as many fertile field moths as were available (ranging from 4-15), and recorded oviposition behavior into a digital voice recorder (Sony ICD-P250). Observers used headlamps with a red filter to observe behavior. The following behaviors were recorded 1) where a moth laid an egg on a _Physalis_ plant (stem, leaf, fruit or flower), and 2) if a moth laid an egg on a weed, how far the weed was from the _Physalis_ plant (15, 30, or 100 cm). Data from a total of 11 observation nights were obtained. Because all observations occurred in one field tent, each night served as a replicate.
**Host finding behavior**

After 11 observation nights with the treatments as described above, we changed the arrangement to include 12 plants with only the 15 cm distance treatment, since most eggs laid on weeds from the previous observations were laid on those that were 15 cm from host plants. Every other *Physalis* plant was covered with a cage constructed from 2 layers of wire mesh screen (mesh size: 1.8 mm) to form a 36 cm (diameter) x 85 cm (height) circular cage (Fig 1b). Foam weather stripping was placed between the two layers of screen; this prevented moths from touching plant material with ovipositors while on the cages. The top of each cage was covered with cheesecloth, which obstructed the view of the plant inside when viewed from above; plants were not very visible from the sides of the cages either (Fig 1b). The same behaviors described above were recorded, as well as any lands or ovipositions on caged plants. A total of 9 observation nights were conducted with this set of treatments.

Results from the above-described experiments suggested that vision may be an important cue for host finding in Hs; this hypothesis was tested in field experiments in 2008. For observations in 2008, a total of 6 *P. angulata* plants were placed in the front 1/3 of the observation tent. Plants were covered with round cages (height: 90 cm, diameter: 48 cm) constructed from chicken wire covered with bridal veil. A second layer of chicken wire was placed inside the cages, which prevented leaf material from touching the outside-most portion of the screen; this ensured that moths that landed on cages could not touch plant material with ovipositors. One layer of bridal veil created a nearly translucent cage, allowing full view of the *Physalis* plant inside, while eight layers was just enough to obstruct the view of the plant inside (Fig 1c). Three of the plants were contained inside cages with one layer of bridal veil (hereafter referred to as 1-layer cages), and the other three plants were contained inside cages with eight layers of bridal veil (hereafter referred to as 8-layer cages). Plants were placed in two rows, with three plants per row (spaced 1.8 m from each other), and covered with 1- and 8-layer cages as shown in Fig 1d. Plants were used for 5 days, after which they were replaced with new plants. For each night of observations, plants were
rotated to receive a different treatment, so that the same plant never received the same
treatment on consecutive observation nights. Observations occurred on 9 nights from 27

At sunset, moths were released into the outdoor field tent. Approximately 15-20 moths were
released into the tent each night; moths were recaptured at the end of the night (with an
~80% recapture rate), and reused the next 2-3 nights if observations were taking place on
those nights. Upon release, activity of moths was immediately recorded using a digital voice
recorder. For each plant in the large field tent, the following activities were recorded: 1)
number of approaches to a plant, 2) number of landings on the cage covering the plant, and
3) whether a landing resulted in an oviposition event or not. A moth was considered to have
approached a plant when it approached an area within approximately 0.5 m of the plant and
hovered in this area for greater than 2 seconds. For each of these observations, the type of
cage covering the plant (1-layer or 8-layer) was noted. Two observers were always present,
with one observer recording observations and the other calling out observations to the
recorder if more than one moth was active at a time. Observers used headlamps to observe
moths approximately 1 hour after sunset; because observations in 2006 and 2007 showed that
there was no difference in moth behavior whether white light or red light was used, we used
white light to observe moths in 2008.

To determine the difference in host finding behavior on plants covered with 1-layer cages
versus those not covered at all, we used the same experimental set up as described above,
however three plants were covered with 1-layer cages and three were left uncovered.
Observations occurred on two nights (9 and 14 Oct 2008), and observations were collected as
described above.

Volatile emission through cages

To test for potential differences in volatile emission from plants through the two types of
cages used in 2008 (covered with 1 versus 8 layers of bridal veil), we used solid-phase
micro-extraction (SPME) sampling, coupled with gas chromatography. A blend of four chemical standards (trans-2-hexenal, 2-ethyl-1-hexanol, benzaldehyde and eugenol) was created (20 µg/mL of each compound, in hexane). 100 µl of this solution was pipetted onto filter paper (Whatman #1, 42.5 mm diameter), and the filter paper was suspended 80 cm above the ground to mimic the average height of the Physalis plants placed in cages during observation periods. Each type of cage was placed over the suspended filter paper containing the blend. The two cages were spaced 1.3 m apart, as they were in the field. The SPME fibers were conditioned in the injector of the GC system, according to the manufacturer's instructions, before use. The SPME holding devices were suspended 2 cm above the top of each cage (7 cm from the filter paper containing the standards), and the SPME fibers were exposed for 60 minutes. Volatile collection took place in a closed room with limited air flow. After a 60 minute aeration period of the room, the procedure was repeated with a second set of SPME devices. During the second run, the position of the two types of cages was interchanged. Each of the four SPME devices were subsequently inserted into the GC injector to desorb the fibers, and GC analysis of each of the volatile components was carried out. This pair of experimental runs was repeated two more times, for a total of 6 replications. Because each syringe may have slightly different adsorption properties, the two syringes used to collect volatiles from the 1-layer cages were used to collect volatiles from the 8-layer cages in subsequent replications.

Each of the four standards used in the experiments was identified in the GC output data based on known retention times. Prior to experiments, six different amounts of each standard compound (1, 2, 10, 20, 50, 100, and 200 ng) was injected into the GC column, and the area under the curves obtained from chromatography data acquisition software for each of these amounts was used to generate standard curves for each of the compounds. The amount of each of the four standards detected by the SPME devices in each of the experimental runs was calculated by using these standard curves (each curve had an R² = 0.999). The average amount of each compound detected from each of the types of cages was calculated. Paired,
one-tailed t-tests were used to determine if the amount of each compound detected was
different for the 1-layer versus 8-layer cages.

Because cages were separated by only 1.3 m (to replicate field set-up conditions), and the
experiments were run in a small room where air flow was kept at a minimum to facilitate
accurate collection of data, additional experiments were run to ensure that volatiles were not
saturating the room and contaminating adjacent SPME detectors. The above described
experiment was repeated with an exposure time of 30 min (instead of 60 min as above), for a
total of 6 more replications. In addition, a separate experiment was run in which only one of
the two cages being tested contained filter paper saturated with 100 µl of the volatile blend.
The other cage contained filter paper saturated with 100 µl of deionized water. SPME
devices were placed over each of the two cages and deployed, and volatile detection above
each of the two cages was analyzed as described above. This was repeated, switching the
cages that received volatile- versus water-infused filter paper.

*Host finding behavior data analysis*

For each observation night, data were pooled for each treatment (each treatment consisted of
three plants). The following was calculated for each treatment within an observation night:
percent of approaches, the percent of approaches that resulted in a landing, and the percent of
landings that resulted one or more eggs being laid. These nightly observations were
averaged, and averages for each treatment were compared using t-tests. One-tailed tests were
used for the comparisons, because our a priori hypotheses were that more approaches,
landings, and ovipositions would occur on 1) 1-layer versus 8-layer covered plants, and 2)
uncovered plants versus 1-layer covered plants.
RESULTS

2006 observations

Moths observed in the field tent in 2006 had oviposition behavior similar to those observed in the field in 2004 and 2005 (Benda 2007). Of all the eggs laid in the field tent during the 2006 observation nights, 90% of eggs on were laid on Physalis plants and the remaining 10% on weeds. No moths laid any eggs on weeds that were 100 cm from Physalis plants. Of all the eggs laid on weeds, 95% were on plants 15 cm from Physalis plants, and the remaining 5% were on weeds 30 cm from host plants. Moths also laid similar proportions of eggs on various parts of Physalis plants in the field tent compared to field observations in 2004 and 2005 (Fig 2). During the 9 observation nights when half of the plants were uncovered, and the other half were covered with cages shown in Fig 1b, no eggs were laid on any of the cages; moths ignored these plants, with all approaches being to plants that were uncovered.

2009 observations: caged versus uncaged plants

On uncaged plants, there was a higher percentage of approaches compared to caged plants (63 ± 6 versus 37 ± 6; \( t_2 = 3.00, p = 0.048 \)), and a higher percentage of lands resulting in oviposition events compared to caged plants (64 ± 9 versus 31 ± 6; \( t_2 = 2.90, p = 0.050 \)) (Fig 3). However, the percentage of approaches resulting in lands on uncaged versus caged plants (70 ± 5 versus 59 ± 2) was not significantly different (\( t_2 = 1.85, p = 0.102 \)). The percentage of approaches that finally resulted in oviposition events was higher for uncaged plants (44 ± 3 versus 18 ± 3; \( t_2 = 5.98, p = 0.013 \)) (Fig 3, dotted lines). Moths laid a total of 173 eggs on plants that were not caged, and only 20 on the cages covering plants in the caged treatment.

2009 observations: 1-layer versus 8-layer plants

1-layer versus 8-layer cages: volatile emission

The two types of cages used in the 2009 observations (those covered by one layer of bridal veil and those covered by 8 layers) did not differ significantly in the amount of volatiles
emitted through the layers of fabric (Fig 4) (trans-2-hexanal: $t_{7} = 0.42$, $p = 0.342$; 2-ethyl-1-hexanol: $t_{11} = 0.25$, $p = 0.402$; benzaldehyde: $t_{11} = 0.192$, $p = 0.426$; eugenol: $t_{11} = 1.20$, $p = 0.128$). Data were combined for both of the collection times (30 min or 60 min) because no differences in volatile detection between the two types of cages were observed for either of these collection times. To verify that the SPME detectors were only detecting chemicals from the cage above which they were placed and that they were not being contaminated by chemicals from the adjacent cage, we ran trials with only one cage within a replicate containing the volatile blend. There was no cross contamination of volatiles; SPME detectors placed over cages with water-saturated pieces of filter paper detected negligible amounts of volatiles (Fig 5).

1-layer versus 8-layer plants: moth behavior

Plants contained in cages with 1 layer of bridal veil (1-layer caged plants) were more attractive than those covered with 8 layers of bridal veil (8-layer caged plants) in approaches, landings, and oviposition events. Average percent of approaches to 1-layer caged plants versus 8-layer caged plants was slightly but significantly higher (56 ± 3 versus 44 ± 3; $t_{16} = 3.07$, $p = 0.004$) (Fig 6). The percent of approaches that resulted inlands was also significantly higher for 1-layer caged plants (47 ± 3 versus 31 ± 6; $t_{16} = 2.47$, $p = 0.012$), as was the percentage of lands that resulted in oviposition events (42 ± 12 versus 9 ± 4; $t_{16} = 2.71$, $p = 0.008$) (Fig 6). The percentage of approaches that finally resulted in oviposition events was also higher (20 ± 6 versus 4 ± 2; $t_{16} = 2.57$, $p = 0.010$) (Fig 6, dotted lines). Moths laid a total of 191 eggs on 1-layer cages covering plants, and 21 eggs on 8-layer cages covering plants.
**DISCUSSION**

**Natural field observation versus field tent observations**

Studies of host finding are most commonly conducted in the laboratory or in cages contained inside greenhouses. Although these areas rarely harbor conditions similar to those in the field, they alleviate the difficulties of obtaining observations in natural field conditions. In this study, we show that female Hs moths observed in a large tent placed in a field setting behave similarly to wild Hs females in the field. The large tent design is clearly advantageous because it allows natural behavior while still being able to control the type and number of the insects being observed.

In the field tent, the proportion of eggs laid on various parts of *Physalis* plants was similar to those found by Benda (2007) (Fig 2), as was the proportion of eggs laid on hosts versus non-hosts. Benda (2007) showed that 80% and 20% of eggs were laid on *Physalis* and non-hosts, respectively; observations from the current study showed that moths laid approximately 90% of eggs on hosts and 10% on non-hosts in the field tent. Our results are also similar to those found by Benda (2007) with respect to where non-host ovipositions occurred; both studies showed that most eggs were laid on weeds no more than 15 cm from the plant. These ovipositions occurred after moths had laid eggs on *Physalis* plants. It is somewhat surprising that Hs lays even a small portion of its eggs on non-hosts, since larvae cannot survive on plants outside of the genus *Physalis*. It is possible that moths may have evolved to lay a portion of eggs on non-hosts as a mechanism for avoiding defenses against eggs by leaves of its *Physalis* host plants (see Chapter 2).

**Host finding and oviposition cues**

Results from the 2006 observations indicated that volatiles emitted from *Physalis* plants were not solely responsible for host location in Hs, since moths never approached or landed on cages covering plants (these cages blocked the view of the plant but allowed for flow of volatiles). Even when all uncovered plants were removed from the cage on two occasions,
leaving only caged plants, moths stopped all oviposition behavior and remained on the walls of the large field tent. Although these cages were made from screen material with holes large enough to allow for emission of volatiles (1.8 mm), plants were not clearly visible from the outside of the cages (Fig 1b). Follow up experiments showed that plants completely covered in one layer of white cheesecloth with no metal caging material were also not attractive to moths; however, plants covered with one layer of clear bridal veil were very attractive to moths, with many approaches to and lands on these plants (data not shown). Moths also actively tried to oviposit on plants covered in bridal veil, curling their ovipositors to try to reach the plant under the bridal veil material.

Observation trials set up in 2008 supported the hypothesis that vision is important in host finding and oviposition in Hs. Significantly more approaches, approaches resulting in lands, and lands resulting in oviposition events occurred on 1-layer covered plants versus 8-layer covered plants. Approaches to 1-layer caged plants resulted in five-fold more oviposition events compared to approaches to 8-layer caged plants (Fig 6). Because experiments showed that volatile flow through both the 1-layer and 8-layer cages did not significantly differ (Fig 4), the difference in oviposition behavior on the two types of cages can be attributed to visual cues. Plants were easily seen through the nearly transparent 1-layer cage, whereas the plant was not visible through the 8-layer cage (Fig 1). Visual cues seemed to be important in all variables measured (approaches, lands, and oviposition events), since these behaviors occurred at a significantly higher numbers on 1-layer cages than those on 8-layer caged plants. Once a moth landed on either of the types of cages, moths were four times more likely to oviposit at least one egg on the 1-layer cages relative to the 8-layer cages (Fig 6).

The set-up of the cages (1- or 8-layers) did not allow a moth to bend her ovipositor through the mesh fabric of the bridal veil and touch the tip of the ovipositor to the plant, thus touching the plant substrate is not necessary for oviposition. However, for both 1-layer and 8-layer caged plants, moths often curled their abdomens for extended periods of time, pushing them through the holes in the fabric and seemingly trying to reach the plant (which
was only about 1 cm away). Indeed, uncaged plants were more attractive compared to 1-layer plants, with significantly more approaches and approaches resulting in oviposition events on uncaged plants versus those covered with the 1-layer caged plants (Fig 3). Thus, contact chemoreception, which can only occur when an insect lands on a plant, is likely very important for oviposition, but not necessary since moths did lay eggs on 1-layer caged plants. Contact chemoreception has been shown to be an important sensory modality involved in host acceptance for a number of insects (Ramaswamy 1988, and references therein). In the case of Hs moths that lay 10-20% of their eggs on nearby non-hosts, it could be that volatiles from the Physalis plants act as general releasers of oviposition behavior.

Although there has been considerable effort in the past decade devoted to understanding the chemical cues involved in host finding and oviposition, there has been a lack of studies that examine the relative role of vision in moth host finding. More studies are necessary for gaining a better understanding of the relative role of vision in host finding for phytophagous insects. Here, we conclude that in addition to chemical cues (Mitchell and Heath 1987, Tingle et al. 1990), visual cues play an important role during host finding in Hs.

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Figure 1: Field tent and cages. a) field tent; b) cages used for 2006 observations; c) from left to right, 1- and 8-layer cages placed over plants in 2008, and d) treatment layout for 2008 cages.
Figure 2: Mean percent of eggs oviposited on stems, leaves, and reproductive parts (flower, calyx) of *Physalis* plants. 2004 and 2005 observations are from Benda 2007, in the field; 2006 observations were taken inside a large tent placed in the field.
Figure 3: Percentage (mean ± SE) of moth approaches, landings, and egg laying events on caged and uncaged plants averaged over each observation night. Arrows and adjacent numbers represent the percent of events to the right of the arrow that resulted from events to the left of the arrow (i.e. of all approaches on plants that were caged, 59% resulted in a land). Dotted arrows represent the percentage of oviposition events that resulted from approaches.
Figure 4: Amount of volatile organic chemicals (mean ± SE) detected by SPME analysis from a blend placed in cages covered by either 1-layer or 8-layers of fabric.

Figure 5: Amount of volatile organic chemicals (mean ± SE) detected by SPME analysis from cages either containing a loaded sample of VOCs (loaded) or filter paper containing water (blank).
Figure 6: Percentage (mean ± SE) of moth approaches, landings, and egg laying events on plants covered with cages made of 1 or 8 layers of wrapped bridal veil, averaged over each observation night. Arrows and adjacent numbers represent the percent of events to the right of the arrow that resulted from events to the left of the arrow. Dotted arrows represent the percentage of oviposition events that resulted from approaches.
COULD HOST PLANT RESPONSE TO EGGS OF ITS SPECIALIZED HERBIVORE SELECT FOR OVIPOSITION ON NON-HOSTS?

JENNIFER PETZOLD, J CONSUELO ARELLANO, FRED GOULD

1 Department of Plant Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

2 Department of Statistics, North Carolina State University, Raleigh, North Carolina 27695-8203 USA

3 Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

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The moth, *Heliothis subflexa*, is a specialist on plants in the genus *Physalis*, but lays approximately 20% of its eggs on nearby non-host plants. We observed that the leaves of *Physalis* plants respond physically to the eggs of *Heliothis subflexa*, and in this study we tested the hypothesis that this plant response could have selected for non-host oviposition. We found that leaves of *P. angulata* and *P. pubescens* respond to eggs by the formation of 1) necrotic tissue, 2) undifferentiated cells that form a bump (neoplasm) under the eggs of this herbivore, or 3) both types of responses. Greenhouse experiments showed that 64% of eggs laid on *P. angulata* elicited a response, and that a response to an egg decreased the probability of hatching. Further experiments in the field with *P. angulata* showed that 25% of eggs elicited a response, and that the probability of an egg eliciting a response increased as temperature increased. Field experiments also confirmed that a plant response to an egg decreased the probability of hatching and increased the probability of removal from the plant (by physical dislodgement or predation). Eggs that elicited a response had a 25% lower probability of hatching, and a 28% lower probability of remaining on the plant. This resulted in a fitness cost of 18% for *H. subflexa*. Thus, the formation of necrotic tissue and/or neoplasms on plant tissue in contact with eggs could be one factor that selected for non-host oviposition by the specialist *H. subflexa*. 
Plants have evolved both constitutive and inducible defenses against herbivores (Karban and Baldwin 1997, Agrawal *et al.* 1999, Dicke and van Loon 2000, Schoonhoven *et al.* 2005). Many studies over the past 40 years have examined plant defenses induced by the feeding of herbivores (Karban and Baldwin 1997, see reviews by Kessler and Baldwin 2002, Howe and Jander 2008). Recently, however, a number of studies have shown that plants can respond to insect eggs, a remarkable defense that targets insect enemies before harm to the plant can be initiated.

Both direct and indirect induced plant responses to oviposition have been observed (Hilker and Meiners 2002). In several systems, plants have been shown to respond indirectly to insect eggs by releasing volatiles that attract egg parasitoids or predators. For example, in response to oviposition by the elm leaf beetle, elm trees emit volatiles that attract the egg parasitoid *Oomyzus gellerucae* to the beetle eggs (Meiners and Hilker 1997). Emission of enemy-attracting volatiles induced by insect oviposition has also been shown for two species of bean (*Vicia faba* and *Phaseolis vulgaris*) (Colazza *et al.* 2004), Scots pine (*Pinus sylvestris*; Hilker *et al.* 2002), and brussels sprout (*Brassica oleracea gemmifera*; Fatouros *et al.* 2005).

Direct, induced plant responses to insect eggs have been shown for several plant species. One way in which plants respond directly to eggs is the formation of tumor-like, or neoplastic, growth that forms directly under the egg. Neoplastic growths, or neoplasms, occur when cell division is stimulated in non-meristematic areas. Pea plants of a specific genotype react by forming neoplasms in response to pea weevil eggs (Doss *et al.* 1995). These neoplasms elevate the egg above the surface of the pea pod, resulting in disorientation of newly hatched pea weevils, which eventually die of desiccation (Doss *et al.* 2000). A second way plants respond directly to insect eggs is by initiating a hypersensitive response.
A hypersensitive response, first described in the context of defense against microbes (Ward and Ward 1902; Klement and Goodman 1967), is characterized as the formation of necrotic tissue at the site of egg placement. In mustard (Brassica nigra), eggs of Pieris brassicae and Pieris napi induce a hypersensitive response in tissue directly under eggs within 24 hours of oviposition, killing eggs within three days (Shapiro and Devay 1987). Similarly, Colorado potato beetle eggs induce the formation of necrotic tissue in leaves of certain potato hybrids in a zone under egg masses, causing detachment from the leaf and subsequent larval death (Balbyshev and Lorenzen 1997). In all the above-mentioned cases, physical responses to eggs result in an increase in egg or larval mortality.

In this study, we examine the effect of physical responses of Physalis leaves to eggs of the herbivore Heliothis subflexa (Hs). Hs is a monophagous Lepidopteran that specializes on plants in the genus Physalis (Brazzel et al. 1953, Bateman 2006). Hs behavior and physiology appears to be highly adapted to feeding on Physalis plants, and many characteristics of the interactions between Hs and Physalis seem to have resulted from a common evolutionary history (see examples in Sisterson & Gould 1999, Oppenheim and Gould 2002, De Moraes and Mescher 2004, Bateman 2006, Benda 2007). One puzzling aspect of this system is that Hs females do not oviposit exclusively on Physalis plants, despite the fact that larvae can only develop on Physalis fruit. Instead, females lay approximately 20% of eggs on non-hosts growing in the immediate vicinity of their Physalis hosts (Benda 2007). Oviposition on non-hosts could simply be non-optimal behavior, or it could be that there would be disadvantages to H. subflexa females that lay 100% of their eggs on Physalis.

Our observations in the field and greenhouse lead to the discovery that at least two species of Physalis plants, P. angulata and P. pubescens, can respond to Hs eggs. Responses include neoplastic growth and/or the formation of necrotic tissue directly under the singly laid eggs, although not all eggs induce a response. Here, we evaluate whether the ability of Physalis plants to physically respond to Hs eggs may be one factor that could have selected for non-
host oviposition. We test the hypothesis that a response by a leaf to an Hs egg decreases the probability of survival for the egg. We first test this hypothesis in a greenhouse study with *P. angulata* and *P. pubescens*, and follow up with a more focused field study using *P. angulata*. We show that response of *Physalis* plants to Hs eggs in the field incurs a substantial fitness cost to this insect and may be one reason for this specialist insect ovipositing a substantial portion of its eggs on non-hosts. While previous studies have demonstrated that some plants have effective defenses against their specialist herbivore’s eggs, this is the first study supporting the hypothesis that herbivores can behaviorally respond to this type of defense.

**Materials and Methods**

*Greenhouse experiment*

*Plants.*—In the fall of 2005, *P. angulata* and *P. pubescens* seeds were sown in a greenhouse once every two weeks. Flats contained potting soil (2 Mix Professional Formula, Fafard), and two teaspoons of fertilizer (Osmocote 14-14-14, N-P-K). Seeds were from a lineage of *Physalis* plants originally collected from wild populations in Orangeburg County, SC, USA (33°33′N, 81°04′W) in 1998. Seedlings were transferred to 0.5-l plastic pots three weeks after germination, and were fertilized with one teaspoon of fertilizer. Ten-week old plants were transplanted into 7.5-l plastic pots and fertilized every four weeks.

*Insects.*—All Hs insects were from our laboratory colony, which was established using larvae collected from natural populations of *Physalis* in Florence and Barnwell Counties, SC, USA, in 1996 (Sheck et al. 2006). Pupae were separated by sex and placed in small buckets (20-30 per bucket). Upon emergence, each female was placed with one male in a single bucket and given sugar water (5% sucrose solution). Single mating pair buckets were placed in a
greenhouse for two to five nights, after which males were dissected to determine if mating took place (Groot et al. 2006). Females paired with males that tested positive for mating were used in the experiment.

Experimental design

This experiment took place between 27 Nov 2006 and 18 Jan 2007. Every few days (as mated females became available), one mated female and one plant were placed in a screened metal cage (40 x 40 x 40 cm) housed in a greenhouse. Plants were either seven to nine weeks old, or 12-16 weeks old (hereafter referred to as young and old plants, respectively). Young and old plants differed substantially in phenology; young plants were approximately 30 cm tall, with five to 10 leaves, and were in early flowering stages with few or no immature fruit. Old plants were approximately 40-70 cm tall, with >30 leaves, and contained several to many mature fruit. Moths were left overnight to oviposit, since *Hs* oviposits from sunset until two hours after sunset (Benda 2007). Moths that did not lay any eggs on a plant were discarded, and the plant was used again the next night with a new, mated moth. Each plant that contained eggs was examined thoroughly. Each leaf that contained an egg was marked with a numbered twist tie, and the side of the leaf containing the egg (abaxial or adaxial), as well as the exact location of the egg(s) was recorded. Eggs that were laid on flowers, calyces, and stems were also recorded. After all eggs were accounted for, plants were moved into a large, screened cage in the greenhouse. For each of the next six days, or until it hatched, each egg was checked using a small hand lens to determine if and when a plant response to the egg occurred (and if so, what type of response occurred). Eggs that hatched were easily identifiable by the presence of the black larval head capsule within the egg one day prior to hatching, and the remaining broken shell post hatching. Plants were gently watered every day throughout the experiment, and discarded after use.

Statistical analysis.—To determine if plant age, species and/or the placement of the egg on
the plant affected the probability of a response to an egg, we used the GENMOD procedure in SAS (SAS 2008) to model a positive response to an egg. This was done using a generalized linear model with the logit of the proportion of response to an egg as the dependent variable, and plant age (young or old) and plant part (calyx, stem, adaxial and adaxial side of leaf) nested within plant species as independent (explanatory) variables. The analysis used a logit link function (binomial distribution). To account for correlations between plant parts on the same plant, a REPEATED statement was used in the model, with the plant ID as the clustering variable, and the working covariance matrix set as independent. This invokes analysis using weighted generalized estimating equations (GEE), which accounts for the correlation among the observations for each subject, and assumes statistical independence of responses from different subjects (Cole, 2001). Contrast statements were used to test for significant differences in the probability of a response to an egg for: 1) eggs laid on the abaxial (underside) versus adaxial (topside) positions (for each species); 2) eggs laid on *P. angulata* versus *P. pubescens* leaves; and 3) eggs laid anywhere on *P. angulata* versus *P. pubescens*. Mean percent of eggs laid on leaves that elicited a response for each of the two species and age classes were calculated.

The GENMOD procedure in SAS was also used to determine what factors affected the probability of an egg hatching. The proportion of eggs that hatched for each plant was calculated and set as the dependent variable. The model included leaf side (abaxial or adaxial), plant species, presence of a response, plant species x presence of a response, and plant age (young or old) as categorical independent variables, and a REPEATED statement was used, with plant ID as the subject effect. To account for over-dispersion across plants, the Pscale option was implemented to estimate the dispersion parameter $\phi$, calculated by dividing the Pearson’s chi-square statistic by the degrees of freedom. Contrast statements were used to test for significant differences in the probability of an egg hatching for eggs that caused a response versus those that did not, for both species. Means for the percent of eggs laid on leaves that hatched when a response to an egg was and was not elicited were
calculated for each species.

Field experiment

Plants.—In order to grow plants as uniformly as possible and without the possibility of infestation from common greenhouse pests, plants used in this experiment were grown in NCSU’s phytotron, a facility that houses controlled-environment chambers. All plants were grown in a chamber kept at 26/22°C day/night, with lights on from 8:00 – 17:00 (fluorescent and incandescent lighting) and a night interruption to promote flowering from 23:00 – 2:00 (incandescent lighting). Light intensity ranged from 550 – 600 μmoles/m²/sec, and humidity ranged from 40 – 65%. Plants were watered twice a day with a nutrient solution (see Saravitz et al. 2008 for nutrient composition). Plants were started from seed as described above, and subsequently transplanted to small Styrofoam cups one to two weeks after germination. Two weeks later, plants were transplanted to 5-l plastic pots, and after three weeks they were moved to an outside field plot (no Hs were present at this location) to acclimate to field conditions. Here, plants were transplanted to 7.5-l plastic pots and watered daily. Plants remained outside until used for the experiment; plants used in the field experiment were eight to 12 weeks old.

Experimental design.—This field experiment was conducted 07 July - 12 July and from 21 July – 22 Aug 2008. Insects were reared and mated as described for the greenhouse experiment. Each week (for a total of six weeks), five to seven P. angulata plants were each placed in a screened cage containing one mated moth as described for the greenhouse experiment, and all eggs laid on leaves were recorded the following morning. Eggs were marked by outlining a circle around each egg on the opposite side of the leaf with a fine-tipped Sharpie pen (i.e., if the egg was laid on the adaxial side of the leaf, we drew a circle around the egg on the abaxial side). To control for any effects the ink may have had, we drew a circle approximately 3 cm from the egg on the same side of the leaf. No visible
effects of the ink were observed, so control circles were only used for the first two weeks of
the experiment. Immediately after eggs were recorded, the plants were taken to a 0.26
hectare field located within the NCDA/NCSU Central Crops Research Station (Clayton, NC),
where natural populations of Hs occur. Because this field site has been used in previous field
experiments, each year volunteer Physalis plants (mostly P. angulata and P. pubescens)
dominate the field from late June until mid-August. Four rows (each 20 m long) of a mixed
planting of P. angulata and P. pubescens were also planted here for use in other experiments.
Our experimental plants were nestled between these planted Physalis plants. We returned
each day to monitor the eggs on our experimental plants, recording whether or not each egg
induced a response, hatched, and/or disappeared from the plant. Each replicate of the
experiment was ended when all fertile eggs had hatched. Hatching occurred within three to
five days with the exception of week five. Due to unusually low temperatures during week
five, eggs took longer to hatch and the experiment continued for seven days during this week.
Weather data (average temperature, maximum temperature, minimum temperature, average
pressure, average windspeed, average relative humidity, average precipitation, and average
solar radiation) were obtained for each of the days of the six weeks that the experiment took
place, from the State Climate Office of NC. The weather reporting station from which we
obtained data was approximately one mile from our field site.

Statistical analysis.—This experiment differs from the greenhouse experiment in that only
one plant species was used, only eggs laid on leaves were included in the experiment, and
each week a cohort of plants was used (instead of individual plants analyzed continuously).
Because weather patterns varied for each of the six weeks of this experiment, a general linear
model (PROC GLM) was used to determine if the week that a cohort of plants was used was
a significant factor in the probability of a response to an egg occurring. The classification
variables in this model were week, presence of a response, and plant ID. Week was treated
as the independent variable and percent of eggs that caused a response by each plant was the
dependent variable. The average temperature, maximum temperature, minimum temperature,
pressure, average windspeed, relative humidity, precipitation, and solar radiation were calculated for each week of the experiment. Each of these variables was analyzed in SAS with PROC CORR to generate Pearson correlation statistics. Weather variables that significantly correlated with response to an egg were further analyzed using PROC REG with the average percent response for each week as the dependent variable, and the weather variable of interest as the independent variable. In addition, because a significant fraction of eggs ‘disappeared’ from the plant in the field (i.e. fell off the plant or were predated upon), we ran the statistical analyses described above to determine if the disappearance of eggs from plants was correlated with weather variables.

Any given egg in the experiment either disappeared from the plant, hatched, or did not hatch. To determine if a response to an egg had an effect on the probability of an egg disappearing, we used a generalized linear mixed model in SAS, implemented by PROC GLIMMIX. This procedure is appropriate for incorporating random effects in the model and allowing for subject-specific (conditional) inference. The proportion of eggs that disappeared from each plant was set as the dependent variable; week, response, and the interaction between week and response were independent fixed variables, and Plant ID nested within week was a random variable. The data was fit to a binomial distribution with a logit link function. Eggs disappeared from the plants each day of the week that the experiment took place; any egg that fell off of the plant before a response occurred was eliminated from this analysis, since our goal was to determine the effect of a plant response on egg disappearance. For each plant, mean percent of eggs that disappeared was calculated for eggs that did and did not cause a response. In this analysis and in the analysis described below, plants used during week five were eliminated from the analysis due to no response to eggs, as were four other plants that had low response to the small number of eggs on these plants (less than 10).

To determine if a response to an egg had an effect on the probability of an egg hatching, we modeled the proportion of hatched eggs (eliminating all eggs that disappeared from the plant
from the analysis) using PROC GLIMMIX. Week and response were independent fixed variables (the interaction between week and response was non-significant and thus not included in the model), and Plant ID nested within week was a random variable. Because of overdispersion in the data, a multiplicative dispersion parameter was added. The data was fit to a binomial distribution with a logit link function. Mean percent of eggs that hatched were calculated over plant for eggs that did and did not cause a response.

**Experimental values used to estimate fitness cost.**—Using the field collected data, the fitness cost of the egg reaction phenomenon was calculated for Hs. The probability of survival for eggs that caused a response was calculated by multiplying the probability of hatching by the probability of staying on the plant. The probability of survival for eggs that did not cause a response was calculated in the same way. The probability of survival for eggs that caused a response was divided by the probability of survival for eggs that did not cause a response. This value represents the relative survival (fitness) for eggs that cause a response, compared to eggs that do not cause a response. This value was subtracted from 1 to determine the fitness cost due to the response. However, since not all eggs cause a response, this fitness cost was multiplied by the percent of eggs that caused a response in the field to compute the overall fitness cost due to plant response.

**RESULTS**

**Greenhouse experiment**

A total of 14 *P. angulata* and 12 *P. pubescens* plants were used in the experiment. Of these plants, 9 were young plants (5 and 4 of *P. angulata* and *P. pubescens*, respectively) and 17 were old plants (9 and 8 of *P. angulata* and *P. pubescens*, respectively). A total of 331 and 348 eggs were laid on *P. angulata* and *P. pubescens*, respectively. Moths laid an average of
26 eggs per plant (standard deviation: 12.9), with a range of 6 and 50 eggs. It is important to note that there was moderate damage to the plants by western flower thrips (*Frankliniella occidentalis*), as there was an infestation in the greenhouse at the time of the experiment.

**Response to eggs.**—There were three types of responses observed for both plant species: necrosis, neoplastic growth, and a more extreme type of response showing both necrotic tissue and neoplastic growth (Fig 1). For all eggs laid on *P. angulata* that elicited a response, 37% elicited the combined response of necrosis and neoplastic growth, 36% elicited a neoplasm, and 27% elicited necrotic tissue. For all eggs that caused a response on *P. pubescens*, necroses were elicited at the highest rate (69%), followed by neoplasms (18%) and the combined response (13%). Responses to eggs were elicited 48-72 hours after egg deposition.

The location of an egg (abaxial side of leaf, adaxial side of leaf, calyx, stem) had an insignificant effect on the probability of a response (d.f.=7, Chi-square=13.38, p=0.0633). For both species, young plants (7-9 weeks) responded at a higher rate compared to old plants (>12 weeks) (d.f.=1, Chi-square=7.38, p=0.0066) (Fig 2).

*P. angulata* and *P. pubescens* did not differ in the percent of responses to eggs when all plant parts were considered (abaxial side of leaf, adaxial side of leaf, calyx, stem) (contrast results: d.f.=1, Chi-square=0.05, p=0.8153). Only 24 and 22 total eggs were laid on calyces and stems, respectively. For eggs laid on calyces, 42% caused a response (SE: 12.6), while 22% of eggs laid on stems caused a response (SE: 11.4). Because so few eggs were laid on calyces and stems compared to leaves, eggs laid on these parts were eliminated from further analyses. When we consider only eggs laid on leaves, *P. pubescens* and *P. angulata* significantly differed in the percent response (contrast results: d.f.=1, Chi-square=7.66, p=0.0056). *P. pubescens* responded to about 50% (SE: 4.9) of the eggs laid on leaves, while *P. angulata* responded to about 64% of eggs (SE: 4.8). *P. angulata* responded to a higher percentage of eggs on the abaxial (lower) side of the leaf (68%) compared to *P. pubescens*
(39%) (contrast results: d.f.=1, Chi-square=7.38, p=0.0066), but percent response to eggs did not differ between species on the adaxial (upper) side of the leaf (contrast results: d.f.=1, Chi-square=0.84, p=0.3605).

Effect of induced responses to eggs on hatching.—The PROC GENMOD analysis indicated that eggs that elicited a response had a significantly lower probability of hatching compared to eggs that did not elicit a response (Table 1). A response to an egg decreased the percent of eggs that hatched by 16% for P. angulata (contrast results: d.f.=1, Chi-square=6.54, P=0.0106), and by 9% for P. pubescens (d.f.=1, Chi-square=3.97, P=0.0464) (Figure 3). There was no significant effect of plant age, egg placement on leaf (abaxial or adaxial), or plant species on the probability of an egg hatching (Table 1). There was also no interaction between plant species and response, indicating that a response to an egg had the same effect on the probability of an egg hatching for both species.

Field experiment

Response to eggs.—Moths laid a total of 781 eggs on 24 P. angulata plants during the six-week field experiment. Although three distinct types of responses occurred in the greenhouse study, the combined response of a necrosis and neoplasm was the most common in the field. Of all eggs that caused a response, 83% induced the combined response, 9% induced a necrosis, and 8% induced a neoplasm.

The overall mean percent response to eggs was 25% (SE=2.34). Week was a significant factor in the probability of a response to an egg occurring (F₅=9.47, P<0.001). Weather varied considerably during the six weeks of the experiment; the most variable factor was temperature. Average percent response to eggs for each week positively correlated with average weekly temperature (r²=0.576, p<0.0001) (Fig 4). There were no other significant correlations with the other weather variables and percent response to eggs. Similarly, there were no significant correlations with any of the weather variables and percent egg disappearance from plants.
Effect of induced responses to eggs on disappearance and hatching.—Over the course of the week-long experiments, a significant number of eggs disappeared from (fell off or were predated upon) plants. The GLIMMIX results showed that a response to an egg significantly increased the probability of an egg disappearing from the plant from ($F_{1,31}= 9.24; P = 0.0048$) (Fig 5a). Week did not significantly effect egg disappearance from the plant ($F_{4,14.52}= 2.90, P = 0.0598$), however, there was a significant interaction effect between week and the response variable ($F_{4,31}= 2.89, P = 0.0384$).

For eggs remaining on the plant, a response to an egg significantly decreased the probability of an egg hatching ($F_{1, 15.01}= 18.57, P = 0.0006$; Fig 5b). Week was also a significant effect in the model ($F_{1, 13.81}= 3.96, P = 0.0303$).

Fitness cost.— The probability of survival for an egg that causes a response was calculated by multiplying the probability of such an egg staying on the plant (1- probability of disappearing) by the probability of such an egg hatching (0.55 x 0.18). This value was divided by the probability of survival for an egg that did not cause a response (0.83 x 0.43), to yield a value of 0.28 (i.e., for eggs that cause a response, 28 are predicted to survive for every 100 eggs that do not cause a response). This value was subtracted from one to get fitness cost, then multiplied by 0.25, the average percent response in the field, to yield a final fitness cost of 18%.

**DISCUSSION**

**Effect of the response on egg viability**

In both the greenhouse and field studies, a response to an egg by a *Physalis* leaf decreased the probability of hatching (Fig 3; Fig 5b). Eggs that did not hatch either shriveled, or remained turgid and stayed milky white or turned black. All of these characteristics are
common for eggs that do not hatch under normal circumstances (i.e. no plant response to an egg, or for eggs raised on laboratory materials). The mechanism by which plant responses to eggs increase egg mortality is unknown. It is possible that necrotic or neoplastic tissue under the egg promotes desiccation of the egg. Shapiro and Devay (1987) found that necrotic tissue on leaves of *Brassica nigra* elicited by eggs of *Pieris rapae* and *P. napi* promoted death of eggs by desiccation within 72 hours of oviposition.

For *P. angulata* in both the field and the greenhouse experiments, eggs that induced a response had a lower overall percent of eggs that hatched than eggs that did not induce a response. However, this difference was greater in the field experiment. This could be due to differing environmental conditions in the greenhouse versus the field. Another possibility for this difference in percent of eggs that hatched is increased mortality in the field from egg parasitoids and predators that leave the eggshell intact. During data collection in the field, we observed a nymph of the egg predator *Orius insidiosus* with its stylet inserted into an egg that had induced a plant response. Generalist egg parasitoids in the genus *Trichogramma*, some of which parasitize eggs of *Hs*, use a number of cues—including visual cues—to locate hosts (Wanjberg and Hassan 1994). At least one *Trichogramma* species uses visual profiles of eggs during short-range searching behavior to locate eggs (Wanjberg and Hassan 1994); thus elevation above the surface of the leaf due to neoplastic growth could make eggs more visible to predators and parasitoids. Increased predation from egg predators could also explain not only why responses to eggs decreased the percent hatch for eggs that stayed on the plant, but also increased the percent of eggs that disappeared from the plants in the field (Fig 5a). Leaf discoloration due to necrosis or elevation above the plant from neoplastic growth could serve as a visual cue for predators; during data collection eggs were much easier to locate if there was necrotic tissue around the egg. Whether such visual cues are important for predators/parasitoids remains to be seen. Further experiments are needed to determine the effect of a response to an egg on egg parasitoids and predators. Lastly, elevation of eggs that induce a neoplastic response could render eggs more vulnerable to dislodging from the plant due to wind, rain, or other weather conditions.
The week that an experiment took place had a significant effect on an egg hatching in the field. Although there was no correlation between temperature and the percent of eggs that hatched for both those that did and did not cause a response, the effect of week on hatching could be due to other differences in field conditions during the experiment. Although the plant subjects used in the experiment were always eight to 12 weeks old and similar in phenology, the non-experimental *Physalis* plants in the field—both planted and volunteer—varied in phenology over the course of the field experiment. *Physalis* plants in the field were small and not yet fruiting during the first several weeks, mature and fruiting during the third to fifth weeks, and senescent during the last week of our experiment. This could have greatly affected the suite of predators, parasitoids, and other insects active in our field site at various times, contributing to differences each week in percent hatch. Although the week the experiment took place was not a significant factor in the percent of eggs that disappeared from plants, there was a significant interaction between week and response on egg disappearance. Again, differences in abundance of predators and parasitoids, which may be more attracted to eggs that cause a response, as well as differences in weather conditions, could explain this. The biggest difference in percent egg disappearance between those that did and did not cause a response occurred during weeks three and four, the weeks with the highest average temperatures; thus temperature may have an effect on the extent to which eggs that cause a response either fall off the plant or are predated upon.

*Plant response to eggs*

Although we have established that responses by *Physalis* leaves to *Hs* eggs results in a decrease in egg viability, there is still much to be uncovered about this phenomenon. Every plant in the experiment responded to eggs (with the exception of plants used during week five, when average temperature was much lower than during other weeks). However, there was a substantial amount of variability in response rate to eggs among individual plants, even though we took care to ensure that each plant grown for the field study had near identical growing conditions. It did not appear that certain leaves had a greater capacity to respond to
eggs compared to other leaves; in fact in a number of cases where multiple eggs were laid on a leaf, one egg would induce a response whereas another egg (from the same moth) millimeters away did not. Plant genotype is likely important in determining a plant’s capacity to respond to Hs eggs based on data from studies of specific potato hybrid and pea plant genotypes’ response to eggs (Doss et al. 1995, Balbyshew and Lorenzen 1997). It is also possible that the order in which the female moth oviposits the eggs has an effect on which eggs induce a response.

Results from the greenhouse study indicated that younger plants (7-9 weeks) had a higher response rate than older plants (12-16 weeks) for both P. angulata and P. pubescens (Fig. 2). Ontogenetic effects on plant-induced defenses are widely recognized, and a number of studies have shown that plants’ allocation of resources to defenses are reduced during reproductive stages (Boege and Marquia 2005, and references therein). Further experiments are needed to determine if leaf age has an effect on the probability of a response. Although using the same plant age classes that were used in the greenhouse experiment would have been ideal, timing and other logistics made this difficult to implement in the field experiment. Thus, only plants in the 8-12 week old range were tested in the field experiment.

It is clear from the field experiment results that temperature is important in the induction of a response to an egg (Fig. 4). Temperature explained 58% of the variation in response to eggs, with response to eggs increasing as temperature increased. During week five, when the average temperature was 22°C, plants did not respond to any eggs. Shapiro and Devay (1987) also found temperature to be important in the induction of a hypersensitive response (necrosis) by Brassica nigra to Pieris rapae and P. napi eggs. Responses to eggs were most pronounced if the foliage was exposed to full sunshine or high temperatures (above 30°C), and were rare under fluorescent illumination at lower temperatures (Shapiro and Devay 1987). In our greenhouse experiment, average temperature was approximately 31-33°C during the day, and 23-25°C at night. The higher overall temperature in the greenhouse experiment, as well as the fact that the age of the field plants was on average greater than the
age of the plants used in the greenhouse experiment, could explain why overall response to eggs for *P. angulata* was greater for the greenhouse experiment. It is likely that factors other than temperature are important for the induction of a response to an egg. Although we tested for effects of a number of other weather variables on plant response during our field experiment, average temperature was the only variable that had a significant effect in explaining variation in response. Indeed, there was substantial variation in average temperature over the six-week experiment. However, variation in humidity and other weather variables was not as large, thus further experiments are needed to examine the role of these variables, especially humidity, in affecting the response rate of plants to eggs.

Three types of responses to plant eggs were observed both in the greenhouse and field experiments: neoplasmic tissue, necrotic tissue, and a combination of both neoplasmic and necrotic tissue under the site of egg deposition (Fig 1). The reason for elicitation of different types of responses is unknown; possible reasons for variation in responses may include slight differences in the microhabitat surrounding each egg, or differences in the microfauna/flora associated with each egg. The three different types of responses were not elicited in similar proportions in the field and in the greenhouse study for *P. angulata*; this could be due to differences in temperature and other environmental conditions in the greenhouse versus the field experiment. In addition, UV wavelengths from sunlight filtered out by the greenhouse glass could have contributed to differences in the elicitation of different responses (Doss *et al.* 2000).

The mechanism by which eggs induce a plant response is unknown. Preliminary data suggests that the elicitor of a response to an egg is not physical, but chemical. Small craft beads the size of *Hs* eggs affixed to *Physalis* leaves did not induce a response (Petzold, unpublished data). The supposition that the elicitor is chemical and not physical concurs with what little information is available on the nature of elicitors of (non gall-forming) plant responses to eggs. Only two elicitors have been identified thus far. Neoplasms on pea pods in response to oviposition by the bruchid beetle *Callosobruchus maculatus* are elicited by
bruchins, esters of long-chain diols (Doss et al. 2000). The elicitor that induces emission of parasitoid-attracting volatiles from Brussels sprout following oviposition by *Pieris brassicae* is the male-derived compound benzyl cyanide, received by females after mating (Fatouros et al. 2008). This compound is found in secretions of the accessory reproductive gland, which is released with the eggs onto the plant surface (Fatouros et al. 2008). Other elicitors that have not been fully identified have also been targeted in oviduct secretions. The release of egg parasitoid-attracting volatiles by pine is elicited by a small, unidentified protein in the oviduct secretion of the pine sawfly (Hilker et al. 2005). Similarly, components in the oviduct secretions of the elm leaf beetle *Xanthogaleruca luteola* are responsible for elicitation of a plant response that results in the release of volatiles that attract an egg parasitoid of this beetle (Meiners and Hilker 1997). Thus, it is likely that the eliciting agent of physical responses of *Physalis* leaves to *Hs* eggs is located in the oviduct secretion that is deposited with the egg during oviposition. We also cannot rule out that microbes present on the eggs may play a role in eliciting a response (Felton and Tumlinson 2008). Future studies will address these questions.

**Conclusions**

Results from this study support the hypothesis that a plant response to an *Hs* egg decreases its probability of survival. In our field experiment, ability of *Physalis* plants to respond to *Hs* eggs incurred a mean fitness cost of approximately 18% for *Hs*. In the warmest week when there was plant response to 59% of the eggs, the fitness cost was calculated to be 42%. It is hard to predict from a single experiment in one location what the historical fitness costs of plant response to eggs have been for this insect that occurs from the northern to southern temperate zones, but for which most of its hosts occur in tropical or semi-tropical areas (Bateman 2006). We can at least conclude from our experiments that the response of *Physalis* leaves to *Hs* eggs may be one significant factor that selected for egg laying on non-host plants.
Both direct and indirect plant responses to eggs have been recorded in a growing number of systems; this has generated increasing evidence that plants can actively respond to insect herbivores before harm to the plant is initiated. Future work in this area will no doubt continue to influence our thinking about plant/herbivore interactions and the selective pressure that insects and plants have on each other.

ACKNOWLEDGEMENTS

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Bateman, M. 2006. Impact of plant suitability, biogeography, and ecological factors on associations between the specialist herbivore Heliothis subflexa G. (Lepidoptera: Noctuidae) and the species in its host genus, Physalis L. (Solanaceae), in west-central Mexico. PhD thesis, North Carolina State University, Raleigh, NC, USA.


Doss, R. P., J. E. Oliver, W. M. Proebsting, S. W. Potter, S. R. Kuy, S. L. Clement, R. T.


Table 1: Output from PROC GENMOD showing the effect of leaf side (abaxial or adaxial), plant species, response, plant species by response interaction, and plant age on the probability of an egg hatching.

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Figure 1: Response of *Physalis* leaves to eggs of *H. subflexa*. a) neoplastic growth, b) a necrotic zone, or c) both necrosis and neoplastic growth directly under the site of egg deposition.
Figure 2: Mean percent eggs on *P. angulata* and *P. pubescens* leaves to which old plants (>12 week old) (white bars) and young plants (7-9 weeks) (shaded bars) responded. Means were calculated over plant; numbers in bars represents number of replicates (plants) for each treatment. N = 488 eggs for old plants, N = 198 eggs for young plants. Error bars represent 1 ± SE.

Figure 3: Mean percent of eggs that hatched on *Physalis* leaves for eggs that induced a response (shaded bars), and for eggs that did not induce a response (white bars). A total of 14 *P. angulata* plants containing 331 eggs, and 12 *P. pubescens* eggs containing 348 eggs were measured. Error bars represent 1 ± SE.
Figure 4: Average percent response to *Hs* eggs by *P. angulata* leaves over a range of temperatures during the six-week field experiment. Each data point represents response to eggs averaged over the cohort of plants used for each week of the experiment; the start date for each week is listed next to the corresponding data point. Error bars represent $1 \pm$ SE.
Figure 5: Mean percent of *Hs* eggs that (a) disappeared (were predated upon or fell off) from *P. angulata* leaves and that (b) hatched; for eggs that did not induce a response (white bars) and for eggs that did induce a response (shaded bars) over the six-week field study. A total of 538 eggs on 23 *P. angulata* plants were used in the analysis. Error bars represent 1 \( \pm \) SE.
Effect of *Heliothis subflexa* herbivory on fruit abscission by *Physalis* species: the roles of mechanical damage and chemical factors

**Jennifer Petzold**, **Cavell Brownie** and **Fred Gould**

1Department of Plant Biology, North Carolina State University, Raleigh, North Carolina, U.S.A., 2Department of Statistics, North Carolina State University, Raleigh, North Carolina, U.S.A. and 3Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina, U.S.A.

**Abstract.** 1. Insect oral secretions are important for the induction of a number of plant responses, but the relative role of mechanical damage in the induction of these responses is often not well understood. Damage from the frugivore *Heliothis subflexa*, a specialist on *Physalis* species, causes herbivore-induced fruit abscission. In this field study, we examined the separate and combined effects of mechanical damage and *H. subflexa* oral secretions on *Physalis* fruit abscission.

2. To determine the relative role of mechanical and chemical factors, the following treatments were administered to fruit: (1) three levels of mechanical damage, (2) natural herbivore damage by control larvae and by larvae surgically treated to inhibit saliva secretion, and (3) injection of *H. subflexa* oral secretions and a water control. Abscission of mechanically damaged fruit with and without the addition of oral secretions was also compared.

3. Mechanical damage was sufficient to cause fruit abscission, and the addition of oral secretions to mechanically damaged fruit did not cause an increase in fruit abscission. Normal caterpillars and those treated to inhibit saliva secretion caused similar abscission rates.

4. Though most studies examining the effects of insect oral secretions on induced plant responses find these chemical stimuli to be important or essential, the results of the present study showed that oral secretions are not necessary for fruit abscission. Future work is needed to determine the relative importance of mechanical damage in herbivore-induced plant responses in other systems.

**Key words.** Fruit abscission, *Heliothis subflexa*, herbivore-induced plant defences, insect oral secretions, mechanical damage, *Physalis*.

**Introduction**

Damage to plant tissue by insect herbivores can cause a significant reduction in plant fitness, and plants have evolved both constitutive and herbivore-induced defences in response to this damage (Summers & Rainher, 1987; Nunez-Farfán & Dirzo, 1994; Karban & Baldwin, 1997). Induced responses to herbivory include increased amounts of toxic or repellent allelochemicals produced at the feeding site or throughout the whole plant, a change in plant nutrient content, and emission of enemy-attracting volatiles (see reviews by Gatehouse, 2002; Dudareva *et al.*, 2006). Another important inducible defence mechanism for some plant species is the abscission of leaves or reproductive structures on which the herbivore is feeding (reviewed by Sallabanks & Courtney, 1992; Marr & Pellmyr, 2003).

Both mechanical damage from herbivore feeding, as well as chemical stimuli in insect oral secretions, have been shown to be important factors in the induction of herbivore-induced plant defences. Insect oral secretions are defined as fluids collected from an insect following disturbances, such as squeezing or pinching, and contain components from the alimentary canal, as well as saliva (Feltin & Eichhorn, 1999). Election of plant responses have been found in both oral secretions and in pure
saliva (Turlings et al., 1990; Mattiacci et al., 1995; Misser et al., 2006). There are a number of plant defences that are not associated with either wounding or artificial damage alone, but can be induced by the application of elicitors from herbivore oral secretions to artificial plant wounds (Alborn et al., 1997; Felton & Korth, 2000). In addition to inducing direct plant defences, application of elicitors in oral secretions of insects has been shown to cause the release of volatile compounds that attract natural enemies of the herbivore (Turlings et al., 1990; Mattiacci et al., 1995; Alborn et al., 1997). There are some cases in which defences are elicited solely by oral secretions, without any mechanical damage to the plant (Turlings et al., 1993). More often, however, both wounding and components of oral secretions have been shown to play important roles in the elicitation of plant defences (Mattiacci et al., 1995; Turlings et al., 1995; McCloud & Baldwin, 1997; De Moraes et al., 1998; von Dahl et al., 2006).

Though much attention has been given to the role of insect oral secretions in the induction of plant defences, there have been several recent reports demonstrating that mechanical damage alone can elicit defences very similar to those elicited by herbivory. The parasitoid wasp Cotesia glomerata, a natural enemy to Pieris brassicae that is recruited by volatiles emitted from Brussels sprouts (Brassica oleracea var. gemmifera) following herbivory by Pieris brassicae, did not distinguish between volatiles emitted as a result of continuous mechanical damage and those emitted as a result of Pieris brassicae herbivory (Connor et al., 2007). Similarly, Mothöfer et al. (2004) showed that lima bean (Phasolus limensis) leaves subjected to continuous mechanical damage emitted volatile profiles that were qualitatively very similar to those induced by Spodoptera littoralis herbivory. The results of these studies suggest that the role of mechanical damage during herbivory in eliciting induced defences in plants may be more important than previously thought.

Although many studies have demonstrated that induced defences in leaves can be initiated by insect oral secretions or mechanical damage, the literature lacks information about the effects of oral secretions on reproductive structures, and especially on abscission of these structures. Two exceptions include studies that show varying results. Shackel et al. (2005) injected small quantities of Lygus bug salivary enzymes into cotton and alfalfa flowers to determine whether abscission of flowers by Lygus bugs on these crops was caused by stylet damage, or biochemical responses to components in saliva. They concluded that components in the saliva caused the withering and abscission of both cotton and alfalfa flowers (Shackel et al., 2005). Levine and Hall (1978) injected protein extracted from boll weevil larvae into cotton flowers and found that the resulting flower bud abscission was most likely not from boll weevil salivary extracts, but rather from molting fluid in larvae (see also Coakley et al., 1969). Other authors attribute bud abscission to mechanical damage by boll weevils (Santos et al., 2003). To our knowledge, the study described below is the first to test for the independent and combined effects of herbivore oral secretions and mechanical damage on fruit abscission.

Herbivory by Heliothis subflexa larvae is known to cause fruit abscission in Physalis species (Benda et al., 2009). This insect is monophagous, feeding only on plants in the genus Physalis (Brazel et al., 1953; Bateman, 2006), which includes the crop, tomato. Larvae feed on the fruit of Physalis plants, where they are enclosed in the inflated calyx that surrounds the fruit, a characteristic shared by all plants in this genus. This inflated calyx provides a structural refuge from natural enemies to H. subflexa (Sisterson & Gould, 1999; Oppenheim & Gould, 2002). Heliothis subflexa larvae chew a hole through the thin membrane of the inflated calyx to reach the enclosed fruit, and consume all or part of the fruit. Larvae must consume several fruit in order to complete development. Physalis plants can decrease fruit herbivory by absescing fruit on which H. subflexa feed, preventing subsequent movement to other fruit on the plant, unless the larva can relocate and re-establish itself onto the plant following fruit abscission. Plants in the genus Physalis have been shown to vary substantially in both the probability and rate of abscission of herbivore-damaged fruit (Benda et al., 2009). However, the specific mechanism (mechanical damage and/or elicitors in oral secretions) that causes fruit abscission in Physalis species has not been studied.

In this study, we sought to determine the specific mechanisms (oral secretions, mechanical damage, or both) that cause fruit abscission in Physalis species damaged by H. subflexa. The three Physalis species used in this study were chosen based on high preference by H. subflexa females for all three (Bateman, 2006), inherent differences in their architecture, and on differences among them in propensity to abscise herbivore-damaged fruit (Benda et al., 2009). Physalis pubescens is a low-lying shrub-like plant with spreading branches that readily abscises herbivore-damaged fruit, while Physalis angulata is a tall, arboreous plant with intermediate fruit abscission. Physalis cordata, a low-lying plant for which most of its branches lay prostrate on the soil surface, has not been observed to abscise fruit in the field (J. Petzold, pers. obs.). We used a number of different treatments on P. angulata, P. pubescens, and P. cordata plants in field plots to independently examine the effects of oral secretions and mechanical damage, allowing us to test for the separate and combined effects of these two factors on fruit abscission.

Our objectives were to (1) determine if fruit abscission is caused by saliva and/or oral secretions from H. subflexa, mechanical damage, or by an interaction between saliva and/or oral secretions and mechanical damage from H. subflexa, and (2) to characterise the differences in patterns of fruit abscission between the Physalis species we examined. The data collected in this study also provide information on the differences in the extent of fruit abscission caused by artificial damage versus herbivory.

Materials and methods

Plants

Seeds of P. angulata, P. pubescens, and P. cordata, collected in 1998 from wild populations in Orangeburg County, South
Mechanical damage from herbivory causes fruit abscission

Carolina, U.S.A., were field-grown in 2004. Seeds from that planting were sown in a greenhouse on 5 May 2005 in flats containing 5–6 cm³ of Metro Mix soil (Sungro Horticulture, Vancouver BC, Canada) and approximately 100 g of Osmocote slow release fertilizer (10–10–10) (Scotts-Sierra Horticultural Products, Marysville, Ohio). Three-week-old plants were transferred to 1-litre pots and fertilised again with one tablespoon of Osmocote, then treated weekly with Peters Professional Liquid fertiliser (20–20–20) (Scotts Fertilizers, Marysville, Ohio). 5- to 8-week-old seedlings of P. angulata, P. pubescens, and P. cordata were transplanted to a filled field in Wake County on the NC State University Campus (Raleigh, NC) between 24 June and 6 July 2005. The seedlings were planted at 0.75 m intervals, in holes cut into black woven plastic ground cover (Lumate, 183 cm wide). Each seedling was fertilised with approximately one tablespoon of Osmocote fertiliser and Ferti-lome Tomato and Vegetable food (7–22–8, with microelements) (Voluntary Purchasing Groups Inc., Bonham, TX) upon planting. Plants were fertilised approximately every 3 weeks until the end of experiments. Plants were watered as needed.

Insects

Larvae were collected from naturally infested Physalis plants in another field approximately 21 miles away, in Clayton (Johton County), NC, U.S.A. Individuals were reared in 30 ml plastic cups containing a cornmeal and soybean-based diet (modified from Burton, 1970) as well as several fresh P. angulata or P. pubescens fruit. The caterpillars fed primarily on the Physalis fruit.

Experimental design

The experimental design was a split plot, with species as the whole plot factor and treatment as a subplot factor. Four blocks of seedlings were planted, with each block containing one 10.5 m row of each of the three Physalis species. Blocks were adjacent to each other, with no separation between blocks. Fourteen seedlings of the randomly assigned species were planted per row.

Seven different treatments were used to determine the factor(s) that caused fruit abscission. Each treatment was applied to two randomly selected plants per row in order to replicate each of the seven treatments twice for each species. All analyses were carried out after combining results for these duplicate plants.

Treated fruit were numbered using small labels attached to the plant with rust-free, 1-inch safety pins (Major Black Safety Pins, size 1, Zipperstop Inc., New York) inserted through the calyx. Each of the plants had four fruit treated with the assigned treatment, and four other randomly chosen fruit on the plant served as controls. The diameter of each fruit was recorded with digital calipers on the first day of the experiment. For treatments that required access to the fruit, calyces were torn in half longitudinally. We tore open the calyces of control fruit and tagged them in the same manner as treated fruit. Plants in each block were checked for fruit abscission the night of the first day of the experiment (day 0), and every day for 7 days thereafter. Day was treated as a repeated measures factor, first by assuming it was a subplot factor, and also by doing a repeated measures analysis of contrasts. The experiment was conducted twice in 2005. Experimental trial 1 was conducted 27 July–5 August, using two of the four blocks. All four blocks were used for experimental trial 2, which took place on 8–18 August. For each experimental trial, one full day was needed to set up a single block. Therefore, in experimental trial 2, for example, blocks 1, 2, 3, and 4 were set up on 8, 9, 10, and 11 August respectively, and data collection ended on 15, 16, 17, and 18 August, respectively.

Treatments

Oral secretion treatment. In order to determine if elicitors in oral secretions (regurgitated) caused fruit abscission, fruit were injected with freshly collected H. subflexa oral secretions. In caterpillars, saliva exits through the spinneret, an organ directly below the mouthpart region, and is inevitably present in the regurgitated mixture (Felton & Eichenseer, 1999). Fourth to fifth instars in individual 30-ml plastic cups were taken to the field in coolers kept at approximately 24–27°C (ice was added as needed). Oral secretions were collected in the field by gently squeezing the caterpillar behind the head and holding a pipette tip to the mouthpart region, as described by Turlings et al. (1993). Each caterpillar produced approximately 1–1.5 μl of secretions, and immediately after collection this amount was transferred to a 1-ml tuberculin syringe (26 gauge) (Becton Dickinson, Franklin Lakes, NJ) and injected into the middle of a fruit.

Water treatment. As a control for the oral secretion injection, approximately 1.5 μl of deionised water was injected into fruit with an identical syringe.

Damage treatment – caterpillars. Natural caterpillar damage causes most species of Physalis to abscise fruit. In order to determine the proportion of fruit abscised for different levels of caterpillar damage, and also to serve as a positive control for our other treatments, four H. subflexa fourth and fifth instar caterpillars were placed on one branch of the Physalis plant and allowed to feed freely on fruit. The branch was encased in cinched mesh bags constructed from non-woven greenhouse floating row cloth (J & M Industries, Inc., Ponchatoula, LA) to prevent caterpillar escape. The caterpillars were placed on the branches from 17.00–20.00 hours the night before the first day of the experiment, and removed from 08.00 to 10.00 hours the next day. Each caterpillar-damaged fruit was visually assessed for per cent area consumed on a scale of one to 10 (hereafter referred to as ‘damage level’), representing damage categories 1–10%, 11–20%, 21–30%, etc. up to 91–100% damage. Damage level assessments were conducted by one individual. However, another individual conducted damage level assessments on a sample of 94 fruit to evaluate

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the accuracy of the assessments. The damage level assigned to each of the 94 fruit by the first assessor was regressed against the damage level assigned to each fruit by the second assessor. In addition, to verify that damage level assessments were precise, the primary individual who did the damage level assessments re-evaluated a sample of 40 fruit after the first evaluation had been conducted (without knowing the identity of each fruit). The first damage level value assigned to a fruit, was regressed against the damage level same assigned during the second assessment for each fruit. Damage level assessments of the two individuals were very similar \( (r^2 = 0.95, y = 1.03x - 0.35) \), as were the damage level assessments conducted by the same individual twice \( (r^2 = 0.97, y = 0.98x + 0.22) \).

**Damage treatment — non-salivating caterpillars.** To determine if elicitors in saliva alone were important in Physalis fruit abscission, we surgically treated caterpillars to inhibit saliva secretion. These caterpillars will hereafter be referred to as ‘non-salivating caterpillars.’ The spineores (saliva-secreting organs) of each caterpillar were cauterized with a small hot probe (Hod Pen B, Electron Microscopy Sciences, Hatfield, PA), as described by Misser et al. (2006). This treatment is known to severely limit saliva secretion, but does not affect other components in the oral secretions. One day prior to the first day of the experiment, field-collected caterpillars were placed on ice under a dissecting microscope, and the probe was touched to the spinneret. Caterpillars were returned to their cups and resumed feeding within 5–10 min after treatment. The treated caterpillars were able to feed normally and pupate, and they emerged as normal adults. These non-salivating caterpillars were placed on plants and bagged in the same manner as non-treated caterpillars, as described above, and the damage level for each fruit eaten was assessed.

**Damage treatment — mechanical.** To determine if mechanical damage was sufficient to cause fruit abscission, three levels of mechanical damage were applied to fruit. For the lowest damage level, we used a sterilized toothpicks to break the surface of the bottom portion of the fruit, and inserted it approximately 5 mm into the fruit, approximating damage by a first or second instar larva. For the medium damage level, we inserted the toothpick through the bottom of the fruit approximately three-quarters of the way to the top of the fruit, and rotated the toothpick slightly to damage tissue and dislodge seeds. For the highest level of damage, we used a metal blade to slice through the equator and remove the bottom half of the fruit (including half of the seeds). This resembles larval damage at the point when 50% of the fruit has been consumed.

**Interaction between oral secretions and damage**

To follow up on results of the experiments described above, an experiment was designed to determine if oral secretions from caterpillars damaged fruit would cause greater fruit abscission than the damage alone. This experiment was conducted from 19 September to 25 September 2005 using 12 healthy feral Physalis angulata plants in a field site used in previous years (NCDAR/NC State University Central Crops Research Station in Clayton, NC). At that time of the season, all of the initially transplanted Physalis plants were senescent or diseased. For each plant, a total of 12 fruit were used: four controls (tagged), four that received the highest damage treatment (cut in half with a blade along the equator), and four that received the highest damage treatment and in addition were treated with either 2 or 4 μl of the oral secretion mixture. We chose to use the highest level of damage for this experiment, based on results of the previously described experiment which showed that the highest level of mechanical damage caused fruit abscission. Both field-collected and laboratory-reared larvae were used. Laboratory-reared larvae were originally collected from natural populations of Physalis in Florence and Barnwell County, South Carolina in 1996 (see Schlaff et al., 2006), and were raised on a cornmeal and soybean-based diet (modified from Burton, 1970) and held at 25 °C, LD 12:12 (lights off from 20.00 to 08.00 hours). The oral secretions were collected as described above, in the field immediately before application to the fruit. The mixture was transferred from the pipette onto the cut surface of the fruit, and gently wiped across the surface with the side of a glass pipette. We monitored the plants for fruit abscission for 7 days. At this point, almost all of the non-control fruit had either abscised or experienced natural infestation from caterpillars in the field. Fruit damaged from natural infestation were eliminated from the analysis.

**Statistical analyses**

All statistical analyses were performed using SAS 9.1 software (SAS Institute, 2003). Physalis angulata plants did not abscise any fruit throughout the experiment, so this species was eliminated from statistical analyses.

**Repeated measures**

To determine if the pattern of fruit abscission over the 8-day observational period was similar for all species, treatments, and experimental trials, we performed a repeated measures analysis. Data were arcsine transformed to conform to the assumptions of normality and equal variances. The analysis was carried out using PROC GLM with a REPEATED statement. Specific TEST statements were used to produce F tests appropriate for a mixed model. Block in experimental trial, and species by block in experimental trial were treated as random effects, and experimental trial, species, treatments, and day, plus all interactions, were considered fixed effects.

**Day-by-day analysis**

Significant interactions with day led to additional analyses to determine if the proportion of fruit that dropped on each
particular day was affected by (1) experimental trial, (2) plant species, (3) treatment, and (4) all interactions. We conducted mixed model anovas with PROC MIXED for each day, where (1) block was nested within experimental trial and (2) species by block within each experimental trial was treated as a random effect. Data were arc sine transformed. Due to the imbalance in the data set, we used the Satterwaite method to compute denominator degrees of freedom, which performs well under these conditions (Littel et al., 1996). As the Satterwaite method provides a numerical approximation of denominator degrees of freedom, these values are not necessarily whole numbers. The least squares mean (L.S mean) for proportion of fruit abscised was calculated for each day, as were probabilities (Tukey adjusted) for pairwise differences in L.S mean per cent fruit abscission between treatments. The following treatments were compared for significant differences ($P < 0.05$): water injection versus oral secretions injection, caterpillar damage versus non-salivating caterpillar damage, low versus medium versus high damage treatments, and high damage treatment versus salivating and non-salivating caterpillar damage treatments. The L.S mean fruit abscission for each treatment and for controls was graphed for each day of the experiment. As we did not observe a systemic effect for any of the treatments on the control fruit on the same plants, the L.S means for control fruit abscission on each separate day were averaged for all seven treatments, for each plant species. For these controls, the standard errors used were those calculated based on pooled data from control fruit for all seven treatments. ESTIMATE statements were used to test for significant differences between the combined control fruit and treatment fruit.

**Caterpillar damage**

Fruit subjected to the caterpillar damage treatments were scored for per cent damage on the first day of the experiment, immediately after caterpillars were removed from plants. To determine how the per cent fruit damage by salivating and non-salivating caterpillars influenced the day that the fruit was abscised, we performed a mixed model anova with species, experimental trial, treatment, and fruit damage score as fixed effects. Block within experimental trial, and the interaction between species and block, as well as between species and block and treatment, were random effects. We also calculated the slope of the line for abscission date of a fruit regressed against damage score, using the SOLUTION command in the model. In addition, we regressed the average proportion of fruit that fell after 7 days against per cent fruit damage, for several damage level classes, for each species.

**Diameter**

The diameter of each fruit used in the two experimental trials was recorded with digital calipers on the first day of the experiment. Fruit from caterpillar treatments that had too much tissue eaten to get an accurate measure of diameter were eliminated from the analysis. To determine if the initial diameter of a fruit influenced the day that the fruit was abscised, we performed a mixed model anova with species, experimental trial, treatment, and initial diameter as fixed effects. Block within experimental trial, and the interaction between species and block, as well as between species and block and treatment, were random effects. We also calculated the slope of the line for abscission date regressed against fruit diameter using the SOLUTION command in the model.

**Mechanical damage plus oral secretions**

To determine if the proportion of *P. angulata* fruit abscised, as well as the day that they abscised, were different for the mechanical damage treatment versus the mechanical damage and oral secretions addition treatment, we performed an anova using PROC GLM and PROC GENMOD. Treatment (damage, damage plus oral secretions, control), amount of oral secretions used (2 μl or 4 μl), and plant number were fixed effects, while plant number by treatment was a random effect. In addition, a linear contrast was used to determine if the two treatments differed from each other. Another anova using PROC GLM was conducted to determine if the type of caterpillar used (laboratory reared or field collected) was an indicator of fruit abscission. Caterpillar type was a fixed effect, while plant number nested within caterpillar type was a random effect.

**Results**

*Physalis cordata* plants did not abscise any fruit for any of the treatments (non-experimental fruit also remained affixed to the plant), so the following results will focus on *P. angulata* and *P. pubescens*.

**Control fruit**

Both *P. angulata* and *P. pubescens* abscise a number of non-damaged fruit under normal field conditions. For control fruit, the mixed model analysis indicated that species was a significant factor in determining the amount of fruit that dropped, with marginal significance at day 3 ($F_{1,7.95} = 3.93$, $P = 0.084$), and increasingly stronger significance for days 4–7 (days 4–7, respectively: $F_{1,3.95} = 11.55$, $P = 0.0279$; $F_{1,3.95} = 14.74$, $P = 0.00188$; $F_{1,3.95} = 19.65$, $P = 0.0015$; $F_{1,3.95} = 33.76$, $P = 0.0046$). There was a linear increase in cumulative fruit drop over the 8 days for both species, but *P. pubescens* always had a higher cumulative proportion of abscised control fruit, with approximately 40% of control fruit abscised by the end of the experiment compared to less than 5% for *P. angulata*. Thus, the interaction between day and species was highly significant (repeated measures analysis $F_{7} = 20.12$, $P < 0.0001$). Treatment was never a significant factor in determining whether a control fruit would drop or the day that it dropped, indicating that there was no systemic effect for any of the treatments. Block within experimental trial was a highly significant factor in control fruit abscission ($F_{2} = 7.69$, $P < 0.0001$); this was probably due to differences in plant ages between blocks.
Experimental fruit

General patterns for both species and all treatments. For experimental fruit (all fruit that received a treatment), the mixed model analysis indicated that species was a significant factor in determining experimental fruit abscission starting at day 1, and became increasingly more significant each day (Table 1). By the end of the experiment, *P. pubescens* abscised a significantly higher proportion of fruit for each respective treatment compared to *P. angulata*, except for the salivating and non-salivating caterpillar damage treatments, where fruit abscission for the two species did not differ significantly due to almost 100% abscission by both species. This higher overall abscission of the experimental fruit, even in the water treatment, was presumably due to the inherent higher rate of *P. pubescens* fruit drop also seen for the control fruit.

The overall effect of treatment was a highly significant factor in fruit abscission for every day of the experiment (\( P < 0.001 \)) (Table 1). However, there was never an overall treatment by species interaction on any day (Table 1). This means that over all of the treatments, both species were affected in the same manner throughout every day of the experiment. Further tests were done to examine whether there was a treatment by species interaction, if treatments were partitioned into those that caused fruit abscission that significantly differed from controls, versus those that did not. Even with this partitioning, there was again no treatment by species interaction for any of the days (\( P > 0.05 \) for all days). However, as discussed below, when the caterpillar damage treatments were examined further by looking at fruit abscission resulting from specific damage levels 1 through 10, there was a species by caterpillar damage level interaction.

Tests of specific hypotheses

Oral secretions injection versus water injection. For *P. angulata*, the amount of cumulative fruit abscission did not significantly differ between the oral secretion injection treatment (which includes saliva and regurgitant) and the water treatment, or between these two treatments and controls at any time during the experiment (\( P > 0.05 \) for all comparisons on all days). For *P. pubescens*, the water and oral secretion injection treatments never differed from each other for any day of the experiment (\( P > 0.05 \) for all days). However, the water injection treatment differed from controls on days 3 (\( F_{(4,89)} = 2.19, P = 0.0326 \)) and 6 (\( F_{(4,89)} = 2.08, P = 0.0440 \)), and the oral secretion injection treatment differed from controls on days 3 (\( F_{(4,89)} = 2.07, P = 0.0431 \)) and 4 (\( F_{(4,89)} = 2.18, P = 0.0343 \)). This difference is presumably due to a small treatment effect from the injections, but since water and oral secretion injections never differed significantly from each other, the increase in fruit abscission was not due to an effect of oral secretions.

Salivating versus non-salivating caterpillar damage. The mixed model analysis indicated that both species abscised a higher cumulative mean amount of fruit for the salivating and non-salivating caterpillar damage treatments compared to control fruit for both species (Fig. 1). However, salivating versus non-salivating caterpillar treatments did not significantly differ for any of the days (\( P > 0.1680 \) for all days). For *P. angulata*, damaged fruit abscised steadily through to day 2, and continued to abscise at a low rate until day 7 (Fig. 1a). For *P. pubescens*, almost all damaged fruit had abscised by day 3 of the experiment (Fig. 1b). The mean damage level was 6.8 for *P. angulata* (i.e. approximately 68% of the fruit volume was consumed), and 5.4 for *P. pubescens*, respectively, although these values were not significantly different (\( P = 0.1678 \)).

Damage level was not important in determining the likelihood that a *P. pubescens* fruit would abscise in the 8-day period, since abscission was close to 100% irrespective of amount of fruit eaten. However, for *P. angulata*, as the per cent of the fruit eaten increased, the likelihood of abscission after 7 days increased (\( r^2 = 0.8736 \)) (Fig. 2) (species by per cent damage interaction: \( F_{1,118} = 4.09, P = 0.0453 \)). For *P. angulata*, caterpillar-inflicted damage appeared to have more of an effect on the fruit abscised than the same amount of mechanical damage. When a caterpillar ingested 40–60% of the fruit volume, 79% of the fruit abscised, however, when 50% of the fruit was removed by a blade, only 39% of the fruit dropped (Fig. 2).

### Table 1. Effects of treatment, species, and their interaction on the proportion of *Physalis angulata* and *Physalis pubescens* experimental fruit abscised over the 8-day experiment (mixed model analysis, type III tests).

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Species</th>
<th>Treatment × Species</th>
</tr>
</thead>
<tbody>
<tr>
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<td>d.f.</td>
</tr>
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<td>6.456</td>
<td>12.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Numerator d.f., denominator d.f.
¹ Significant P-values are boldfaced.
For *P. angulata*, the per cent fruit eaten by the caterpillar also affected the specific day that a fruit dropped. As per cent fruit eaten increased, the number of days it took a fruit to fall decreased (slope = $-0.4038$, $t_{110} = -5.03$, $P < 0.0001$). For example, if 10–20% of a fruit was consumed (damage level 2), the fruit is predicted to fall between days 5 and 6, while a fruit with 70–80% damage (damage level 8) is predicted to fall between days 2 and 3. A similar trend was observed for *P. pubescens*, however the slope was closer to zero (slope = $-0.2027$, $t_{115} = -1.55$, $P = 0.0282$), indicating that most fruit fell within the first few days.

**Mechanical damage: low, medium, and high damage levels.**

The highest level of mechanical damage caused significantly more fruit abscission than controls, starting at day 2 for *P. angulata* (Fig. 3a) and day 1 for *P. pubescens* (Fig. 3b). By day 3 of the experiment, almost all of the *P. pubescens* fruit that received the highest damage treatment had abscised (Fig. 3b), while *P. angulata* fruit abscised at a steady rate for the first 5 days of the experiment then levelled off (Fig. 3a). For both species, the low and medium damage treatments did not differ from each other throughout the experiment, but fruit with these treatments abscised at a higher proportion compared to controls, beginning with day 3 and continued increasing through to day 7 for *P. pubescens* (Fig. 3b). Abscission for *P. angulata* fruit that received the low and medium damage treatments increased throughout the experiment, however these treatments never significantly differed from control fruit for any day (Fig. 3a).

For almost all of the days, there was no significant difference in mean abscission between the fruit that received the highest damage level treatment, and fruit that received natural herbivory (from both salivating and non-salivating caterpillars). The exceptions occurred on day 0 and 1. For *P. pubescens*, natural herbivory from both salivating and non-salivating caterpillars caused significantly higher fruit abscission compared to the highest damage treatment on day 0. On day 1, only non-salivating caterpillars caused a significantly higher mean fruit abscission compared to the highest damage treatment. For *P. angulata*, on day 0, salivating caterpillars caused significantly higher mean fruit abscission compared to highest damage treatment. For all other days, mean fruit abscission due to natural herbivory was not significantly different from that caused by the highest damage treatment ($P > 0.05$ for all other days).

**Interaction between effect of oral secretions and damage on fruit abscission.** Fruit subjected to the highest damage treatment (cut in half) with and without the application of oral secretions on the cut surface, did not differ in abscission for any day during the experiment (contrast, $F_1 = 0.99$, $P = 0.3304$) (Fig. 4). The amount of oral secretions used (2 µl vs 4 µl) did not have an effect on the proportion of fruit abscised. For fruit that received the damage plus oral secretions treatment, mean proportion of fruit abscised did not significantly differ depending on whether the oral secretions came from a laboratory-reared caterpillar or a field collected caterpillar ($F_1 = 1.43$, $P = 0.2599$).

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Fig. 1. Comparison of the cumulative proportion (mean ± SE) of (a) *Physalis angulata* and (b) *Physalis pubescens* abscised fruit for control fruit (closed bars), salivating caterpillar damage treatment (open bars), and non-salivating caterpillar damage treatment (tinted bars).

Fig. 2. Proportion of *Physalis angulata* fruit that abscised by the last day of the experiment for various levels of *Heliothis subflexa* feeding (mean ± SE). $n$ for categories are: 17, 10, 14, 4, and 25 for damage levels 1–20%, 21–40%, 41–60%, 61–80%, and 81–100%, respectively.

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Diameter. When abscission was averaged over all treatments, the diameter of an experimental fruit was a significant factor in determining the day a fruit abscised for *P. angulata*. Larger fruit abscised slightly later than smaller fruit (slope = 0.1728, $t_{33} = 2.63, P = 0.0087$). For example, a fruit with a diameter of 6 mm would be predicted to fall 1 day earlier than a fruit with a diameter of 12 mm. The diameter of *P. pubescens* experimental fruit was not correlated with the day the fruit abscised.

In contrast, the diameter of *P. pubescens* control fruit did significantly affect the day a fruit dropped: larger fruit abscised earlier than smaller fruit (slope = -0.2441, $t_{34} = -4.76, P < 0.0001$). For example, a fruit that had a diameter of 8 mm would be predicted to fall 1 day later than a fruit with a 12 mm diameter. The diameter of *P. angulata* control fruit did not affect the day that a fruit abscised.

Discussion

The effect of oral secretions and damage on fruit abscission

Fruit abscission in response to fruit or seed predators is a common phenomenon and has been recorded in numerous studies (reviewed by Sallabanks & Courtney, 1992; Rupert et al., 2000; Marr & Pellmyr, 2003; Kravchenko & Boros, 2004; Jolivet & Bernasconi, 2006). However, we are unaware of any other studies that have tested the separate and combined effects of mechanical damage and insect oral secretions on fruit abscission. There was never a difference in abscission between the oral secretion-injected and water-injected fruit for the *Physalis* species we studied, indicating that oral secretions (regurgitant and small amounts of saliva) are not needed for abscission. Mechanical damage alone was sufficient to cause the two *Physalis* species to abscise fruit. Furthermore, normal caterpillars and those treated to inhibit saliva secretion caused similar abscission rates, indicating that saliva is not important in fruit abscission. Finally, there was no difference in the abscission of mechanically damaged fruit whether or not caterpillar oral secretions were applied to the damaged surface, showing that there was no interactive effect of damage and oral secretions. These results suggest that *H. subflexa* oral sections do not have an effect on fruit abscission in the *Physalis* species we examined. Our results are similar to those of Marr and Pellmyr (2003), who found that abscission of yucca flowers was due to mechanical damage from moth ovipositors and not to chemical factors from eggs laid inside flowers or an interaction of damage and chemical factors.

Our finding that mechanical damage was sufficient to cause fruit abscission was unexpected, considering that *Heliothis* spp. oral secretions appear to induce *Physalis* to produce parasitoid-attracting volatiles (Oppenheim & Gould, 2002; De Moraes & Mescher, 2004), and the salivary enzyme glucose oxidase, from the closely related insect *Helicoverpa zea*, inhibits a defense
response in tobacco (Masser et al., 2005). In addition, elicitors in oral secretions of herbivores have been found to be important in eliciting many plant-related defences (Feltton & Tumlinson, 2006, and references therein).

Interestingly, De Moraes and Mescher (2004) found that the regurgitant of *H. subflexa* individuals that fed on *Physalis* fruit lacked the elicitor volicitin, which is present in the regurgitant of other closely related species. Volicitin in the regurgitant of *Heliotis virescens*, a closely related insect that can develop on the leaves of *Physalis* species, was responsible for the elicitation of volatile production that attracted natural enemies to *P. angulata* plants. If the oral secretions of *H. subflexa* lack a signal recognized by *Physalis* plants, evolution of response to mechanical damage from *H. subflexa* herbivory could have been favoured by selection. It is also plausible that natural selection could have favoured plants that absorbed fruit in response to the more general stimulus of mechanical damage, rather than an insect-specific signal. If *Physalis* plants are indeed absorbing fruit to rid the plant of insects feeding on the fruit (Benda et al., 2009), as opposed to a more targeted response such as recruitment of a specific enemy, a general stimulus such as mechanical damage would allow the plant to respond to any insect damaging the fruit.

Over the 8 days of the experiment, the highest level of mechanical damage elicited fruit abscission similar to that caused by the natural herbivory treatments. The only days that natural herbivory caused statistically higher mean fruit abscission were days 0 and 1 for *P. pubescens*, and day 0 for *P. angulata*. This could be due to the fact that larvae were placed on the plants the night before day 0 of the experiment, in order to have all treatments completed for the first day of the experiment. Thus, because larvae were allowed to feed overnight, elicitation could have occurred 12–17 h before the mechanical damage treatment was executed, which could explain the differences in fruit abscission on day 0 and 1 for these treatments.

As our high mechanical damage treatment consisted of removing half of the fruit, we were able to compare fruit that received this treatment to the subset of fruit that had approximately 50% fruit area removed by natural herbivory. Abscission for these two treatments did not differ for *P. pubescens*. However, *P. angulata* abscised a higher proportion of herbivore-damaged fruit (when 50% of the fruit area was removed) after the 8 days of the experiment compared to mechanically damaged fruit (Fig. 2). This result appears to be counter to our conclusion that *H. subflexa* oral secretions are not important for fruit abscission. However, a number of studies reported in the literature indicate that the differences between our one-time mechanical injury and the continuous mechanical injury inflicted by *H. subflexa* are the likely cause of this difference in fruit abscission. Studies that have used continuous mechanical damage to mimic injury inflicted by herbivory, have shown that this type of damage induced plant defence responses that resemble those induced by natural herbivory, much more closely than those induced by one-time mechanical damage and are typically more pronounced (Mithöfer et al., 2005; Conner et al., 2007). It is also possible that other factors associated with herbivory could play a role in fruit abscission induction, such as insect-associated microbes (see review by Feltton & Tumlinson, 2008). However, the results of the experiment testing for abscission of mechanically damaged fruit with and without the application of oral secretions, show abscission is primarily driven by mechanical damage, and that there does not appear to be any additive effect of oral secretions (Fig. 4).

**Differences among Physalis species in fruit abscission**

There was a dramatic difference in the amount of natural abscission that occurred between *P. angulata*, *P. pubescens*, and *P. cordata*. *Physalis cordata* did not abscise fruit for any of the treatments. Even if the fruit had been completely removed or consumed, the empty calyx remained on the plant. In our field observations, even under conditions with very high levels of caterpillar damage, no fruit abscission occurred. Thus *P. cordata* apparently does not use abscission as a way to defend against these herbivores. Future experiments aimed at understanding the physiological/chemical pathway leading to abscission may determine why *P. cordata* does not abscise fruit. In addition, it is unclear whether the seeds of unabsceded, damaged fruit of any *Physalis* species can mature and germinate; future work will address this issue.

*Physalis pubescens* abscised control fruit at the highest rate. Approximately 40% of *P. pubescens* control fruit were abscised by the end of the experiment, while less than 5% of *P. angulata* control fruit abscised (most of the control fruit that dropped for both species were ripe and contained mature seeds). There appeared to be no systemic effect for the treatments, since control fruit abscission on a plant was not affected by the treatment applied to the other fruit on the same plant. Although *P. pubescens* abscised significantly more fruit than *P. angulata* for each day of the experiment except the day the treatments were administered (day 0), there was no interaction between species and treatment for any of the days (Table 1). Overall, the data are consistent with the hypothesis that *P. pubescens* has a higher baseline rate of fruit abscission than *P. angulata*, and that the damage treatments raise the abscission rate above the baseline rate by the same amount for each species.

The inherent differences in fruit abscission between these two species may be related to differences in their architecture. Benda et al. (2009) assessed the ability of *H. subflexa* larvae to relocate the plant after they were dislodged from the tall, arboresecent *P. angulata* plants, and the low, shrub-like *P. pubescens* plants along with an abscised fruit. Larvae dislodged from *P. pubescens* located and gained reestablishment more effectively than those dislodged from *P. angulata*, and this difference is likely due to architectural differences between the two plant species (Benda et al., 2009). The higher inherent rate of fruit abscission for *P. pubescens* shown in this study may have evolved in response to the relative ease with which *H. subflexa* relocates this plant compared to *P. angulata* after dislodgement from fruit abscission.

**Correlation analysis**

Fruit size was a small but significant factor in the likelihood of abscission for all experimental *P. angulata* fruit. The fact that larger *P. angulata* experimental fruit took longer to abscise, may reflect a longer length of herbivory needed
to achieve the same per cent area loss for a smaller fruit. Additionally, damage to larger fruit may require a longer time for signals to reach the abscission zone. A similar trend was observed in cotton bolls for both mechanically damaged buds and those damaged by Lygus hesperus feeding. For both treatments, smaller cotton squares were more likely to abscise compared to larger squares (Strong, 1970). This difference could also reflect evolution on the part of the plant to retain fruits with the highest energy investment.

Conclusion

Based on results from increasing numbers of studies that show the significance of oral secretions in inducing plant responses, and the fact that components in the oral secretions of a closely related Heliothine induced defence responses in *Physalis* species, we developed this study with the expectation that *H. sultana* oral secretions would be important in the induction of *Physalis* fruit abscission. However, our results showed that (1) injection of fresh oral secretions did not cause fruit abscission, (2) the highest level of mechanical wounding induced levels of fruit abscission similar to levels achieved by herbivory, and (3) there was no difference in abscission rates between fruit damaged by normal caterpillars and caterpillars with cauterised spinnerets. Our results, along with several other recent studies (Marr & Pellmyr, 2003; Mithöfer et al., 2005; Connor et al., 2007), emphasise the importance of mechanical wounding in plant-induced defences in these systems, and demonstrates the need for more studies examining the relative importance of chemical, mechanical, and other stimuli in inducing plant responses to herbivores.

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References


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THE GENETICS OF HOST RANGE IN A SPECIALIST AND GENERALIST HERBIVORE: LARVAL HOST PLANT PREFERENCE AND SURVIVAL

JENNIFER PETZOLD¹, FRED GOULD²

¹Department of Plant Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

²Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA
ABSTRACT

The genetic basis of host range in insects is largely unknown. In this study, we examined larval feeding behavior of the generalist *Heliothis virescens*, the specialist *Heliothis subflexa*, and progeny of F₁s backcrossed to each of these species. Larvae were assayed for preference and survival in both choice and no choice tests using leaf discs of *Physalis angulata*, a host of the specialist, and tobacco, a host of the generalist. *Heliothis subflexa* and progeny of F₁s backcrossed to *H. subflexa* both preferred *Physalis* leaf discs in choice tests, and ate very little tobacco. These insects also had high mortality when given only tobacco leaf discs in no choice tests. *H. virescens* showed some variation in preference and survival on the two plant species, but in general preferred tobacco over *Physalis*. However, progeny of F₁s backcrossed to *H. virescens* had variable feeding behavior, with some individuals preferring *Physalis* and others preferring tobacco leaf discs. The results support the hypothesis that at least one major locus is involved in feeding preference between the two host plants in *H. subflexa* and *H. virescens*, and that preference for *Physalis* is a dominant trait.
INTRODUCTION

Although host ranges of insects can vary from extremely broad to very narrow, more than 80% of phytophagous insects specialize on a limited number of host species (Chapman 1982, Price 1983, Bernays 1988). Understanding the genetic factors that are involved in a change in host range is essential for an overall understanding of the evolution of host range. A deeper knowledge of the genetic basis of host range also has important implications for pest management strategies, and may aid in our understanding of mechanisms of speciation (Futuyma and Peterson 1985; Tang et al. 2006). Although there have been a number of studies examining the genetics of host use by hybridizing species or subspecies with different host ranges (e.g. Huettel and Bush 1972; Hanson 1976, van Drogelen and van Loon 1980; Scriber 1983, Tauber and Tauber 1987, Thompson 1988), relatively few studies have crossed species with extreme differences in host ranges to elucidate the genetic basis of larval feeding behavior.

The closely related species Heliothis subflexa and Heliothis virescens are excellent models for studying how much of a genetic change is necessary for a change in host range. H. subflexa (hereafter referred to as Hs) is a monophagous insect, feeding only on plants in the genus Physalis (Solanaceae) (Laster et al., 1982), while H. virescens, (hereafter referred to as Hv) is oligophagous and can feed on plants from 14 different families (Sheck and Gould, 1993). Phylogenetic studies have shown that these two species recently diverged from a common polyphagous ancestral species (Mitter et al. 1993, Fang et al. 1997). However, despite the drastic difference in host ranges of these two species, they can be hybridized in the laboratory (Laster 1982, Sheck and Gould 1993).

The ability to hybridize these two species and the distinct differences in their host ranges provide a unique opportunity to examine the genetic basis of differences in host use between generalists and specialists. Sheck and Gould (1993) crossed Hs and Hv to produce F₁
hybrids and a backcross to Hs. Hybrid and backcrossed larvae were assessed for larval performance and mortality on soybean, tobacco, cotton (hosts of Hv, the generalist), and Physalis pubescens (a host of Hs, the specialist) relative to parental performance. Genes from Hs were found to be overdominant for larval survival and dominant for weight gain on P. pubescens, although epistatic or gene-environment interactions were also likely involved. Genes for Hv were partially dominant for survival and weight gain on cotton and tobacco, and additive for these traits on soybean (Sheck and Gould 1993). In addition, weight gain and survival were concluded to be controlled by autosomal, and not sex-linked, genes. Later experiments sought to determine if loci from Hv conferring utilization of soybean also controlled utilization of other hosts (Sheck and Gould 1996). This was accomplished by moving these loci from Hv into the genetic background of Hs. Although performance on soybean and cotton was partially correlated in individuals with this genetic makeup, larvae did not perform well on cotton or tobacco, and it was concluded that performance on these plants had an independent genetic basis (Sheck and Gould 1996). This finding is important, because it suggests that a single locus is not responsible for the extreme differences in host range between the specialist Hs and the generalist Hv. Tang et al. (2006) also investigated the genetic basis of host use using the generalist Helicoverpa armigera and the closely related specialist H. assulta. They too found that feeding preference was controlled by autosomal genes; however, there was strong evidence for the presence of at least one major locus affecting feeding preference (Tang et al. 2006). Clearly, more studies examining the genetics of larval feeding behavior and host preference are necessary for a more complete understanding of the genetics of host use.

In the current study, the behavior and survival of backcross generation neonates in both the Hs and Hv direction was examined. Although Sheck and Gould (1993) tested growth and survival of hybrid neonate larvae on Physalis and tobacco, they did not study feeding behavior or examine larvae backcrossed to Hv. Using choice and no choice tests for survival on and preference for Physalis angulata and tobacco, host plants of Hs and Hv, respectively,
we were able to draw important conclusions on the inheritance of larval feeding preference and survivorship.

**Materials and Methods**

*Insects and backcrossing procedure*

All Hs and Hv neonates used in the feeding experiments were from laboratory colonies. The Hs colony originated from larvae collected from *Physalis angulata* fruits in Florence and Barnwell Counties, SC, USA, in 1996 (Sheck et al. 2006). The Hv laboratory colony (YDK strain) was established in 1988 using eggs from flowering tobacco in Yadkin County, NC (Gould et al. 1995). Both colonies have been maintained continuously in the laboratory using the methods outlined by Sheck and Gould (1993).

All three of the backcross families in this study originated from a single Hv female (7V-X) that was mated to a single Hs male (Fig 1). Since host use genes in this system had not previously been found to be sex-linked (Sheck and Gould 1993) we did not perform reciprocal crosses. We used an Hv female and an Hs male because crosses in this direction are usually more fertile. Six of the hybrid daughters from this cross were backcrossed to Hs males in single-pair matings to generate backcross individuals in the Hs direction (hereafter referred to as BC₁S). Hybrid females were used in these crosses because F₁ generation males are typically sterile. The most fecund backcross family (produced by female 7V-X-D) was chosen for experimental trials 1 and 2. Similarly, seven hybrid daughters from the original cross were each mated with one Hv male in single-pair matings (hereafter referred to as BC₁V), and the two most fertile families (7V-X-I, 7V-X-M) were chosen for experimental trials 3-6 (Fig 1).
Plants

*P. angulata* seeds were from a lineage of *Physalis* plants originally collected from wild populations in Orangeburg County, SC, USA (33°33'N, 81°04'W) in 1998. The seeds were sown in a greenhouse in flats containing 5-6 cm$^3$ of Metro Mix soil (Sungro Horticulture, Vancouver BC, Canada) and approximately 100 g of Osmocote slow release fertilizer (10-10-10) (Scotts-Sierra Horticultural Products, Marysville, Ohio). Seedlings were singly transferred to 0.5-l plastic pots three weeks after germination, and were fertilized with one teaspoon of fertilizer. Ten-week-old plants were transplanted into 7.5-l plastic pots and fertilized every four weeks. Tobacco seeds (NC 71) were sown in a similar manner; three-week-old seedlings were transferred into small Styrofoam cups containing soil and fertilizer, and ten-week old plants were transplanted and fertilized as described above. Greenhouse temperature was kept at approximately 30/25°C day/night; there was a mild thrips infestation in the greenhouse during the time when plants were grown. Plants were not treated with any pesticides, herbicides, or other chemicals besides fertilizer.

Preliminary experiments showed that plant quality (as judged by percent leaf area eaten by Hs and Hv from their respective host plants) differed markedly between plants, and even between different leaves on the same plant. In order to avoid variation in neonate leaf consumption due to differing levels of plant quality, tests were performed to assess preference of Hs and Hv for leaves from different age classes and of their host plants, as well as different leaf positions for tobacco. Hs was also tested on what appear to be two different varieties of *P. angulata* (purple veined and green veined). Results showed that Hs preferred the purple-veined *P. angulata* plants, and Hv preferred leaves closer to the apical tip of budding tobacco plants (data not shown); all leaves used in experiments were of this nature.

**Feeding experiments**

Each experimental trial consisted of a choice and no choice experiment. Neonates used were newly hatched and no more than 12 hours old. For choice experiments, backcross neonates
were each placed using a small paintbrush in a sealable petri dish (50 mm diameter, BD Falcon) containing moistened filter paper and one leaf disc (10 mm diameter) each of Physalis and tobacco cut immediately prior to experiments using a cork borer. Leaf discs were placed at opposite ends of the petri dish, and larvae were placed in the middle of the two discs. For no choice experiments, each neonate was placed on the edge of the filter paper in a petri dish containing only a Physalis leaf disc. This was repeated for tobacco leaf discs. Fifteen neonates each of Hs and of Hv were used as controls for each choice experiment, and ten Hs and Hv neonates per plant species were used as controls in the no choice experiments. To decrease variation in larval feeding due to differences in leaf quality between leaves, as few leaves as possible were used in each experiment; two to four tobacco leaves and 20-30 Physalis leaves (which are much smaller in size) were used for each experimental trial. All petri dishes were placed on trays in a room kept at approximately 25°C. Preliminary experiments showed that there was substantial variation between tobacco leaves in feeding preference; thus, all tobacco leaves used within an experiment were also given to the Hv and Hs controls in equal proportions. For example, if three tobacco leaves were used in a choice experimental trial, five leaf discs of each of these three leaves would be given to the 15 Hv and to 15 Hs larvae. It was not possible to test Hs and Hv controls against all Physalis leaves used since the number of Physalis leaves used was much higher.

For both choice and no choice experiments each petri dish was examined under a dissecting microscope 1, 3, 6, 12, 24, 48, 72, and 96 hours after the start of the experiment, to determine if neonates were on a leaf disc, eating a leaf disc (as indicated by a green stomach), or not occupying a leaf disc. Leaf discs were then removed with forceps and sandwiched between glass discs for analysis of percent leaf area eaten. Leaf discs were scanned, and measured with a computer image analysis system (CIAS Inc., Seattle, WA) to determine the percentage area removed from each leaf disc.

Two experimental trials (trials 1 and 2) were run for backcross larvae in the Hs direction (BC1S) individuals. All individuals used in these trials were from the same family (i.e., all
larvae used had the same parents) (Fig 1). Four experimental trials (trials 3-6) were run for the backcross larvae in the Hv direction (BC₁V); trials 3 and 4 were run with individuals from the 7V-X-I family, and trials 5 and 6 were run with individuals from the 7V-X-M family (Fig 1). Each experimental trial began on a new day, and consisted of 60 individuals for choice experiments, and 80 individuals for no choice experiments (40 each, for tobacco and Physalis), with these exceptions: 50 neonates were used in choice experimental trial 1, 40 neonates were used in no choice experimental trial 2 (20 each on Physalis and tobacco), and 49 neonates were used in choice experiment 3, due to lack of hatched neonates on the morning of the experiment. A total of 110 and 120 BC₁S neonates, and a total of 229 and 320 BC₁V neonates were used in choice and no choice experiments, respectively.

Data analysis

For each choice and no choice experiment within an experimental trial, the number of BC₁ individuals on a plant at a given time point was summed. The same was done for the Hs and Hv controls. For all choice experiments, the average percent area of Physalis leaf discs consumed by BC₁ individuals was calculated, as was the average percent area of tobacco leaf discs consumed. These averages were also calculated for the Hs and Hv controls. For no choice experiments, averages were calculated in the same way for BC₁, Hs, and Hv individuals. Differences between the percent of Physalis and tobacco leaf area eaten were analyzed with t-tests.

Although we used leaves of generally homogeneous quality for the experiments, and as few leaves as possible to decrease variation in feeding preference due to leaf quality, there was still a considerable tobacco leaf effect in certain experimental trials. If Hv controls discriminated against a particular tobacco leaf relative to other leaves (i.e. when all larvae given leaf discs from a particular tobacco leaf ate only or mostly Physalis in choice experiments, or ignored a particular tobacco leaf in a no choice experiment), all larvae on the unsuitable leaf were eliminated from the analysis. Thus, 41 BC₁V and 5 Hv larvae in the choice experiments were removed from analyses, as were 27 BC₁V, 4 Hs and 8 Hv larvae in
the no choice experiments, in experimental trials 3, 4 and 5. There did not appear to be any unfavorable tobacco leaves in the other experimental trials.

**RESULTS**

*Backcross in the Hs direction*

*Choice experiments*

*Controls.*—Hs individuals preferred *Physalis* leaf discs and ate very little or none of the tobacco leaf discs in both trials (Fig 2 a,b), and after 24 hours the majority of larvae were occupying *Physalis* discs (Fig 2 e,f); however, percent mortality by the end of the experiment for Hs was high in trial 2 (Fig 2 f). In experimental trial 1, Hv larvae preferred tobacco leaf discs when given a choice between tobacco and *Physalis* (Fig 2 a). Nearly all Hv individuals in this trial were present on tobacco leaf discs for each time point, and mortality was 0% in this trial (Fig 2 g). However, there was no significant difference between the percent leaf area of tobacco and *Physalis* consumed in experimental trial 2 (t = 1.05, d.f. = 28, p = 0.303) (Fig 2 b). Larvae initially occupied tobacco leaves in this trial, but gradually moved towards *Physalis* leaf discs 48 hours after the start of the experiment (Fig 2 h).

*BC1S.*—The feeding behavior of BC1S larvae closely resembled that of Hs individuals (Fig 2 a,b). In choice experiments, none of the BC1S larvae fed on tobacco leaf discs in experimental trial 1 (Fig 2 a), and only two individuals fed on tobacco leaves in trial 2 (consuming an average of 0.16% of the total leaf area) (Fig 2 b). After the first 24 hours of the choice experiments, the majority of BC1S larvae were on *Physalis* leaf discs in both trials, and percent mortality was low (Fig 2 c,d; Table 1).
For Hs and BC$_1$S, there was very little variation between larvae in preference for *Physalis* and tobacco; most larvae only ate *Physalis* (Fig 3 a,c).

*No choice experiments*

*Controls.*—Hs larvae given *Physalis* in no choice experiments ate amounts of leaf area comparable to those in the choice experiments (Fig 4 a,b), and the majority of larvae were occupying *Physalis* leaf discs after 24 hours (data not shown). However, Hs larvae given only tobacco leaf discs consumed very little or 0% of the leaf area in both trials (Fig 4 a,b). Most larvae were not present on tobacco leaves throughout the experiment (data not shown), and mortality rates were very high (Table 1). Hv individuals in no choice experiments consumed the species of leaf disc they were given, and there was no significant difference in the average percent leaf area of tobacco and *Physalis* eaten for either experimental trial (trial 1: $t = 1.40$, d.f. = 18, $p = 0.178$; trial 2: $t = 0.992$, d.f. = 18, $p = 0.334$) (Fig 4 a,b). Hv larvae given tobacco were present on these leaf discs throughout the experiment (data not shown), and had low mortality (Table 1). Mortality was higher for Hv given only *Physalis* leaf discs (Table 1), although larvae typically occupied the discs after 24 hours and throughout the rest of the experiment (data not shown).

*BC$_1$S.*—In the no choice experiments, feeding was very low for BC$_1$S individuals given only tobacco leaves (1.5% and 0.3% leaf disc area consumed in experimental trial 1 and 2, respectively) (Fig. 4 a,b). The majority of the larvae were not occupying the leaves at any given time point, and percent mortality for these larvae was high (Fig. 4 c,d; Table 1). For larvae given *Physalis* leaf discs in no choice experiments, larvae ate a significant portion of the leaf discs (Fig 4 a,b), and most of the larvae were found on the leaf discs approximately 24 hours after the start of the experiment (Fig 4 e,f). Mortality was much lower for these individuals compared to larvae given tobacco in no choice experiments (Table 1).
Backcross in the Hv direction

Choice experiments

Controls.—Hv larvae consumed a higher percentage of tobacco than Physalis in all trials of the choice experiments, but percent leaf area consumed varied greatly across trials (Fig 5 a-d). The majority of larvae were occupying tobacco leaves over the 96 hours of each trial; however, percent mortality was variable among trials, ranging from 0-60% (Fig 5 m-p). Hs larvae behaved as they did in the BC₁S experiments, preferring to eat Physalis leaf discs over tobacco (Fig 5 a-d), and gradually occupying Physalis leaf discs over the course of the experiments (Fig 6 i-l). Mortality was also variable for Hs larvae, ranging from 0-47% throughout the four trials.

BC₁V.—Unlike the feeding behavior of BC₁S larvae, the behavior of BC₁V larvae did not resemble that of the parent to which they were the most genetically similar (Hv). In choice experiments, BC₁V larvae consumed more tobacco in experimental trial 3 (Fig 5 a), almost identical amounts of tobacco and Physalis in trial 4 (Fig 5 b), and more Physalis than tobacco in trials 5 (t = 2.37, d.f. = 38, p = 0.023) and 6 (t = 3.60, d.f. = 59, p = <0.0001) (Fig 5 c,d). Although most of the BC₁V larvae occupied tobacco leaf discs during the first several time points during the experiments in all four trials (Fig 5 e-h), they remained on tobacco leaf discs in trials 3 and 4 (Fig 5 e,f), but gradually occupied Physalis leaf discs instead of tobacco in trials 5 and 6 (Fig 5 g,h).

Unlike BC₁S and Hs, BC₁V showed some variation in feeding behavior. In general, larvae fell into two categories: those that consumed mostly Physalis and those that consumed mostly tobacco when given a choice (Fig 3 d). Hv larvae showed some variation, with most preferring tobacco, but some consuming intermediate amounts of Physalis or only Physalis (Fig 3 b).
No choice experiments

Controls.—Hv larvae in no choice experiments consumed a higher percentage of tobacco leaf disc area in experimental trial 3 (Fig 6 a), similar amounts of tobacco and Physalis in trials 4 (t = 1.75, d.f. = 17, p = 0.050) (Fig 6 b) and 6 (Fig 6 d), and a higher percentage of Physalis leaf disc area in trial 5 (Fig 6 c). Most Hv larvae given only tobacco occupied these leaf discs throughout the experiment in trials 3 and 6, but gradually left tobacco leaf discs and had higher mortality rates in trials 4 and 5 (data not shown). Mortality rates for Hv larvae given only Physalis leaf discs was also higher for trials 4 and 5 (data not shown). As in the trials with the BC₁S larvae, Hs individuals consumed very little tobacco in no choice experiments (Fig 6 a-d), and gradually occupied Physalis leaf discs throughout the experiment for larvae given Physalis leaf discs, while those given tobacco spent little time on these leaf discs and had high mortality rates (data not shown).

BC₁V.—In the no choice experiments, BC₁V larvae consumed the same amount of Physalis and tobacco in trial 4 (Fig 6 b), and significantly more Physalis leaf disc area than tobacco leaf disc area in all other trials (Fig 5 a,c,d). For larvae given only tobacco, most remained on these leaf discs throughout the experiment in trials 3 and 4 (Fig 6 e,f), but larvae in trials 5 and 6 gradually abandoned leaf discs after the 24-hour time point (Fig 6 g,h). For individuals given only Physalis leaf discs, the number of larvae occupying leaf discs gradually increased until the 12 and 24-hour time points, and then either remained steady or gradually decreased throughout the experiment (Fig 6 i-l). Mortality for BC₁V larvae ranged from 10-72% on the two plant species in the different trials, but in general mortality was higher regardless of the assigned plant species in trials 9 and 10 (Fig 6).

Mortality

Larval mortality was variable between trials, but general patterns existed. Hs and BC₁S larvae had much lower mortality in choice experiments, and in the no choice experiments with Physalis leaf discs. A similar trend was observed with Hv: lower mortality in choice
experiments and no choice experiments with tobacco leaf discs. However, BC$_1$V larvae had similar mortality values across all types of experiments.

DISCUSSION

Studies that utilize crosses between two closely related insects with extreme differences in host ranges may prove to be the most useful in elucidating the genetic architecture of host range. In this study, we report on the differences in feeding preference and survival of reciprocal backrosses of the specialist Hs and the generalist Hv on host plants of these two species, a necessary step for more detailed genetic analyses that aim at understanding what genetic factors contribute to the differences in host range for Hs and Hv.

Our results show that the BC$_1$S larvae (progeny of hybrids backcrossed to the specialist, Hs) exhibit feeding behavior very similar to Hs. These larvae consumed Physalis leaf discs (host of Hs) when given a choice between Physalis and tobacco (host of Hv), spent most of their time occupying these leaf discs in favor of tobacco discs, and had much higher survival on Physalis (Fig 2, 3) (Table 1). When given only tobacco in no choice experiments, larvae avoided leaf discs and ate very little, if any, plant material, resulting in high mortality rates. These results are congruent with the studies that have shown that genes from Hs are overdominant for survival and dominant for weight gain on Physalis (Sheck and Gould 1993). In contrast, BC$_1$V larvae (progeny of hybrids backcrossed to the generalist, Hv) did not closely resemble Hv in feeding behavior. These larvae showed variation in feeding behavior (Fig 3d), with some individuals preferring tobacco and some preferring Physalis when given a choice. This bimodal distribution that existed for BC$_1$V larvae for feeding preference (Fig 3d) is not what would be expected if feeding preference for tobacco and Physalis were controlled by many loci. These results contrast with those found by Sheck and Gould (1996), who found that there was not one major locus that controlled feeding on
Physalis, tobacco, soybean and cotton. Although more thorough genetic studies are needed to draw conclusions on the inheritance of genes for feeding and survival on tobacco and Physalis, our results suggest that there is dominance for feeding on Physalis in choice tests, and that there is at least one major locus involved in feeding preference on Physalis and tobacco.

BC$_1$S and Hs larvae showed little capacity for surviving on tobacco leaves. Mortality was 81% and 83% for BC$_1$S and Hs individuals, respectively, in no choice tests with only tobacco, and most larvae that survived throughout the 96-hour experiments died later on artificial diet. Scheck and Gould (1993) found similar patterns in survival for BC$_1$S larvae, with an 80% mortality rate for BC$_1$S larvae given only tobacco plants. During observations of BC$_1$S larvae, very few showed any sign of ‘testing’ the plant material, as would have been indicated by green digestive tracts. Other studies have shown that Hs is more likely to reject at first encounter food containing chemical deterrents, compared to Hv (Bernays et al., 2000). It is possible that chemoreceptors on the mouthparts or antennae of Hs allowed detection of deterrent compounds in tobacco leaves without ingestion. Interestingly, although Hs and BC$_1$S larvae had higher percent occupation on Physalis leaf discs in choice experiments, this only occurred after approximately 24 hours of exposure to leaf discs. Sheck and Gould (1996) also report this behavior for Hs larvae. It appears that a substantial amount of time is needed for larvae to accept Physalis as a host plant and begin consumption. This could be due to the fact that Hs are primarily frugivores. Although they can survive to adulthood on leaves of Physalis angulata, Hs always consume fruits over leaves if they are available (Petzold, pers. obs.). Hs larvae ate a smaller percentage of leaf area compared to BC$_1$S larvae; in general, BC$_1$S larvae appeared healthier and more active than Hs larvae. Prior work with our Hs and Hv laboratory strains has shown that the Hv strain is in general more vigorous than the Hs strain in many respects, including lower levels of mortality and higher fecundity. Also, Hv are primarily leaf feeders, while Hs are primarily fruit feeders. Because BC$_1$S contains approximately 25% of its genetic material from Hv, higher levels of
feeding compared to Hs may be due to the contribution of these factors by the presence of Hv chromosomes.

Patterns in feeding behavior for BC1V larvae were much less consistent than those observed for Hs and BC1S larvae. The variable behavior of BC1V larvae can be partially attributed to variation in tobacco leaves, as was indicated by very low levels of feeding by Hv and BC1V on leaf discs from the same leaf for several different leaves. A number of studies have also shown that substantial variation can exist between different leaves of the same plant (Denno and McClure 1983, Watson and Casper, 1984, Bowers and Stamp 1992, Winn 1996). Although these leaves were eliminated from the analysis, we cannot be sure that other leaves included in the analysis were of equal nutritional value or attractiveness to larvae. Variation in leaf quality could explain why in some trials BC1V larvae consumed more Physalis or ate similar amounts of Physalis and tobacco. However, our experimental design ensured that Hv larvae were exposed to the same leaves as backcross larvae, and Hv larvae still consumed more tobacco in all choice trials, and similar or greater amounts of tobacco in all but one of the no choice trials. BC1V larvae did not show the same preference for tobacco as Hv, and the high levels of variation between individuals within trials and between trials cannot be attributed to variation in leaf quality and is most likely due to the genetic make up of BC1V larvae. Unlike BC1S larvae, the BC1V larvae used came from two different families (Fig 1). Although more data would be needed to determine if there were clear differences between the two families, it appears that neonates from the 7V-X-M family (used in trials 5 and 6) had a higher preference for Physalis than tobacco overall, as indicated by higher levels of percent leaf area eaten (Fig 5 c,d) and more larvae on Physalis leaf discs by the end of the experiment in choice experiments (Fig 5 g,h). This could be due to difference in the genetic makeup of the two hybrid mothers used in the experiments, or the two Hv males to which they were mated.

In a study similar to ours, Tang et al. (2005) examined the feeding preference of progeny from interspecific crosses (F1, F2 and backcrosses) of a specialist (Helicoverpa assulta) and a
generalist (*H. armigera*). Feeding preferences of hybrids resembled those of the generalist. Backcrosses to the generalist exhibited feeding behavior nearly identical to the generalist, while backcrosses to the specialist had more variable feeding behavior and were grouped into two classes: those that fed primarily on pepper, the specialist’s host plant, and those that fed primarily on cotton, a host plant of the generalist. The findings in this study suggested that larval feeding preference was determined by autosomal genes, *H. armigera*-derived alleles were partially dominant to *H. assulta* alleles, and that at least one major autosomal gene was involved in larval feeding on cotton (Tang *et al.* 2006). In several respects, results from this study reflect patterns seen in our system. Survival for hybrids of Hs and Hv is inherited as an autosomal trait, although hybrids can survive on both *Physalis* and tobacco in no choice tests (Sheck and Gould 1993). Backcrosses in our study showed similar patterns to those reported by Tang *et al.* (2006); however, in our study it was the backcrosses to the generalist that showed variation in feeding behavior, and backcrosses to the specialist that consumed only the specialist’s host (*Physalis*). It appears that at least one major locus is involved in host choice, although more work with our system is necessary to confirm this finding.

Because our system involves closely related species that have extreme differences in host range and thus strong phenotypic differences in feeding behavior, it is an ideal system for determining the genetic changes responsible for differences in host range. The information provided by this study is necessary for the design of a more thorough genetic study involving the use of QTL mapping to determine the number of changes that are necessary for a shift in host use. Although we reared insects that were used in this experiment to pupation for a QTL analysis, the small number of larvae that survived yielded insufficient numbers for a thorough QTL analysis. A QTL analysis of feeding behavior of backcross larvae would provide information on whether an accumulation of many small changes or a small number of large genetic changes are necessary for a shift in host use to occur. QTL analyses have been performed using this system as a model for studying the evolution of changes in the multi-component pheromone blends by determining the genetic basis of the blends (Sheck *et al.* 2006). Data provided by this study indicate that BC1V larvae would be the most useful
cross to use for a QTL analysis, since the most variation in feeding behavior exists for these larvae. Also, because variation in leaf quality of tobacco was still a factor in this experiment despite the effort put forth to minimize this variation, it is essential that care be taken to ensure that tobacco leaves used in future experiments are as uniform as possible by growing plants in an environmental chamber with tightly controlled conditions.

Hs and Hv serve as an excellent model system for studying the evolution of host range, because of the ability to hybridize these insects and their extreme difference in host range. Although further genetic studies employing molecular techniques will be necessary to gain a more complete understanding of the genetic factors involved in changes in host range, the data here provide evidence supporting the hypothesis that at least one major locus is involved in host range shifts in our system. More experiments studying the feeding behavior, as well as the oviposition behavior, of insects with different host ranges are necessary for a general understanding of what genetic factors are involved with a change in host range.

ACKNOWLEDGMENTS

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LITERATURE CITED


Table 1: Percent mortality for larvae used in choice and no choice feeding experiments (averaged over each type of experiment). Standard deviation is shown in parentheses. Hs: *Heliothis subflexa*, Hv: *Heliothis virescens*, BC₁S: progeny of F₁ individuals backcrossed to *H. subflexa*, BC₁V: progeny of F₁ individuals backcrossed to *H. virescens*.

<table>
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<td>24.29 (35.80)</td>
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Figure 1: All larvae used in experiments originated from the same set of grandparents: Hv female 7V-X, and an Hs male. The resulting female F₁ offspring were backcrossed to either Hs or Hv males; the offspring from females that were most fecund (7V-D-D, 7V-X-I, and 7V-X-M) were chosen for experiments (experiment number indicated in parentheses under each set of backcross larvae).
Figure 2: Feeding and behavioral data for BC$_1$S and control (Hs, Hv) larvae in choice experiments. The first column of graphs (i.e. a, c, e, g) and the second column (i.e. b, d, f, h) each represent one replicate of the experiment. *First row:* Average percent leaf area eaten from *Physalis* (solid bars) and tobacco (gray bars) leaf discs over a 96-hour period, in (a) trial 1 and (b) trial 2. Error bars show 1 SE of the mean. *Remaining rows:* Number of BC$_1$S (c,d),
Hs (e,f), Hv (g,h) neonates on *Physalis* (black line) and tobacco (gray line) leaf discs in choice experiments over a 96-hour period. Dotted line represents number of larvae that were not on a plant at the respective time point. Percentages listed at the top of the graphs represent percent larval mortality 24, 48, 72, and 96 hours after the start of the experiment.

Figure 3: *Physalis* leaf area consumed as a percent of the total leaf area consumed for each larva in choice experiments: (a) Hs, (b) Hv, (c) BC₁S, and (d) BC₁V. Larvae that did not consume any leaf material were excluded from the analysis.
Figure 4: Feeding and behavioral data for BC₁S larvae and controls (Hs, Hv) in no choice experiments (larvae were either given Physalis or tobacco leaf discs). Each column of graphs represents one replicate of the experiment. First row: Average percent leaf area eaten from Physalis (solid bars) and tobacco (gray bars) leaf discs over a 96-hour period, in (a) trial 1 and (b) trial 2. Error bars show 1 SE of the mean. Remaining rows: Number of BC₁S neonates on (c,d) tobacco (gray line) and (e,f) Physalis (black line) leaf discs over a 96-hour period, for each of the trials. Dotted line represents number of larvae that were not on a plant at the respective time point. Percentages listed at the top of the graphs represent percent larval mortality 24, 48, 72, and 96 hours after the start of the experiment.
Figure 5: Feeding and behavioral data for F$_1$ larvae backcrossed to Hv (BC$_1$V) and controls (Hs, Hv) in choice experiments. Each column of graphs represents one replicate of the experiment. First row: Average percent leaf area eaten from Physalis (solid bars) and tobacco (gray bars) leaf discs over a 96-hour period, in (a) trial 3, (b) trial 4, (c) trial 5 and (d) trial 6. Error bars show 1 SE of the mean. Remaining rows: Number of BC$_1$V (e-h), Hs (i-l), Hv (m-p) neonates on Physalis (black line) and tobacco (gray line) leaf discs in choice experiments over a 96-hour period. Dotted line represents number of larvae that were not on a plant at the respective time point. Percentages listed at the top of the graphs represent percent larval mortality 24, 48, 72, and 96 hours after the start of the experiment.
Figure 6: Feeding and behavioral data for BC1V larvae and controls (Hs, Hv) in no choice experiments (larvae were either given Physalis or tobacco leaf discs). Each column of graphs represents one replicate of the experiment. **First row:** Average percent leaf area eaten from Physalis (solid bars) and tobacco (gray bars) leaf discs over a 96-hour period, in (a) trial 3, (b) trial 4, (c) trial 5 and (d) trial 6. Error bars show 1 SE of the mean. **Remaining rows:** Number of BC1V neonates on (e-h) tobacco (gray line) and (i-l) Physalis (black line) leaf discs over a 96-hour period, for each of the trials. Dotted line represents number of larvae that were not on a plant at the respective time point. Percentages listed at the top of the graphs represent percent larval mortality 24, 48, 72, and 96 hours after the start of the experiment.
THE GENETICS OF OVIPOSITION BEHAVIOR IN A SPECIALIST AND GENERALIST HERBIVORE

JENNIFER PETZOLD\textsuperscript{1}, STEPHANIE GORSKI\textsuperscript{2}, FRED GOULD\textsuperscript{3}

\textsuperscript{1}Department of Plant Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

\textsuperscript{2}Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA

\textsuperscript{3}Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina 27695-7613 USA
ABSTRACT

There is little information on how many genes control differences in oviposition behavior between species and races, or on the mode of inheritance of this behavior. The heliothine moths *Heliothis subflexa* (Hs) and *H. virescens* (Hv) serve as an attractive system for studying the genetic architecture of host range because Hs is a specialist on plants in the genus *Physalis*, while the closely related Hv is a broad generalist. Hs and Hv were hybridized, and F₁S were mated to each parent species to produce backcrosses. A large outdoor cage in a field setting was used to study the oviposition behavior of Hs, Hv, hybrids, and offspring resulting from backcrosses to Hs (BC₁S) and Hv (BC₁V). Females were given a choice of *Physalis angulata* (a host of Hs) and tobacco (a host of Hv). Hs and BC₁S had almost identical oviposition behavior, laying most eggs on *Physalis angulata* and spending the majority of both flying and non-flying time on this plant. The oviposition behavior of Hv and BC₁V was also very similar, with the majority of eggs laid on tobacco and the most time spent on and around tobacco plants. The F₁ hybrids had oviposition behavior that was near identical to Hv and BC₁V. Specific behaviors observed during oviposition in this field setting, as well as modes of inheritance of genes controlling oviposition behavior, are discussed.
INTRODUCTION

A major pattern that has emerged from studies of insect host-plant associations is that the vast majority of phytophagous insects are specialists, feeding on a small number of species that are often closely related taxonomically (Janz et al. 2001, and references therein). Why so many insects have such narrow host ranges, and what genetic factors control shifts in host range, are not well understood.

Because the neonate larvae of many phytophagous insects are small and largely immobile, female oviposition behavior is one of the most important components of evolutionary modification of host range. However, detailed information on the genetic architecture of host use and oviposition behavior is scarce (Fox et al. 2004). For studies that have used line crosses to examine the genetics of species or population differences in oviposition behavior, there are varying results regarding the number of genes controlling host use, the degree to which dominance and epistasis are involved, and whether genes are additive or non-additive. A number of studies have detected some dominance and epistasis of genes controlling oviposition behavior (Guldemond 1990, Lu and Logan 1995, Sheck and Gould 1995, Craig et al. 2001), while other studies have shown evidence for an additive genetic basis governing oviposition preference (Sezer and Butlin 1998). Fox et al. (2004) showed that differences in oviposition preference of different populations of the seed beetle Callosobruchus maculates were explained by complete additivity, however dominance and epistasis were responsible for differences in egg dispersion. In some systems, oviposition genes are thought to be sex-linked (Thompson 1988, Scriber et al. 1991, Janz 1998), whereas other systems show no evidence of sex-linkage (Nylin et al. 2005, Hora et al. 2005). Finally, the number of genes controlling oviposition behavior is also variable, with some systems showing monogenic control (Guldermond 1990, Lu and Logan 1995, Sezer and Butlin 1998), and others showing polygenic control (Sheck and Gould 1995).
It is surprising that studies on the genetics of host use are not more abundant, given its importance in our understanding of significant evolutionary questions such as ecological specialization, sympatric speciation, and host range formation. A significant obstacle in studying the genetic architecture of host range is lack of appropriate systems (Hora et al. 2005). Systems that enable crosses between two species with broad differences in host range and that have recently diverged are the most favorable systems for studying the genetics of host use (Hora et al. 2005).

*Heliothis subflexa* (hereafter referred to as Hs) and *H. virescens* (hereafter referred to as Hv) serve as an excellent model system for studying the genetics of host range. Hs is an extreme specialist, feeding only on plants in the genus *Physalis* (Laster, Pair and Martin 1982), while Hv is a broad generalist, feeding on at least 37 species of plants in 14 plant families (Sheck and Gould 1993). Phylogenetic analyses suggest that although they are not sister species these two noctuid moths have evolved quite recently from a shared, generalist ancestor (Fang et al. 1997, Mitter et al. 1993, Poole et al. 1993), and, despite the vast difference in host range between these two species, they can be hybridized in the laboratory. Thus, the Hs/Hv pair should be an exceptional tool for studying the genetic factors involved in host use.

Sheck and Gould (1995) examined oviposition preference of Hs, Hv, and reciprocal F₁ hybrids in oviposition cages in a greenhouse. Four hosts were supplied as oviposition substrates: cotton, tobacco, soybean (hosts of Hv), and *Physalis angulata* (host of Hs), and these hosts were ranked for preference. Hs females preferred *Physalis*, while Hv and both reciprocal hybrids preferred tobacco. There was no evidence of sex-linkage, since both reciprocal hybrids had similar patterns of preference across hosts (Sheck and Gould 1995). Feeding studies using this system have also shown that host utilization traits are not sex-linked (Sheck and Gould 1993).

In this study, we provide a detailed behavioral analysis of oviposition preference of Hs and Hv in a field setting within large cages. We also examine the behavior of F₁ hybrids, and offspring from backcrosses to each of the parent species. By observing oviposition behavior
of each of these five strains on host plants of the two parents species in a large outdoor cage, we were able determine host preference for each of these strains and draw conclusions about modes of inheritance of host preference genes in this system.

**MATERIALS AND METHODS**

**Plants**

Plants used in this study were grown in NCSU’s phytotron, a facility that houses controlled-environment chambers. Seeds of tobacco and *Physalis angulata* were started in flats containing potting soil, and were subsequently transplanted singly to small Styrofoam cups one to two weeks after germination. All plants were grown in a chamber kept at 26/22°C day/night, with lights on from 8:00 – 17:00 (fluorescent and incandescent lighting) and a night interruption from 23:00 – 2:00 (incandescent lighting). Light intensity ranged from 550 – 600 μmoles/m²/sec, and humidity ranged from 40 – 65%. Plants were watered twice a day with a nutrient solution (see Saravitz *et al.* 2008 for nutrient composition). Two weeks later, plants were transplanted into 5-l plastic pots. Three and five weeks later, respectively, *Physalis* and tobacco plants were moved to an outside field plot to acclimate to field conditions. Here, plants were transplanted to 7.5-l plastic pots and watered daily. Plants remained outside for 1-3 weeks, and were then placed in the outdoor field cage, where they remained for one week. *Physalis* plants used in the experiments had mature fruit, and tobacco plants were in the budding/flowering stage. Seeds of both species were sown every week, so that a continuous supply of plants was available throughout the field season.

**Insects and crossing procedures**

*Control moths.*—Observations occurred over two field seasons in 2007 and 2008. Hs and Hv released into the field for observation were taken directly from laboratory colony stocks in
2007. The Hs colony originated from larvae collected from *Physalis angulata* fruits in Florence and Barnwell Counties, SC, USA, in 1996 (Sheck *et al.* 2006). The Hv laboratory colony (YDK strain) was established in 1988 using eggs from flowering tobacco in Yadkin County, NC (Gould *et al.*, 1995). Both colonies have been maintained continuously in the laboratory using the methods outlined by Sheck & Gould (1993). Briefly, moths of each species are mated in groups of 20-30 in large buckets, with cheesecloth used as an oviposition substrate, and neonates are reared on a corn/soymeal diet (Burton 1970). On observation nights, 4 females each of Hs and Hv were taken from mating buckets containing females that were laying eggs, and used for observation in the cage. In 2008, Hs and Hv used in the observation cage were taken as pupae from colony stocks every week. Single pair matings were set up upon emergence of moths; fertile females (determined as described below) were chosen for observation in the cage. Five to 8 females of each species were released in the cage each observation night. In both 2007 and 2008, females’ wings were marked with Sharpie markers for identification in the field cage as described below, and they were used during consecutive observation nights until they died or were lost in the cage.

*Overview of crossing procedure.*—All Hv and Hs insects used in the experiments were from established laboratory colony lines (described above). Each year, an Hv female was crossed with an Hs male to produce hybrids. Because host preference genes in this system had not previously been found to be sex-linked (Sheck and Gould 1993) reciprocal crosses in which the initial female was Hs were not conducted. F$_1$ females from this cross were mated singly with Hs males to produce backcross families in the Hs direction (hereafter referred to as BC$_1$S), and with Hv males to produce backcross families in the Hv direction (BC$_1$V). Hybrid females were used in these crosses because F$_1$ generation males are typically sterile. For each year, all backcrossed moths originated from the same two grandparents. Additional crosses were not set up because the original intention for this study was to conduct a quantitative trait locus (QTL) analysis, for which offspring originating from one set of grandparents is ideal for data analysis. However, insufficient numbers of moths provided behavioral data, so a QTL analysis was not possible.
On 6 April 2007, 10 Hv females were mated to 10 Hs males. All matings were set up with one female and one male in a small plastic containers containing sugar water (5% sucrose solution) and covered with cheesecloth. Containers were placed in a greenhouse kept at approximately 30/25°C day/night. Each pair was kept in the mating container for production of eggs until one of the moths died; eggs were collected daily. After four to five days, eggs began to hatch and the most fertile mating was chosen as the F_1 family to be used for observation in the field cage and to produce backcrosses. Neonates from this family were placed singly in individual cups containing a corn/soymeal artificial diet (Burton 1970); and were removed after pupation occurred. Male pupae were discarded and female pupae were placed in plastic containers covered with cheesecloth (18-25 per container). Some containers were placed in a cold room held at 15°C to delay emergence. Upon emergence, females kept at room temperature were prepared for release into the field observation cage (described below), with the exception of females that were mated with Hs and Hv to produce the backcross families. Thirty-three F_1 females were singly mated to Hs males as described above, and the 10 most fertile matings were chosen for backcrosses in this study. Similarly, 44 F_1 females were singly mated to Hv males and the 9 most fertile matings were selected. Matings occurred over a period of two months, using F_1 females that emerged from pupae kept at room temperature in the first two weeks, and gradually removing F_1 pupae from the cold room for later matings. The resulting backcross neonates were set on diet and reared to pupation, and female pupae were placed in plastic containers as described above.

In order to extend the emergence of BC_1 females over the summer months so that they could be observed in the outdoor cage for as long a period as possible, the plastic cups containing the pupae were placed in the cold room (kept at 15°C) to initiate diapause. Cups were removed gradually throughout the summer months, thus allowing for large numbers of progeny with the same parents to be observed over the course of the field season. No differences were observed between behavior of females kept in the cold conditions as pupae and those that never experienced cold conditions.
On 9 May 2008, Hv females and Hs males were mated in single pairs as described above to produce F$_1$ offspring. Eighteen and 10 of the female F$_1$s from the most fertile cross were mated with Hs and Hv males, respectively, to produce backcross families as described above. Larvae from 5 families (3 BC$_1$S and 2 BC$_1$V) were produced for use in experiments. Larvae were reared as described above.

*Moth preparation for observations*

After removal from the cold room of plastic cups containing female pupae, the cups were placed on a lab bench in a room that ranged from approximately 24-26°C, and females emerged within 1-2 weeks. Cups were checked for emergent females daily, and each adult female was mated singly with one male. Male Hs and Hv moths used for matings were taken as pupae directly from our laboratory colonies, and placed on the same lab bench as BC$_1$ pupae. Since we were not interested in the offspring produced from these matings but only interested in achieving mating so that we could observe oviposition behavior of females, the male species to which females were mated was not a major concern. However, since spermataphore size other factors may likely be different between Hs and Hv males, we mated BC$_1$S females with Hs males, and BC$_1$V females with Hv males. This was the case unless there was a day on which many (>15) females emerged, and not enough males of the respective species were available for mating. For example, if many BC$_1$S females emerged on one day, each was mated to an Hs male until depletion of Hs males, and the remaining were mated with Hv males; this was not common.

The pairs of moths were allowed to mate in plastic containers covered with cheesecloth and containing sugar water as described above, and placed in the greenhouse. Moths were removed from the greenhouse after 2-3 nights, and males were dissected to determine if mating had occurred (Groot et al. 2006), since females can lay infertile eggs. If mating did not occur, females were mated with new males if they were available, and checked again for mating 2 days later. Mated females were gently removed from their container, and marked with dots or numbers on their wings using Sharpie pens for identification in the outdoor cage.
Females were placed in plastic containers with a supply of sugar water and returned to the greenhouse until release into the large outdoor cage that night.

Observations

All observations took place in one of two 7.6 x 15.2 x 2 m outdoor cages. One cage was placed in a tilled field in Wake County on the NC State University campus (Raleigh, NC, U.S.A). In September of 2007, due to very low levels of moth activity in this cage, another cage was placed in a field plot on an NCSU experimental farm (Central Crops Research Station) in Johnston County, NC, U.S.A., where natural populations of Hs and Hv were present. The cages consisted of a mesh screen made of nylon, supported by a frame constructed from PVC pipe. The ground was covered by black shade cloth to deter growth of other plants. Each week, a new group of plants was added to the cage that was being used; there was always an equal number of Physalis and tobacco plants present in the cage. Plants were placed in two rows that ran the length of the cage, with tobacco and Physalis plants interspersed evenly between each other. A total of 14 -18 plants were in the cage at a given time with an equal number of plants in either row. Plants were watered daily.

Because batches of female pupae were removed from the cold room and mated gradually throughout the summer, we were able to release 10-15 females every 1-4 days, ensuring a steady supply of moths for observations. At sunset, moths were released from the small plastic containers into the cages. Observers initially used headlamps with red light to observe moths when conditions were too dark (about 1 hr after sunset); however, because behavior of moths did not differ whether white or red light was used, observers did not use a red filter to observe moths during the last half of 2007 and 2008. Two observers were present each night, with one observer closely following an active moth and recording activity on a digital recorder (Sony ICD-P250), and the other assisting with keeping track of the moth and checking plant parts for eggs to confirm oviposition in cases where moths moved too fast after a supposed oviposition for the primary observer to see an egg. During nights when activity was high, the second observer would also observe moths and report behaviors into a
recorder. As soon as an active moth was sighted, its behaviors were recorded continuously for as long as possible until the moth was inactive or lost. The methods used here are similar to those of Benda (2007) in which Hs moths were observed under completely natural conditions. The following information was recorded:

- The location of the moth during flight. This included four locations: 1) *Physalis* plants, 2) tobacco plants, 3) the wall, and 4) neutral areas. A moth was considered to be flying in the vicinity of a plant when it approached an area within approximately 0.5 m of the plant and hovered in this area for greater than 2 seconds. Moths that were not flying around plants were either flying along the length of the wall of the cage or flying in the space between the plants (neutral areas).

- The location of the moth when it was not in flight. This included the same four locations: 1) *Physalis* plants, 2) tobacco plants, 3) the wall, and 4) neutral areas. Neutral areas consisted of the ground or the observers.

- The number of times a moth curled its abdomen on a plant, the number of times this abdomen curl was followed by an oviposition, and where the abdomen curls and ovipositions took place.

If a moth rested for more than several minutes on a plant without moving, the observation period for the moth was ended if that moth had already been observed for more than four minutes and another moth was active in the cage. Observers collected as many moths as could be found after activity ceased (usually 1.5 h after sunset) or after 45 minutes of no observed behavior. Moths were returned to plastic containers, given fresh sugar water, and placed in the greenhouse for use on the next night of observations. Approximately 80% of the released moths were recovered from the cage at the end of each night. Moth age ranged from 3-8 days, although for moths that were active, most were only active on the first few nights. Thus, most moths were 3-5 days old at time of observation.
Data analysis

All data were transcribed from voice recorders to spreadsheets. For each moth, a single cell in the spreadsheet was designated for each action (i.e. flying in the vicinity of a plant, landing on a plant, ovipositing, resting, etc.), with the exact time in seconds that the action occurred listed in the adjacent cell. Time spent doing each action was calculated. For each moth, the total time spent flying in the four areas (Physalis, tobacco, the wall, or neutral areas) was calculated, and time spent in each of the four areas as a percentage of total flying time was calculated for each moth. Means and standard errors for these percentages were determined for each strain (Hs, Hv, F$_1$, BC$_1$S, BC$_1$V). Similarly, the total time each moth spent during non-flight time was calculated, and time spent in each of the four areas as a percentage of this time was calculated for each moth, and averaged per strain. Also, the total number of eggs laid on the wall, tobacco plants, and Physalis plants was summed for each moth, as were abdomen curls on these areas that did not result in an oviposition.

All statistical analyses were performed with SAS 9.1 (SAS 2008). Average number of eggs laid on Physalis and tobacco per night was calculated for each strain, as was percent of eggs laid on Physalis and tobacco. ANOVAs were used to determine if there were differences between the five strains in 1) average number of eggs laid per night, 2) percent eggs laid on Physalis, and 3) percent eggs laid on tobacco. An ANOVA was used to determine if there were differences between strains in the percent of abdomen curls that resulted in ovipositions on each plant species. Additionally, an ANOVA was used to determine if there were differences between strains in the percent of time moths spent flying. CONTRAST statements were used to test specific hypotheses about differences between the strains for the ANOVAs.

To determine if there were differences in the flying patterns between the five strains, we used a multivariate ANOVA (MANOVA) using PROC GLM with a MANOVA statement, with time spent flying in each of the four areas (as a percentage of total flying time) as the dependent variables and strain as the independent variable. Similarly, a MANOVA was used
to determine if there were differences between strains in the time spent in each of the four areas (as a percentage of total non-flying time). CONTRAST statements were used to test for hypothesized differences between particular strains.

RESULTS

A total of 17.2 hours of recorded behavioral observations were collected from Hs, Hv, F₁, and backcross moths in 2007 and 2008. In 2007, although over 700 backcross and F₁ moths were released into the tent, observational data were only collected from 51 of these moths (Table 1). All other moths either remained motionless at the time of release, or flew very briefly along the walls of the cage, where they remained until they were collected at the end of the night. Although control moths (Hs, Hv) were more active in 2008 compared to 2007, most BC₁ moths were inactive. Of the 136 BC₁ moths released in 2008, only 8 of these moths were active. Even fewer F₁ moths showed any type of behavior; behavioral data were only collected from 7 F₁ moths. Thus, conclusions regarding F₁S should be considered preliminary.

Moths laid an average of 8-15 eggs per night (Fig 1). Contrast results showed that Hs laid significantly more eggs per night than BC₁V (F₁ = 4.52, p = 0.036), however, there were no differences in average eggs laid per night among the other strains. Hs and BC₁S laid most of their eggs on Physalis (Fig 1), and did not significantly differ in the percent of their eggs laid on Physalis (F₁ = 0.10, p = 0.754), or tobacco (F₁ = 0.70, p = 0.406). BC₁S and Hs moths laid a significantly higher percent of eggs on Physalis than BC₁V, F₁, and Hv moths (F₁ = 128.94, p < 0.0001). Hv, F₁, and BC₁V moths laid a significantly higher percentage of eggs on tobacco plants compared to Hs and BC₁S moths (F₁ = 147.72, p < 0.0001) (Fig 1), and laid very few eggs on Physalis. These three strains (Hv, BC₁V, and F₁) did not significantly
differ from each other in the proportion of eggs laid on *Physalis* or tobacco (p > 0.05 for all comparisons).

Before ovipositing, moths would curl their abdomen and touch their ovipositor to the plant surface, sometimes dragging their ovipositor across the substrate. Most often, abdomen curls would result in an oviposition. For Hs and BC1S moths, most abdomen curls on *Physalis* plants resulted in an oviposition, while ovipositions after abdomen curls on tobacco occurred less than 20% of the time (Fig 2). These two strains did not differ from each other in the percent of abdomen curls resulting in an oviposition on *Physalis* plants or tobacco plants (*Physalis*: F1 = 3.58, p = 0.062; tobacco: F1 = 0.26, p = 0.613). Most abdomen curls on tobacco resulted in ovipositions for Hv, BC1V, and F1 moths (Fig 2). These three strains laid significantly more eggs on tobacco following abdomen curls compared to eggs laid on *Physalis* after abdomen curls, and they did not differ in the percent of abdomen curls resulting in ovipositions on tobacco or *Physalis* (p > 0.05 for all comparisons).

There was a considerable amount of variation in the time moths spent flying as a percentage of total observation time, and strains did not differ in average percent time spent flying (F1,102 = 0.90, p = 0.4650) (Fig 3). However, strains differed considerably in their profiles for percent time spent in the four areas during flight (Fig 4). The multivariate test of differences between the strains using the Wilks Lambda criteria was statistically significant (F5.59,16 = 296.98; p < 0.0001). Follow-up multivariate comparisons using contrasts showed that Hs and BC1S were significantly different from BC1V, Hv, and F1 in their flying profiles (F4,97 = 22.52; p < 0.0001), but Hs did not differ from BC1S (F4,97 = 0.36, p = 0.836), and BC1V, F1, and Hv also did not differ from each other (p > 0.05 for all comparisons). Separate univariate ANOVAS showed that strains did not differ significantly in the percent time flying in neutral areas (F4,104 = 0.64, p = 0.6330) or on the wall (F4,104 = 1.02, p = 0.4015) (Fig 4), so the multivariate test was rerun using the combination of percent time spent flying around tobacco and *Physalis*. This test was statistically significant (F8,198 = 11.31, p < 0.0001), showing that there were differences in time spent flying around the two plant species.
between the five strains. Contrast results showed that Hs and BC₁S moths spent significantly more time flying around *Physalis* plants compared to Hv, BC₁V and F₁ moths (F₁ = 55.14, p < 0.0001), whereas Hv, BC₁V, and F₁ moths spent significantly more time flying around tobacco compared to Hs and BC₁S moths (F₁ = 40.85, p < 0.0001) (Fig 4). Multivariate tests showed the profiles for percent time flying around tobacco and *Physalis* did not differ between Hs and BC₁S (F₂,₉₉ = 0.43, p = 0.649), nor did they differ between BC₁V, Hv, and BC₁S (all comparisons p > 0.05).

Strains also differed considerably in their profiles for percent time spent in the four areas during non-flight time (Fig 5). Patterns were similar to those that were observed for flight behavior, except less time was spent on the walls and in neutral areas (moths were in neutral areas during non-flight times very seldom, since these areas only included the ground and the two observers). The multivariate test of differences between the strains in non-flying time using the Wilks Lambda criteria was statistically significant (F₁₆, 2₈₇.₈₁ = 6.43, p < 0.0001), showing that patterns in areas occupied by moths during non-flying time differed between the five strains. Separate univariate ANOVAS showed that strains did not differ significantly in the percent time flying on the wall (F₄,₁₀₁ = 2.10, p = 0.087) or in neutral areas (F₄,₁₀₄ = 1.21, p = 0.311), so the multivariate test was rerun using the combination of percent non-flying time on tobacco and *Physalis*. This test was statistically significant (F₈,₁₉₂ = 12.77, p < 0.0001), showing that the strains differed in non-flying time spent on *Physalis* and tobacco. The profiles of percent non-flying time spent on *Physalis* and tobacco did not differ for Hs and BC₁S moths (F₂,₉₆ = 1.48, p = 0.2337), nor did they differ between Hv, BC₁V, and Hv moths (all comparisons, p > 0.05). Hs and BC₁S moths spent significantly more time on *Physalis* plants compared to Hv, BC₁V and F₁ moths (F₁ = 67.61, p < 0.0001), whereas Hv, BC₁V, and F₁ moths spent significantly more time on tobacco compared to Hs and BC₁S moths (F₁ = 106.06, p < 0.0001) (Fig 5).
DISCUSSION

Because oviposition behavior is generally difficult to observe in the field, most studies involving the collection of oviposition data occur in laboratory settings. By using a large outdoor cage, we were able to collect behavioral data during oviposition bouts on Hs, Hv, hybrid and backcrosses of these two species in a field setting. For active female moths in our study, flight and oviposition behavior was generally similar to that recorded for wild Hs moths observed in the open field (Benda 2007). To our knowledge, this is the first study involving the genetics of host range to collect detailed information on oviposition behavior in a natural field setting for two species with extreme differences in host range, as well as on their hybrids and backcrosses.

Behavior was difficult to extract from the backcross strains, and to a higher degree, F1s. The reason for the lack of behavior from the hybrids and backcrosses that were released into the cage is unknown. These moths laid fertile eggs on the cheesecloth that covered the plastic containers in which they were housed during the daytime, so the observed behavior was not due to infertility. Even when F1s and BC1 moths were manually placed on plants or propelled into the air to promote oviposition behavior, the vast majority of the moths remained motionless and did not fly. A lack of normal oviposition behavior from the hybrids and backcrosses could be due to genetic reorganization of the genome and novel interactions between genes resulting from hybridization and subsequent backcrossing. It is useful to note that in 2008 some males from the BC families were used to test their responses to pheromones in a wind tunnel. These backcross males were just as active as the parental species (N. Vickers, U. of Utah, pers. comm.).

The females of the two parent species behaved as expected in the cages, ovipositing most of their eggs and spending the largest proportion of both flying and non-flying time around their respective host plants (Fig 1,4,5). Although its offspring cannot survive on tobacco, Hs did
lay a small percentage of eggs on tobacco plants; the generalist Hv laid eggs only on tobacco (Fig 1). Sheck and Gould (1995) also observed that Hs laid a percentage of its eggs on non-hosts, and that, contrary to what would be expected, the generalist Hv had a more narrow niche breadth than Hs, laying most of its eggs on tobacco. Similarly, Benda (2007) found that Hs lays approximately 20% of its eggs on non-hosts. This behavior may have evolved as a mechanism for avoiding defenses against eggs by leaves of its Physalis host plants (Petzold 2009).

All strains spent similar amounts of time flying, and although Hs laid significantly more eggs than BC1V, all other strains laid comparative amounts of eggs. The plant on which a moth curled her abdomen had a significant effect on whether or not an egg was laid (Fig 2). These results are in concordance with Ramaswamy (1990), who found that abdomen curls by Hv on cotton plants resulted in ovipositions more often than those on a screen, although results from an ablation study did not show any evidence that ovipositor receptors were involved in host-plant perception (Ramaswamy et al. 1987). Our results differ from those of Benda (2007), who found that oviposition was just as likely to occur on host and non-host plants once an abdomen curl took place.

The F1 line was indistinguishable from Hv and BC1V in oviposition behavior; however, caution should be used when drawing conclusions from F1s in this experiment since observations are based on only 7 individuals. Another study has also shown that F1 hybrids preferred tobacco as an oviposition substrate when given a choice between Physalis, tobacco, cotton and soybean in a smaller cage (Sheck and Gould 1995). Reciprocal F1 crosses displayed similar oviposition patterns on all hosts, and it was concluded that genes controlling oviposition were not sex-linked (Sheck and Gould 1995). Thus, it is unlikely that oviposition on tobacco in our study was a result of maternal effects or sex-linkage (F1s were produced from female Hv and male Hs). Results with our system differ from several other studies, in which oviposition traits were determined to be sex-linked (Thompson 1988, Scriber et al. 1991, Janz 1998).
Data from F₁ moths would indicate that simple dominance governs oviposition behavior on tobacco and *Physalis*, with the gene(s) for oviposition on tobacco being dominant. However, this model is not consistent with data from the backcrosses. Each of the two backcross lines displayed oviposition behavior nearly identical to the parent species it most closely resembled genetically (Fig 1,2,4,5). If oviposition genes were governed by simple dominance on tobacco, BC₁S would not have behavior that mimics Hs. Two plausible explanations could account for our results. First, it may be that oviposition behavior is controlled by multiple genetic loci, and a threshold needs to be met for the behavioral phenotype to resemble Hv (Lander and Schork 1994, Roff 1996). If this were the case, it would account for why F₁ moths behaved like Hv and why BC₁S moths (which contain approximately 25% Hv genes and thus may not meet this threshold) resembled Hs in oviposition behavior. Conversely, oviposition behavior could be controlled by a single locus, and heterozygosity at this locus could impart a survival disadvantage (Arnold and Hodges 1995) or could impair oviposition behavior in some way. In this case, individuals that were heterozygous at this locus (all of the F₁S and half of all the BC₁S) may have been among those females that never laid any eggs in the cages. Moths from which data were obtained would be BC₁S that are homozygous at this locus; oviposition behavior of these moths would mirror that of the parent to which they were backcrossed, which is what this study showed.

Further genetic studies employing the use of molecular markers would allow us to determine if there is one or few major loci that govern oviposition behavior, or if oviposition behavior is controlled by many loci. Sample sizes from each of the crosses in our study were too small to perform a QTL (quantitative trait locus) analysis. Future studies aimed at obtaining phenotypic data on oviposition behavior from a large sample of backcross moths would allow us to use our system to determine how many loci control oviposition behavior and are therefore important for a major change in host range to occur.
LITERATURE CITED


Poole, R. W., C. Mitter, and M.D. Huettel. 1993. A revision and cladistic analysis of the *Heliothis virescens* species groups (Lepidoptera: Noctuidae) with a preliminary morphometric analysis of *H. virescens*. In Mississippi Agriculture and Forestry Experiment Station Bulletin.


Table 1: Night observations of female Hs, Hv, hybrid, and backcross moths. Observations occurred during a total of 55 and 23 nights in 2007 and 2008, respectively.

<table>
<thead>
<tr>
<th>Year</th>
<th>Strain</th>
<th>Total observation time (min)</th>
<th>Total # moths observed</th>
<th>Observation time/moth (min)</th>
<th>Total eggs</th>
</tr>
</thead>
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<tr>
<td>2007</td>
<td>Hs</td>
<td>69.05</td>
<td>10</td>
<td>6.91</td>
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</tr>
<tr>
<td></td>
<td>BC1S</td>
<td>305.83</td>
<td>22</td>
<td>13.90</td>
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<tr>
<td></td>
<td>F1</td>
<td>47.48</td>
<td>5</td>
<td>9.50</td>
<td>89</td>
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<tr>
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<td>24</td>
<td>8.56</td>
<td>315</td>
</tr>
<tr>
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<td>Hv</td>
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<td>2</td>
<td>7.98</td>
<td>68</td>
</tr>
<tr>
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<td>TOTAL</td>
<td>643.68</td>
<td>63</td>
<td>10.22&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1047</td>
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<tr>
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<td>0.98</td>
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<td>1.18</td>
<td>8</td>
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<td>17</td>
<td>6.21</td>
<td>247</td>
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<tr>
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<td>TOTAL</td>
<td>388.00</td>
<td>42</td>
<td>9.24&lt;sup&gt;1&lt;/sup&gt;</td>
<td>788</td>
</tr>
</tbody>
</table>

<sup>1</sup>Average of observation minutes
Figure 1: Average number of eggs laid per moth per night for Hs, Hv, hybrids (F₁), and backcrosses (BC₁V, BC₁S). Error bars (grey=tobacco; black=Physalis) show 1 SE of the mean.

Figure 2: Average percent of abdomen curls on a plant resulting in an egg being laid on that plant, for Physalis and tobacco. Error bars show 1 SE of the mean.
Figure 3: Average percent of total observation time that each moth strain spent flying. Error bars show 1 SE of the mean.
Figure 4: Time spent flying in the vicinity of *Physalis* plants, tobacco plants, the wall, or neutral areas, as a percentage of total flying time for Hs, Hv, their hybrids (F₁), and backcrosses BC₁S and BC₁V. Error bars show 1 SE of the mean.
Figure 5: Time spent on or around *Physalis* plants, tobacco plants, the wall, or neutral areas, as a percentage of the total observation time minus time spent flying for Hs, Hv, their hybrids ($F_1$), and backcrosses $BC_1S$ and $BC_1V$. Error bars show 1 SE of the mean (error bars for neutral and wall were very small, and thus are omitted for clarity).
APPENDICES
APPENDIX A. Supplementary information for:

Chapter 2. COULD HOST PLANT RESPONSE TO EGGS OF ITS SPECIALIZED HERBIVORE SELECT FOR OVIPOSITION ON NON-HOSTS?
Table 1: Insects present in field during data collection. Insects were collected by sweep netting during week 6 of the experiment. Seven unidentified arachnids were also collected.

<table>
<thead>
<tr>
<th>Order</th>
<th>Suborder</th>
<th>Family</th>
<th>Quantity</th>
<th>Predator/parasitoid?</th>
</tr>
</thead>
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<td></td>
<td>Cimicidae</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coreidae</td>
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</tr>
<tr>
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<td></td>
<td>Hebridae, nymph</td>
<td>3</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hebridae</td>
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<td>*</td>
</tr>
<tr>
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<td></td>
<td>Lygaeidae</td>
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<td>*</td>
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<td>Pentatomidae</td>
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</tr>
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<td></td>
<td>Pisesmatidae</td>
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<tr>
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<td>Tingidae</td>
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<td>Chrysomelidae</td>
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APPENDIX B. Supplementary information for:

Chapter 3. EFFECT OF HELIOTHIS SUBFLEXA HERBIVORY ON FRUIT ABSCISSION BY PHYSALIS SPECIES: THE ROLES OF MECHANICAL DAMAGE AND CHEMICAL FACTORS
Figure 1: Average size of *Physalis angulata* and *Physalis pubescens* fruit used in both experimental trials. Size classes represent fruit diameter. Size class 1 includes fruit less than 2 cm; size class 2 includes fruit greater than or equal to 1 cm and less than 2 cm; size class 3 includes fruit greater than or equal to 3 cm and less than 4 cm, etc.
Figure 2: Average size of *Physalis angulata*, *Physalis pubescens*, and *Physalis cordata* fruit on mature plants. All fruit on one mature branch of each plant species were measured. Sample sizes are as follows: for *P. pubescens*, a total of 289 fruit were measured from 6 plants; *P. cordata*: 189 fruit, 3 plants; *P. angulata*: 291 fruit, 5 plants. Size class 1 includes fruit less than 2 cm diameter; size class 2 includes fruit greater than or equal to 1 cm diameter and less than 2 cm diameter; size class 3 includes fruit greater than or equal to 3 cm diameter and less than 4 cm diameter, etc.
APPENDIX B.2. CAN LARVAE ‘SENSE’ WHEN FRUIT WILL ABSICISE?

METHODS

Because many Physalis fruit that are inflicted with H. subflexa herbivory are only partially eaten, I hypothesized that larvae consuming fruit could ‘sense’ when fruit would fall, and leave just prior to abscission of the fruit. To test this hypothesis, 3 third to fourth instar H. subflexa larvae were each placed on a Physalis plant inside a greenhouse. Every three hours, each plant was checked for fruit that contained a larva inside. If a fruit contained a larva on the previous time point, that fruit was checked during each consecutive 3-hour time point to determine if the larva still occupied the fruit. If a fruit contained a larva that later abandoned it, the fruit was checked at each 3-hour time point to determine the time of abscission. Plants were arranged on a greenhouse table, and a rotating fan set on low speed was angled so that each plant would receive a small gust of air simulating wind in the field (this created the stimulus necessary for fruit abscission). This experiment was repeated for a total of three trials. For the first, second, and third trial, the experiment was run for 48, 72, and 96 hours respectively; 3, 6, and 8 plants respectively were used in each trial. For each fruit that was occupied by a larva and not fully consumed, we calculated the amount of time between when the larva abandoned the fruit, and when the fruit abscised.

RESULTS

A total of 13 P. pubescens and 4 P. angulata plants were used in the three trials, and data were collected from 141 fruit. Of these fruit, 40% were completely consumed. The remaining 60% (84) were partially consumed. For fruit that were partially consumed, 31% contained larvae at the time of abscission, and the remaining 69% were empty. For fruit that were consumed then abandoned, most larvae left fruit approximately 3-5 hours before abscission occurred; this was not dependent on the amount of time the larva spent feeding on
the fruit (Fig 1). This suggests that *H. subflexa* larvae can ‘sense’ when the fruit on which they are feeding will fall.

![Figure 1: For larvae that spent various amounts of time feeding inside of a fruit (shown in gray bars), the average amount of time between when a larva exited fruit, and when the fruit abscised. Larvae exited fruit several hours before abscission; this was not dependent on the amount of time a larva fed on a fruit.](image)
APPENDIX C. Supplementary information for:

Chapter 4. THE GENETICS OF HOST RANGE IN A SPECIALIST AND GENERALIST HERBIVORE: LARVAL HOST PLANT PREFERENCE AND SURVIVAL
Figure 1: Experimental trial 1 (BC1S larvae) feeding profiles. a) Proportion of time that each neonate spent on *Physalis*, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.
Figure 2: Experimental trial 2 (BC₁S larvae) feeding profiles. a) Proportion of time that each neonate spent on *Physalis*, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.
Figure 3: Experimental trial 3 (BC₁V larvae) feeding profiles. a) Proportion of time that each neonate spent on Physalis, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.
Figure 4: Experimental trial 4 (BC₁V larvae) feeding profiles. a) Proportion of time that each neonate spent on *Physalis*, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.
Figure 5: Experimental trial 5 (BC$_1$V larvae) feeding profiles. a) Proportion of time that each neonate spent on *Physalis*, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.
Figure 6: Experimental trial 6 (BC₁V larvae) feeding profiles. a) Proportion of time that each neonate spent on Physalis, tobacco, or no plant. b) Proportion of leaf area that each corresponding neonate consumed.