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**BEHAVIORAL RESPONSES OF MALE PARASITIC WASPS TO PLANT
CUES: A COMPARISON OF TWO HOST-PLANT COMPLEX SOURCES
OF *COTESIA CONGREGATA* (SAY)**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

by

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Acknowledgments

I thank my advisor, Dr. Karen Kester, for the opportunity as an undergraduate to intern in her lab which led to my fascination with insect behavior and to, well, an awesome research project. Her support and advice through the ups and downs of this project has been invaluable. I would not have been able to complete this project without the small army of interns and volunteers who helped raise hundreds of caterpillars and wasps for my research project. They not only made spending time in the lab more enjoyable, but also gave me the chance to help encourage and mentor their involvement in research. I also would like to thank Buster Tyson for the use of his catalpa grove and especially Paul Semtner who helped me locate tobacco fields and spent hours wandering up and down rows of tobacco collecting hornworms with me. I thank my fellow graduate students, especially my labmates Jessica Bray and Justin Bredlau, for their friendship and support during my project. You made spending all day (and more than a few nights) at the lab running assays more entertaining than stressful. Lastly, I thank Sean Burns, for helping me stay sane throughout my graduate years and supporting my love of insects and research; and being okay with having a freezer full of dead insects.

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Abstract

BEHAVIORAL RESPONSES OF MALE PARASITIC WASPS TO PLANT CUES: A COMPARISON OF TWO HOST-PLANT COMPLEX SOURCES OF *COTESIA CONGREGATA* (SAY)

By Megan E. Ayers

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2015

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Prior exposure to plants cues can enhance assortative mating in insects. We hypothesized that, as previously reported for females, males of *Cotesia congregata* would display inherent responses to plant cues that could be modified by postemergence experience and further, that males originating from two different host-plant complexes (HPCs) would display different behavioral responses to these HPCs. In no-choice contact assays with a non-host plant, searching responses of males and females increased sharply at Day 2 and remained stable through Day 4. In no-choice assays with potential host plants, males

searched longer on catalpa than tobacco; responses were not modified by postemergence experience. In choice assays with both HPCs, naïve males did not display orientation preferences; however, males experienced with their natal plant preferred their natal HPC. Results indicate that postemergence experience on the natal host plant induces an orientation preference for the natal HPC and thus, can facilitate assortative mating.

INTRODUCTION

Insect parasitoids exhibit high levels of speciation and host race formation due to the intimate relationships they share with their hosts. These relationships rely on adaptations of parasitoids to the host and also the plant on which their hosts feed. Typically, parasitoids show host fidelity by attacking the same host species on the same host plant as their mothers (Feder et al. 1994; Stelinski & Liburd 2005). Host races may form when a parasitoid population shifts to a closely related host species and gene flow is restricted if the timing of host emergence also shifts (Feder, et al. 1993). Selection toward the extremes of different host races may also be driven by habitat selection and assortative mating (Smith 1966). Assortative mating may be further refined through conditioning or learning of various hosts and plant cues (Smith 1966).

Female parasitoids use plant olfactory cues to locate food sources and potential hosts. These chemical cues are emitted by plants, both damaged and undamaged, and are detectable by the parasitoids (Turlings et al. 1990b, 1995). Parasitoid responses to these cues can be inherent or modified through different methods of learning, such as associative learning with oviposition (Vet 1983), postemergence experience (Kester & Barbosa 1991), and pre-imaginal learning (Gandolfi et al. 2003; Konig et al. 2015). Learning can enhance the fitness of parasitoids by increasing the probability of finding potential hosts (Vet et al. 1990), food sources (Lewis & Takasu 1990; Wackers & Lewis 1999; Dukas & Duan 2000; Sato & Takasu 2000), and by allocating more female progeny to hosts on previously encountered plants (Lentz-Ronning & Kester 2013).

Many studies on parasitoid responses to plant cues have focused on the role of learned responses in host location of females. Experienced female *Cotesia marginiventris* typically show a preference for the experienced host plant and host (Turlings et al. 1990a). For both *C.*

glomerata and *C. rubecula*, a larger proportion of experienced wasps respond to plant odors in a wind tunnel and when provided an ovipositional experience with the host, their flight response also increases (Bleeker et al. 2006). Females of *C. congregata* show enhanced responses to their host plants through postemergence learning (an experience immediately after emergence from cocoons) (Kester & Barbosa 1991); further ovipositional learning affects their inherent searching and sex ratio allocation responses (Lentz & Kester 2008). Postemergence learning has been demonstrated in female *C. congregata* and has been hypothesized to promote assortative mating on the natal plant leading to host race formation (Kester & Barbosa 1991).

Studies involving male parasitoids have focused mainly on foraging for food (Sato & Takasu 2000) or the use of female pheromones in the process of mate selection (Danci et al. 2006; Bredlau & Kester in press) and only a few have considered whether males can learn. For example, through associative learning, male *Nasonia vitripennis* exhibited learning of colors when the reward of mating increased (Baeder & King 2004). Both male and female *Diachasma alloeum* that experienced a blueberry infested with their host, *Rhagoletis mendax*, had increased responses to an uninfested blueberry compared to wasps that had not received a blueberry experience (Stelinski & Liburd 2005). Males of the aphid parasitoid, *Aphidius ervi*, learn both novel odors and ones from their host-plant complexes (Villagra et al. 2005, 2008). Males learn novel odors when paired with the reinforcement of copulation with virgin females (Villagra et al. 2005). A copulation experience in the presence of their host-plant complex (HPC) stimulus results in a preference for their HPC over an alternative HPC (Villagra et al. 2008).

The braconid wasp, *Cotesia congregata*, is a gregarious larval endoparasitoid that parasitizes various species in the family Sphingidae (Krombein et al. 1979). *Cotesia congregata* is an arrhenotokous parasitoid: males develop from unfertilized eggs and females from fertilized

zygotes. Therefore, all male broods develop if females do not mate. Females oviposit eggs into host caterpillars, larvae develop within the host, and feed on nutrients in the hemolymph. Wasp larvae egress from their hosts in 2-3 weeks, spin cocoons in which they pupate, and emerge as adults in ca. 7 days. Males tend to emerge before females, and may mate multiple times whereas females mate once. Immediately after emergence, females will antennate on the surface of the leaf on which their hosts resides, resulting in a learned positive response to the plant on which they emerged (Kester & Barbosa 1991). Females will then search for potential hosts using the learned plant cues and males may fly to other plants in search of additional females.

Two locally abundant host-plant complexes of *C. congregata* are *Manduca sexta* (L.) on tobacco (“MsT”) and *Ceratomia catalpae* (Haworth) on catalpa (“CcC”). The tobacco hornworm (*M. sexta*) is a solitary species that feeds on a variety of solanaceous plants. They have adapted to plant alkaloids, including nicotine, as have populations of *C. congregata* (Kester & Barbosa 1994). The catalpa worm (*C. catalpae*) is a gregarious species that specializes on trees in the genus *Catalpa* (Lamiales: Bignoniaceae). Catalpa produces iridoid glycosides, catalpol and catalposide, which are unpalatable to most generalist herbivores (Bowers 1991). However these plant chemicals appear to have little effect on *C. congregata* (Lampert et al. 2010). The MsT and CcC host-plant complexes of *C. congregata* have diverged into incipient species (Kester et al. in press; Bredlau & Kester in press). This is thought to result from different selective pressures exerted by the combination of host life history traits (solitary vs. gregarious host), host plant chemistry (alkaloids of solanaceous plants vs. iridoid glycosides of catalpa trees), as well as the polydnavirus, an underlying post-mating barrier that inhibits the host’s immune system (Bredlau & Kester in press).

This study focuses on the mechanisms of learning in males of *Cotesia congregata*, with respect to host-plant complex origin. First, I investigated male *C. congregata* inherent responses to plant cues for comparison to those of females. We hypothesized that the male wasps would either a) respond to plant cues immediately after emergence when they typically mate or b) respond similarly to females, which have been previously reported to show peak responses 48 hours after emergence (Kester & Barbosa 1991). We also investigated the effects of postemergence experiences with the host plant on male searching and orientation responses and how this experience may affect their responses to an alternate host plant. We hypothesized that male *C. congregata* would search longer and have a greater preference for the host plant experienced immediately after emergence over the alternate host plant. We also hypothesized that responses would differ with respect to the host-plant complex origin of the wasps in that males would show increased searching and orientation responses to their natal plants. If males are capable of learning and utilizing host-plant cues for locating potential mates, this would promote assortative mating on the natal host-plant, which could lead to host-race formation and ultimately, serve as a mechanism of speciation.

MATERIALS AND METHODS

Parasitoids

Wasps from a colony originating in 2005 from the Southern Piedmont Agricultural Research and Extension Center site in Blackstone, VA were used for mechanistic work with a non-host plant (Experiment 1) before caterpillar appearance in the field. This colony was reared on *M. sexta* fed on a laboratory diet. Once larvae egressed, all experimental caterpillars were placed into individual plastic rearing cups (4 oz.) and held at ambient laboratory conditions (temperature and humidity ranges: 22±2°C; 30-50% RH) until wasp emergence. Wasps had no contact with plant material other than experimental exposure.

Tobacco hornworms and catalpaworms were collected from, tobacco and catalpa, respectively, with and without parasitoid cocoons from three field sites between late July and early October 2014. Tobacco hornworms (*M. sexta*) were collected on tobacco (MsT wasps) from two locations in Blackstone, VA; 1) a privately owned tobacco farm (37.01499, -78.03598) where tobacco has been grown for over 100 years and at the collection plot, for 3 or 4 rotations in the past 10 years and 2) the Southern Piedmont Agricultural Research and Extension Center (37.09432, -77.95365) where tobacco has been grown since 1974. Catalpaworms (*C. catalpae*) were collected on catalpa (CcC wasps) from a private property in Columbia, Virginia (37.71273,-78.16388) where a catalpa grove of 23 mature trees is annually infested with catalpa worms to the point of complete defoliation.

All field collected caterpillars were kept in plastic shoe boxes (28 x 16 x 11 cm) with approximately 10-15 caterpillars and host plant leaves collected from the appropriate field site. Once larvae egressed, all field collected caterpillars were placed into individual plastic rearing cups (4 oz) and held at ambient laboratory conditions (22±2°C; 30-50% RH) until wasp

emergence (parent generation) was observed. Parent generations from each field site were then placed into plastic rearing containers (20cm x 20cm x20cm) with water and agar-honey. Lab reared *M. sexta* fed on a laboratory diet were then used to rear out the first (F₁) and second (F₂) lab generations for both MsT and CcC HPC's used in Experiments 2 & 3. F₁ and F₂ wasps had no contact with plant material other than experimental exposure.

Plants

All host plants used were grown from seeds in a greenhouse controlled environment free of pesticides and other insects. Tobacco ("NC95") seeds were planted every two weeks from May to October to ensure plants of equal size and age for each test. The three youngest fully grown leaves from tobacco plants between 10 cm to 30 cm tall were used for experiments. Catalpa trees used were 2 years of age and started from seeds collected from a local tree (Monument site, 37.595,-77.531). Healthy catalpa leaves of approximately the same size as the tobacco leaves were used. Cabbage plants ("Flat Dutch Cabbage") free of apparent pests and insect damage were purchased locally. Healthy full-grown leaves were removed from one of 15 cabbage plants of approximately equal age grown under grow lights in ambient laboratory conditions (ranges: 22±2°C; 30-50% RH) free of pesticides. The novel non-host plant elicits moderate searching responses from female wasps (Kester & Barbosa 1992). Both tobacco and cabbage plants were fed Miracle-Gro® on a weekly basis and the catalpa trees were supplemented with Osmocote every 2 months.

Postemergence experience

Wasps from each treatment group, consisting of 1-4 broods emerging at approximately the same time (±1 hour), were given a postemergence experience with a plant or a Kimwipe (naïve control wasps). The ripped piece of leaf or Kimwipe of approximately the same size was

introduced into the rearing cup, containing a single brood and host, prior to emergence of the wasps and left for approximately 4 hours. This ensured all emerged wasps had contact with the ripped piece of leaf or Kimwipe experience. Wasps were then released into plastic candy jars (approximately 1.12L) with thin fabric mesh secured over the opening and offered water and agar-honey, separated by treatment group until testing.

No-Choice Searching Assays

Wasps were transferred individually to a glass vial (1 dram) with a cork stopper and for 5-10 minutes prior to testing. A single leaf disk (12.7 cm in diameter) was placed inside the vial for 2 minutes. Searching time, described as the active antennation on the leaf disk, was recorded as described by Kester and Barbosa (1991). Each wasp was assayed once between 10am and 3pm. Wasps that searched for less than 1 second were considered nonresponsive and therefore excluded from analyses. All searching data were transformed using (square root (time + 0.5)).

Choice Orientation Assays

Orientation of male *C. congregata* to the host-plant complexes was measured using a T-tube olfactometer (Fig. 1) designed by Christopher Crockett. Air was pumped into a flask containing activated carbon to first purify the air then passed through a flask containing one of two odor sources consisting of the host plant, host, and host frass, with the plant mechanically crushed to stimulate the plant chemicals. The odors were then pumped into either side of the T-division of the olfactometer at a flow rate of 0.2 LPM. Wasps were removed from candy jars and held individually in capsules (size 00) for 5-10 minutes before being introduced into the end of the T-tube olfactometer. Assays were run between 10am-3pm or 10pm-3am; intervals chosen to coincide with periods of peak activity (Kester pers. obs.) on days where the barometric pressure was within the range of 30.05 ± 0.35 inHg. The divided portion of the T-tube was held under light

at $29.5 \pm 3.0^\circ\text{C}$ and the relative humidity running through the olfactometer increased over the course of each night (range: $85 \pm 15\% \text{RH}$) which was accounted for by running 5 tests for each treatment group and then repeating this over the course of each testing interval. Wasps were given 5 minutes to choose between the two odors, defined as the wasp moving into one side of the T-division and spending at least 10 uninterrupted seconds in that side. Wasps that spent less than 10 uninterrupted seconds on either side of the divided portion of the olfactometer were counted as non-responders and excluded from any analyses. Both flow rates and time to choice were based on preliminary work.

Experiment 1: Comparison of male and female inherent responses to plant cues

To compare inherent responses of males and females, the no-choice searching assay (described above) was used to quantify responses to cabbage across five testing days. Naïve wasps were set up on a rolling basis and placed into treatment groups by emergence date, e.g., wasps set up were tested on Day 0, then Day 1, through Day 4. Four to six males and an equal number of females from a treatment group were assayed on the same day to allow for comparisons of wasps from the same broods (number of male wasps between 35 and 52 per treatment group; female wasps between 36 and 54). Assays were run only on days when both males and females were available from a treatment group; however not all treatment groups were run on the same day, e.g., if male and female wasps were available from Day 0, 1, 2, & 4, but not from Day 3, assays were run using wasps from those treatment groups only (Day 0, 1, 2, & 4). Assays were performed on days when environmental conditions were in the following ranges: barometric pressure 30.2 ± 0.4 inHg, relative humidity $32.5 \pm 12.5\%$, and temperature $26 \pm 2.0^\circ\text{C}$. An ANOVA followed by a Tukey's post-hoc test was run to compare searching responses across sex and days. Separate linear regressions were run to compare searching times of male and

female wasps in response to barometric pressure, temperature, and humidity to test for correlations between environmental factors and searching times (JMP 2013).

Experiment 2: Effects of a postemergence experience on searching responses of male wasps

To investigate the effects of postemergence experience on searching responses, male wasps from the MsT and CcC complexes were given a postemergence experience with either tobacco, catalpa, or a Kimwipe (as described above). Based on the results of Experiment 1, wasps were assayed on Days 2-3 after emergence. Males from the three treatment groups (postemergence experience with tobacco, or catalpa, or no experience) were offered a tobacco or catalpa leaf disc. Individuals were tested once. Due to time constraints, MsT males (n between 48 and 62 per treatment group) were assayed on different days from CcC males (n between 46 and 76 per treatment group). MsT males were assayed on days when environmental conditions were in the following ranges: barometric pressure 30.05 ± 0.35 in, relative humidity $65 \pm 15\%$, temperature $27 \pm 3.0^\circ\text{C}$. CcC males were assayed on days when environmental conditions were in the following ranges: barometric pressure 30.0 ± 0.3 in, relative humidity $65 \pm 25\%$, and temperature $28 \pm 3.0^\circ\text{C}$. An ANOVA compared male searching responses for each host-plant complex comparing the postemergence experience, the plant assayed, and the interaction between the two. A Tukey's post-hoc test was used to compare means. Separate linear regressions were run to compare the searching times of the wasps to the barometric pressure, temperature, and humidity to look for correlations between environmental factors and searching times (JMP 2013).

Experiment 3: Effects of a postemergence experience on orientation responses of male wasps

Wasps from the treatment groups described in experiment 2 were also used in Experiment 3. Based on the results of experiment 1, 2-3 day old wasps were used in all

olfactometer tests. Individual wasps were tested once. Due to time constraints, MsT males (n between 30 and 50 per treatment group) were assayed on different days than CcC males (n between 44 and 50 per treatment group). The first odor flask contained an early 4th-instar tobacco hornworm with its frass and a crushed tobacco leaf, and in the second flask, an early 4th-instar catalpa worm was placed with its frass and a crushed catalpa leaf. Odor sources consisted of the host-plant complex to mimic olfactory cues wasps might use to locate plants in the field. Air was run over the odor sources and through tubing that led into the olfactometer tube. After five wasps (either MsT or CcC) were assayed from each postemergence treatment group, the olfactometer tube was cleaned in a bleach, soap, and hot water solution. After air drying, the tube was reversed to control for external factors that may have induced bias, e.g., if the first set of assays was set up with the MsT odor source on the left and the CcC odor source on the right, after the flip, the CcC odor source would then be on the left and MsT odor source on the right). Wasp responses were analyzed using two methods. First, a likelihood chi-square goodness-of-fit test was used to compare the percent choices within each postemergence group (JMP 2013), e.g., we compared the percent of naïve MsT males that choose the MsT odor source to those that choose the CcC odor source. Second, a Z-test for independent proportions was used to compare the proportion of wasps choosing the host-plant complex experienced compared to the naïve wasps, e.g., the proportion of tobacco experienced wasps choosing the MsT odor source compared to the proportion of naïve wasps choosing the MsT odor source.

RESULTS

Experiment 1: Comparison of male and female inherent responses to plant cues

Male *Cotesia congregata* responses showed a similar pattern to those of females although searching times of males were lower (Table 2). Both male and female responses peaked at Day 2 and held steady through Day 4 (Fig. 2) giving an optimal time period for additional tests. Searching responses of male wasps were similar across days within the optimal testing period (Day 2 vs Day 3 $P=1.0$; Day 3 vs Day 4 $P=0.999$; Day 2 vs Day 4 $P=0.959$) with female responses also similar across days within the optimal testing period (each comparison $P=1.0$). Searching times of males were not correlated with either the temperature or humidity ($r^2=0.001$, $P=0.534$ & $r^2=0.002$, $P=0.479$ respectively). Male searching times were positively correlated with the barometric pressure ($r^2=0.039$, $P=0.0034$). Searching times of female wasps were not correlated with the barometric pressure, temperature, or humidity ($r^2<0.001$, $P=0.909$; $r^2=0.01$, $P=0.074$; $r^2=0.004$, $P=0.319$ respectively).

Experiment 2: Effects of a postemergence experience on searching responses of male wasps

Searching responses of MsT males differed with respect to the plant assayed but were not affected by postemergence experience (Table 2a, Fig.3 a). With respect to the plant assayed, males searched longer on the non-natal plant (catalpa) than the natal plant (tobacco) regardless of postemergence experience (Naïve: $P<0.0001$, Tobacco: $P=0.0006$, Catalpa: $P<0.0001$). When males were given an experience with either their natal plant (tobacco) or the alternate plant (catalpa) searching times on tobacco did not differ from naïve males (Tobacco: 6.66 ± 0.94 s vs Naïve: 4.47 ± 0.396 s, $P=0.643$; Catalpa: 3.95 ± 0.4 s vs Naïve: 4.47 ± 0.396 s, $P=0.9886$). Likewise, when given an experience with either their non-natal plant (catalpa) or the alternate plant (tobacco) searching times on catalpa did not differ from naïve males (Catalpa: 14.78 ± 1.37 s vs

Naïve: 13.61 ± 1.39 s, $P=0.978$; Tobacco: 12.43 ± 1.51 s vs Naïve: 13.61 ± 1.39 s, $P=0.9365$). For MsT males, searching times were not correlated with either temperature or humidity ($r^2=0.009$, $P=0.197$ and $r^2<0.001$, $P=0.685$ respectively). Searching times were positively correlated with the barometric pressure ($P=0.0049$); however, the association between the two was low ($r^2=0.048$).

CcC males were similar to MsT males in that searching responses also differed with respect to plant assayed and were not affected by postemergence experience (Table 2b, Fig. 3b). Regardless of postemergence experience, CcC males searched longer on their natal plant (catalpa) than the alternate plant (tobacco) ($P<0.0001$). When males were given an experience with either the alternate plant (tobacco) or their natal plant (catalpa), searching times on tobacco did not differ from naïve males (Tobacco: 3.96 ± 0.44 s vs Naïve: 2.71 ± 0.24 s, $P=0.7498$; Catalpa: 2.57 ± 0.21 s vs Naïve: 2.71 ± 0.24 s, $P=1.0$). In contrast, males experienced with their natal plant (catalpa), displayed a significant increase in their response compared to naïve males (Catalpa: 14.19 ± 1.39 s vs Naïve: 9.60 ± 0.81 s, $P=0.0046$) and no difference when given an experience with the alternate plant (tobacco) (Tobacco: 9.69 ± 1.05 s vs Naïve: 9.60 ± 0.81 s, $P=1.0$). Similar to MsT males, searching times of CcC males were not correlated with either the temperature or humidity ($r^2=0.004$, $P=0.38$ & $r^2=0.003$, $P=0.43$ respectively), but searching times were positively correlated with the barometric pressure ($r^2=0.029$, $P=0.025$).

Experiment 3: Effects of a postemergence experience on orientation responses of male wasps

Male MsT wasps experienced with their natal plant (tobacco) oriented towards their natal host-plant complex (MsT odor source) more frequently than naïve males (Fig. 4a, $P=0.063$). Males experienced with the alternate plant (catalpa), displayed no preference for the CcC odor source when compared to naïve males ($P=0.429$). No significant difference was found between

preference for MsT odor source versus the CcC odor source for each postemergence treatment group (Table 3a).

Male CcC wasps when experienced with their natal plant (catalpa) also oriented towards their natal host-plant complex (CcC odor source) significantly more than naïve males (Fig. 4b, $P=0.039$). Males experienced with the alternate plant (tobacco), displayed no preference for the MsT odor source when compared to naïve males ($P=0.364$). Catalpa experienced males showed a significant preference for the CcC odor source versus the MsT odor source ($P=0.0208$), in contrast to the naïve and tobacco experienced males, which displayed no preference between odor source (Table 3b)

DISCUSSION

This study is one of only a few to examine learning in male parasitoids and the first to focus on male *C. congregata*. In the field, male wasps emerge on their natal host plant and mate with their sisters before leaving the emergence site to locate potential food sources and possibly, additional mates. The use of plant cues in mate location may be particularly important for males from all male broods. This study offers support for our first hypothesis that males display inherent responses to plant cues and that their responses mirror those of females. Our second hypothesis, that postemergence experience with the natal or alternate plant would affect male searching and orientation preferences was partially supported in that experience with the natal plant resulted in a preference for the natal host-plant complex. Results did not support our third hypothesis that responses would differ with respect to host-plant complex. Overall, male wasps responded similarly to females, and a postemergence experience with the natal plant increased male orientation preferences for their natal host-plant complex and not the alternate complex.

We first compared the inherent searching responses of *C. congregata* by timing the searching responses of both male and female wasps to cabbage, an attractive non-host plant. Male responses mirrored those of females, peaking at day 2 and holding stable through day 4, when female responses were similar to those previously reported (Kester & Barbosa 1991). The delay in male responses coincides with the preovipositional period in females, suggesting that these responses may be limited by a shared time-dependent physiological constraint. There was a weak negative correlation between the barometric pressure and searching times of male wasps but no correlation with female searching responses.

Postemergence experience had no effect on the searching responses of male wasps. Both MsT and CcC males showed similar responses, searching longer on the non-toxic catalpa than

the toxic tobacco (Fig. 3). CcC males experienced with their natal plant (catalpa) search longer on catalpa than naïve wasps, whereas MsT wasps experienced with their natal plant tobacco did not search longer on tobacco. Experience with the alternate plant did not result in increased searching responses for either MsT or CcC males. However, searching responses (no-choice assays) did not reflect orientation responses (choice assays).

When experienced with their natal host plant, both MsT and CcC males tended to orient toward their natal HPC odors more frequently than naïve males (Fig. 4); thus, MsT males experienced with tobacco chose the MsT odor source more frequently than naïve males and CcC males experienced with catalpa chose the CcC odor source more frequently than naïve males. Alternately, when males were experienced with the non-natal host plant, we did not see a preference for the experienced plant. The effects of postemergence experience on orientation responses were specific to the natal host plant, which suggests sensitization of inherent male responses to the natal plant. These results do not provide conclusive evidence that male *C. congregata* orient towards host plant cues specifically because orientation assays were performed using the two HPCs rather than natal plants alone. Also, due to limited availability of wasps from the field, choice assays were run with F₁ and F₂ generations raised in *M. sexta* hosts for both MsT and CcC HPC's.

Irrespective of the underlying mechanism, our results suggest that experienced males would be more likely to orient toward their natal host-plant complex. Further work needs to focus on assessing whether the wasps are orienting toward the natal host or host plant or a combination of both. These responses may also be further enhanced with the addition of females, e.g., male *C. plutellae* are more likely to find females and successfully mate in the

presence of the host plant (Kawaguchi & Tanaka 1999). Further work needs to be done focusing on the effect of an interaction of female pheromones with the host-plant complex cues.

This work demonstrates that males of *C. congregata* display inherent searching responses to plant cues similar to those previously reported for females and that postemergence experience with the natal plant may enhance their orientation responses to the natal host-plant complexes. The preference for the natal host-plant complex may be a result of different selective pressures of host life history traits and host plant chemistry, and when combined with mating barriers, such as the polydnavirus and courtship behavior, reinforce the incipient speciation of the different host-plant complexes (Kester et al. in press; Bredlau & Kester in press). Postemergence experience with the natal host plant increases the preference of males seeking the natal host-plant complex in search of mates, ultimately promoting assortative mating on the natal host plant.

ACKNOWLEDGMENTS

This research was funded by the Thomas F. Jeffress and Kate Miller Jeffress Memorial Trust. Thanks to the VCU Bug Lab undergraduate interns for help in collecting and raising caterpillars and wasps and a big thanks to LeAynne Miller who helped rear a large portion of my research caterpillars. Thanks to Christopher Crockett who developed the T-tube olfactometer and its methods used in this study. Thanks also to Mr. and Mrs. Buster Tyson for allowing access to their catalpa grove and to Dr. Paul Semtner, VA Tech Southern Piedmont Agricultural Research & Extension Center, for help in collecting tobacco hornwoms. Thanks also to my labmates Jessica Bray and Justin Bredlau for help and support throughout graduate school.

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Table 1. Main and interactive effects of age (Days 0-4) and wasp sex on the inherent searching responses of *Cotesia congregata*.

Source of Variation	df	F value	P
Age	4	108.38	<0.0001
Sex	1	1158.71	<0.0001
Age * Sex	4	23.52	<0.0001
Error	458		
Total	467		

Table 2. Main and interactive effects of postemergence experience (naïve, tobacco, or catalpa) and the plant assayed (tobacco or catalpa) on searching responses of male *Cotesia congregata* from two host-plant complexes (MsT = *Manduca sexta* on tobacco or CcC = *Ceratomia catalpae* on catalpa).

Source of variation	df	F Value	P
<i>a) MsT</i>			
Experience	2	0.13	0.875
Plant assayed	1	121.84	<0.0001
Plant assayed* Experience	2	3.64	0.0274
Error	335		
Total	340		
<i>b) CcC</i>			
Experience	2	2.45	0.088
Plant assayed	1	163.55	<0.0001
Plant assayed *Experience	2	6.20	0.0023
Error	334		
Total	339		

Table 3. Orientation Responses of male *Cotesia congregata* with a postemergence experience (naïve, tobacco, or catalpa) in a T-tube olfactometer to odors from two potential host-plant complexes (MsT = *Manduca sexta* on tobacco or CcC = *Ceratomia catalpae* on catalpa).

Postemergence experience	<i>N</i>	Wasps choosing MsT HPC odors (%)	Wasps Choosing CcC HPC odors (%)	Chi-squared value	p-value
a) MsT					
Naïve	50	43	57	0.79	0.3757
Tobacco	50	59	41	1.66	0.1973
Catalpa	30	41	59	0.86	0.3532
b) CcC					
Naïve	50	51	49	0.02	0.8815
Tobacco	44	55	45	0.38	0.5368
Catalpa	49	33	67	5.34	0.0208

"Non-responsive" wasps were excluded from data analysis

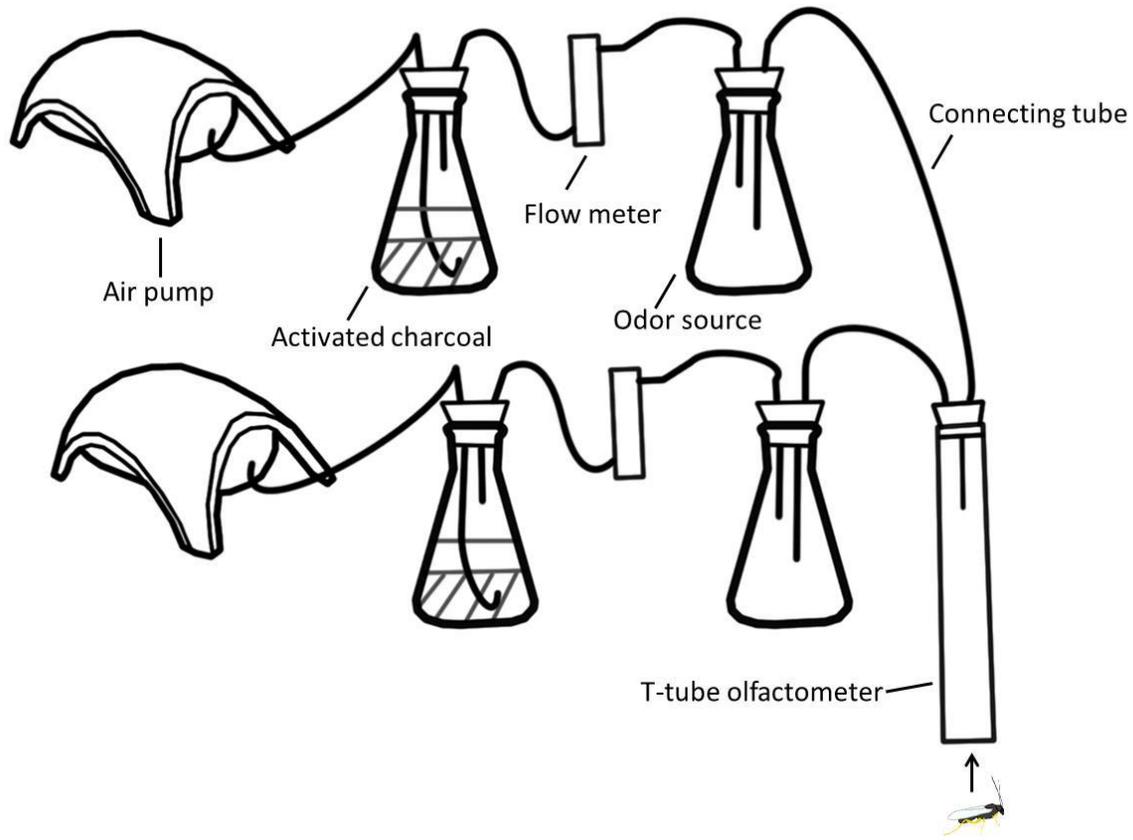


Figure 1. T-tube olfactometer (designed by Christopher Crockett). Air was pumped into a flask containing activated charcoal. Next, the clean air moved through the flow meter and into the flask containing the host-plant complex odor before being pushed into either side of the divided portion of the T-tube. Wasps were introduced into the tube at the opposite end from the odor source.

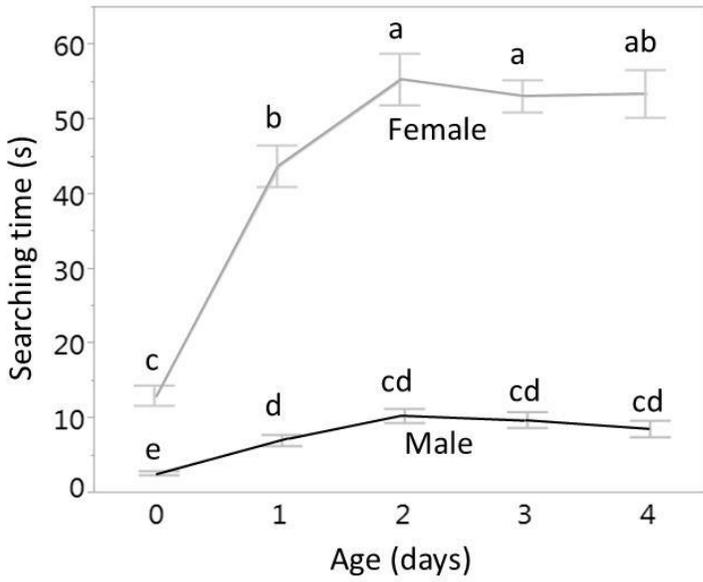


Figure 2. Mean searching times \pm SE of naïve male and female *Cotesia congregata* to cabbage (an attractive non-host plant) (Days 0-4). Both female and male responses peaked at Day 2 and then remained steady through Day 4. Male responses were significantly lower than female responses ($P < 0.0001$).

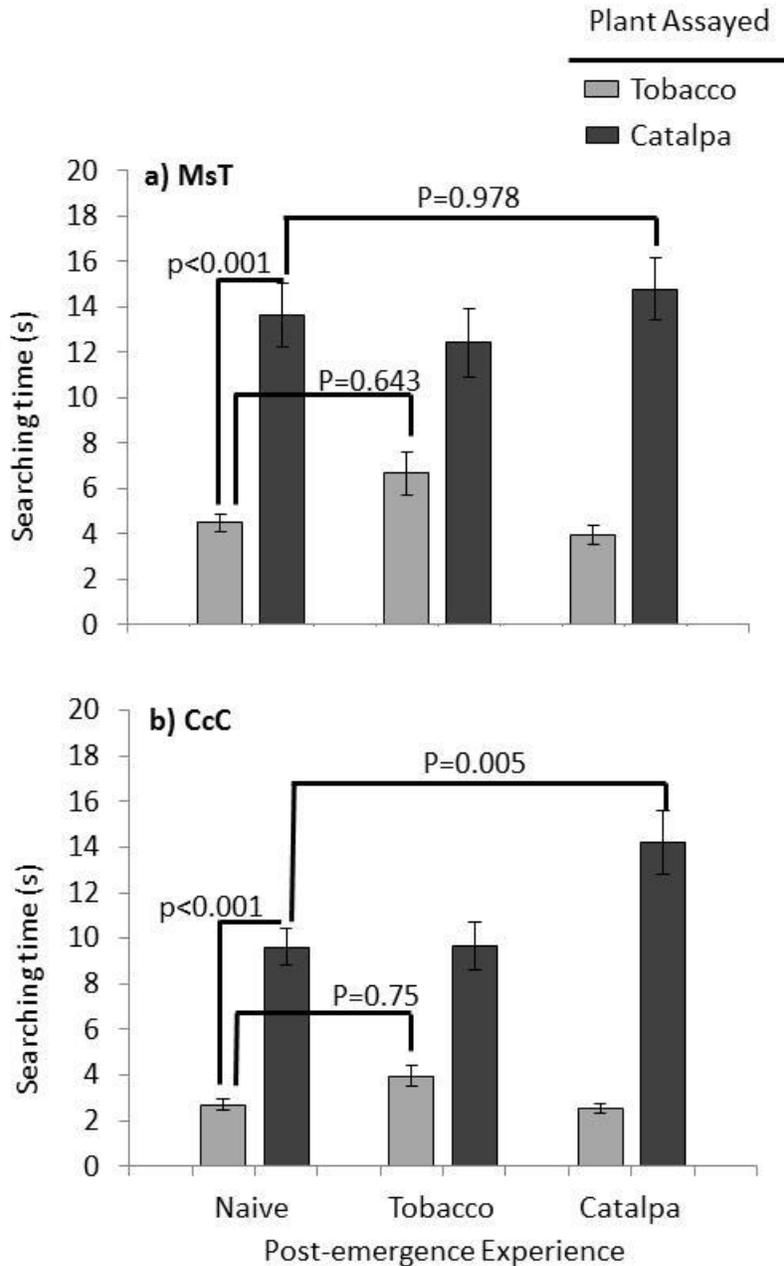


Figure 3. Effects of postemergence experience (naïve, tobacco, or catalpa) on searching responses of male *Cotesia congregata* to two potential host plants (tobacco or catalpa). Each bar represents the mean searching time \pm SE. **a)** MsT (*Manduca sexta* on tobacco) host-plant complex. Searching time was affected by the plant assayed ($P < 0.0001$) where males searched longer on catalpa than tobacco **b)** CcC (*Ceratomia catalpae* on catalpa) host-plant complex. Searching time was also affected by the plant assayed ($P < 0.0001$) where males searched longer on catalpa.

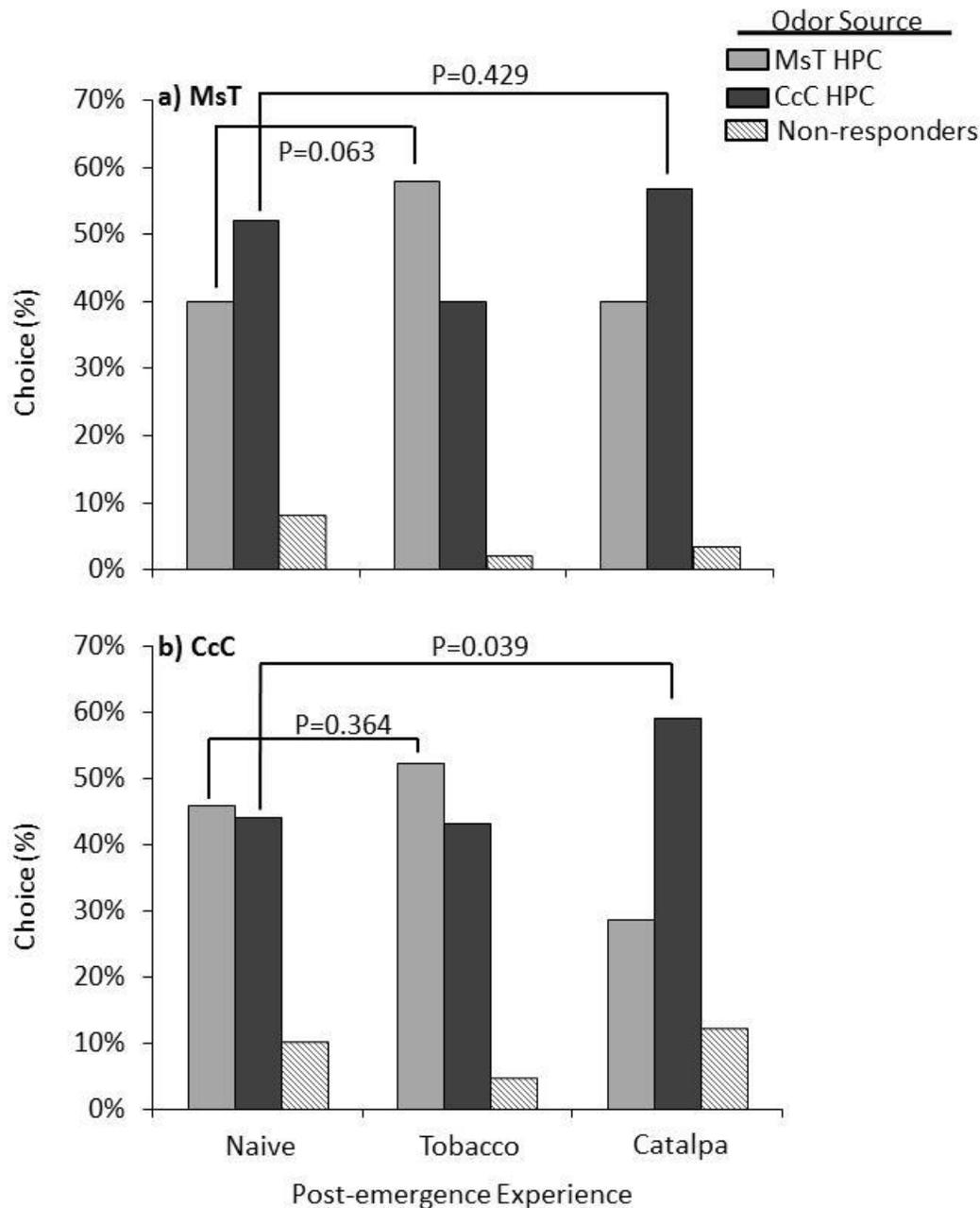


Figure 4. Effects of postemergence experience (naïve, tobacco, or catalpa) on orientation responses of male *Cotesia congregata* to two potential host-plant complex odor sources: MsT (*Manduca sexta* on tobacco) or CcC (*Ceratomia catalpae* on catalpa). Each bar represents the percent of wasps making a choice. Non-responders were not included in data analyses. **a)** MsT host-plant complex. Orientation preference was affected by the plant experienced where males choose to orient towards their natal HPC odor more frequently when experienced with tobacco ($P=0.063$) **b)** CcC host-plant complex. Orientation preference was affected by the plant experienced where males choose to orient towards their natal HPC odor more frequently when experienced with catalpa ($P=0.039$).

Vita

Megan Elizabeth Ayers was born on August 5, 1988 in Norfolk, VA. Coming from a Navy family she moved around a fair bit, living in South Carolina for several years before moving back to Virginia. At age 7, her family moved to Japan and were immersed in Japanese culture for 3 years before returning to the states to spend 2 years in North Carolina before making the final move back to Virginia. Megan spent the next 6 years in Stafford, VA, attending Brooke Point High School and graduating in 2006. She completed her B.S. in Biology at Virginia Commonwealth University in 2011 and worked on a research project focusing on male wasp behavior during her last year. Megan continued working with wasp behavior at VCU during her M.S. degree in Biology. Over the years, she has developed a love for the creepy crawlies, keeping tarantulas, spiders, and insects as pets, and tries to constantly encourage people to overcome their fear and hold a cockroach. Megan plans on teaching Invertebrate Zoology and Entomology Labs at VCU over the summer and fall while continuing research after which she will pursue a PhD in insect behavior or taxonomy and eventually a career in museum work and outreach.