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ORIGINAL PAPER

K. M. Kester · P. Barbosa

Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say)

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Abstract To test the hypothesis that natural enemy populations differ in their behavioral responses to plants or to plant allelochemicals, we compared two populations of the gregarious larval endoparasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae) that differed in their historical and present exposure to tobacco. The major hosts for both populations were *Manduca sexta* L. and *M. quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), but these hosts were typically encountered on tobacco by parasitoids in one population (Upper Marlboro) and on tomato by parasitoids in another population (Wye). Early in the season, Wye parasitoids preferred to oviposit in *M. sexta* on tomato rather than on tobacco and Upper Marlboro parasitoids showed no preference; neither population showed any preference later in the season. Neither of the strains originating from the two populations showed a landing preference for tobacco or tomato in flight chamber trials, but Upper Marlboro parasitoids searched longer on tobacco than on tomato, and Wye parasitoids searched longer on tomato. When nicotine solutions were applied to tobacco leaf, searching responses of Upper Marlboro parasitoids were enhanced by 0.001–1.0% nicotine, and searching responses of Wye parasitoids were decreased by 0.01–1.0% nicotine. We speculate that population differences in searching responses to tobacco and nicotine may explain the differential parasitism responses found early in the season.

Key words Local adaptation · Population differences
Parasitoid behavior · *Cotesia congregata*
Manduca sexta

Introduction

Population differentiation in the behavioral responses of insect parasitoids to plants has rarely been considered experimentally. This lacuna in our knowledge is surprising, since just as phytophagous insects can become locally adapted to abundant foodplants (Fox and Morrow 1981), parasitoids may become locally specialized to search specific plants, thus increasing their ability to exploit their hosts (Kester and Barbosa 1991a). To experimentally explore this possibility, we focused on *Cotesia congregata* (Say) (Hymenoptera: Braconidae), a gregarious endoparasitoid that attacks larvae of *Manduca sexta* L., *M. quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), and other sphingids (Krombein et al. (1979). Both *M. sexta* and *M. quinquemaculata* are restricted to solanaceous foodplants (Hodges 1971) and are often very abundant in areas where these plants are cultivated.

The survival of larval *C. congregata* is directly affected by host-ingested nicotine (the major alkaloid of tobacco) (Parr and Thurston 1972; Thurston and Fox 1972; Thorpe and Barbosa 1986; Bentz and Barbosa 1990; Barbosa et al. 1986, 1990, 1991). The presence of this alkaloid may explain lower rates of parasitism of *M. sexta* on tobacco than on other solanaceous plants (Morgan 1910; Gilmore 1938a, b), or differential rates of parasitism of *M. sexta* on flue and burley tobaccos (Thurston and Fox 1972), or on breeding lines containing high compared to low nicotine concentrations (Thorpe and Barbosa 1986). However, direct behavioral responses of *C. congregata* to tobacco compared to other solanaceous plants, or to nicotine, have never been examined.

In related studies involving two populations of *C. congregata* which differed in their present and historical exposure to tobacco, we found that the population with greater exposure to tobacco showed greater tolerance to dietary nicotine, suggesting at least some degree of adaptation to tobacco (Kester 1991; Kester and Barbosa 1991a). In this paper, we address three questions relating to population differences in the behavioral responses of *C. congregata*: (1) Do females from the two populations

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differ in their ovipositional preferences for hosts on different plants? (2) If so, do they differ in their behavioral responses to plants in the absence of host cues? (3) Do they differ in their behavioral responses to nicotine? Finally, we consider the heritability of a differential behavioral response.

Materials and methods

Study sites and plots

The two study sites used in our work were located within two regions of Maryland that differ in their historical and present abundance of tobacco. One site (Upper Marlboro, "tobacco site") was located west of the Chesapeake Bay at the Tobacco Research Farm, Upper Marlboro, Prince George's County, Maryland (38° 52' N), in a region where tobacco has been intensely cultivated for over 350 years (Heimann 1960; C. McKee, personal communication). The other site (Wye, "tomato site") was located 56 km away and on the eastern shore of the Chesapeake Bay (Delmarva Peninsula) at the Wye Research and Education Center, Wye, Queen Anne's County, Maryland (38° 55' N), in an agriculturally diverse region where tobacco has been rarely cultivated for the past 250 years (Heimann 1960; C. McKee and G. Dively, personal communication). The most abundant host species of *C. congregata* (c. 50–75% of all available sphingids) at both sites were *M. sexta* L. and *M. quinquemaculata* (Haworth) (Lepidoptera: Sphingidae), as determined by black-light trapping (Kester and Barbosa 1991a). Although the two *Manduca* species share the same range of host plants, they have typically been encountered by *C. congregata* on tobacco at the Upper Marlboro site and on tomato at the Wye site. The phenologies of both *Manduca* species were similar at both sites.

Identical study plots were established at each of the two sites. Tomato ("Westover") and tobacco ("NC95") plants were started from seed in a greenhouse and then transplanted to each site. Plots consisted of 16 patches each of tomato and tobacco, each containing 16 plants arranged in four by four rows (512 plants per site). Location of the tomato and tobacco patches within a four by eight arrangement of patches was randomly assigned within the plots. Spacing between plants was 0.46 m; spacing between patches was 0.92 m. Recommended fertilizer, disease and herbicide programs were adjusted to provide a single program for both plant species where possible (McKee 1982; Maryland Cooperative 1985). Nitrogen (18.0 kg/ha in 4-8-12 fertilizer) was applied prior to transplanting and 3 weeks later (9.0 kg/ha in ammonium nitrate). Ridomil (0.59 l/ha) and Devrinol (1.17 l/ha) were applied prior to transplanting. Bravo (0.93 kg/ha) was applied to tomatoes at the green fruit stage, and at weekly intervals thereafter. Insect and weed control were achieved through cultivation and manual removal.

Parasitism preferences of Upper Marlboro and Wye *C. congregata* for *M. sexta* on tobacco or tomato in the field

We tested the hypothesis that the two populations of *C. congregata* differ in their parasitism preferences for *M. sexta* encountered on tomato or tobacco in two separate field experiments by providing similar densities of same-aged larvae on the two plant species. By manipulating the diet on which hornworm larvae were fed prior to their release into study plots, we were also able to control (in the first field experiment) or to test (in the second field experiment) the effects of plant-derived stimuli associated directly with the host on ovipositional choices. The first experiment was initiated in mid-July, when populations of potential hosts and *C. congregata* were relatively small (Kester 1991), both plant species were vegetative and similar in height (8.3±1.2 cm), and available *C. congregata* were most likely those which had overwintered in proximi-

ty to the study plots. The second experiment was initiated in early August, when host and *C. congregata* populations were relatively large (Kester 1991) and both plant species were reproductive. Prior to each experiment, wild hornworms were manually removed from the study plots.

Third-instar hornworms were used in all experiments because they are preferred for oviposition by *C. congregata* (Barbosa et al. 1986), readily switch to tobacco or tomato after feeding on laboratory diet, and do not molt to the fourth instar for 2–3 days after release (K.M. Kester, unpublished). Hornworms used in the first field experiment were reared on a standard laboratory (BioServ Hornworm) diet from hatching and until their release; those used in the second experiment were transferred to tomato or tobacco foliage after the first instar. Hornworms were released (one per plant), recovered 48 h later and then returned to the laboratory where they were held individually until emergence of parasitoids (c. 12–15 days later). Parasitization status of dead hornworms was determined by dissection.

The first field experiment was designed as a two-way analysis of variance (ANOVA) to test the main and interactive effects of parasitoid population (Upper Marlboro or Wye) and plant species (tobacco or tomato) on the number of hornworms parasitized by *C. congregata* per patch. To control for potential host density effects, we adjusted the number of parasitized larvae per patch by entering the number of hornworm larvae recovered per patch as a covariate into the model. This adjustment is consistent with our observations that some released hornworms dropped onto the ground and were attacked by ants within the first 24 h. Insignificant interaction terms involving the covariate were dropped from the model. Prior to analysis, data were transformed by square root (number of parasitized hornworms per patch + 0.5) to correct for normality of residuals; all other assumptions for ANOVA were met. Data were analyzed using SAS GLM (SAS Institute 1985). Means within each site were compared with the PDIF function of SAS GLM. All reported means are actual least squares means (LSMs)±1 SE, generated by performing the described analysis with untransformed data, unless stated otherwise.

The second field experiment was designed as a two-way split-plot ANOVA to test for the main and interactive effects of parasitoid population (Upper Marlboro or Wye), plant species (tobacco or tomato) at the whole-plot level, and pre-release diet (tobacco or tomato foliage) at the split-plot level, on the number of hornworm larvae parasitized by *C. congregata* per patch. Analyses and means comparisons within each site were performed as described above.

Behavioral responses of Upper Marlboro and Wye *C. congregata* to tobacco and tomato

We tested the hypothesis that *C. congregata* originating from the Upper Marlboro and Wye populations would differ in their inherent behavioral responses to tobacco or tomato in the absence of host cues by comparing their landing preferences and searching responses to these plants. The Upper Marlboro strain of *C. congregata* originated from *M. sexta* collected on tobacco at the Upper Marlboro site and the Wye strain originated from *M. sexta* collected on tomato at the Wye site (Kester and Barbosa 1991a); both strains were reared on laboratory diet as described by Barbosa et al. (1991) except that parasitoid cocoons were not detached from their host. Females used in landing and searching experiments were 48–96 h old and had no prior exposure to plants.

Landing preferences were assayed by offering a tomato and tobacco plant in a pull-type flight tunnel (203.2 cm in length×76.2 cm in width×76.2 cm in height), as described in Kester and Barbosa (1992). Prior to each flight trial, plants were artificially damaged by cutting a single 0.8 cm (diameter) hole with a cork borer in the third fully expanded leaf. Plants were arranged to provide overlap in the central area of the odor plume, as visualized in preliminary smoke tests with ethylenediamine and acetic acid (1:1), and adjusted so that their heights, area of artificial damage and presentation to test insects were similar. Left-right orientation of the two plants was switched after every 2–3 trials. All trials were

conducted with a wind speed of 35 cm/s, at $30 \pm 1^\circ\text{C}$ and $62 \pm 7\%$ relative humidity (RH), as determined by periodic hygrometer readings.

In flight trials, a single female was released upwind of the plants by inverting the glass vial (3.7 ml) in which she had been held for 3–5 min. Individual females were tested once only. The experiment was replicated twice, using females from two consecutive generations. Responses for the two generations tested were grouped, because they were not significantly different. The proportion of females choosing to land on the two plants was compared, among and within the two strains, using a generalized Fisher's exact test (Mehta and Patel 1983).

Searching responses of females from the Upper Marlboro and Wye strains of *C. congregata* were compared by offering individual females tomato or tobacco leaf in no-choice trials as described in Kester and Barbosa (1991b). Briefly, each female was held in a 3.7 ml glass vial for 2–6 min and then offered a single leaf disk (0.8 cm diam) cut with a cork borer from the terminal area of the third fully expanded leaf of tobacco or tomato. Total time spent searching on the leaf disk within a 2-min period was measured with a digital stopwatch. Searching was defined as active antennal palpation of any area of the leaf disk. Females were tested once. All searching assays were conducted at $30 \pm 1^\circ\text{C}$ and $67 \pm 7\%$ RH. The experiment was replicated over four generations.

Searching responses were analyzed in a three-way ANOVA to test the main and interactive effects of parasitoid strain (Upper Marlboro or Wye), plant species (tobacco or tomato) and experimental replication (one replication at each of four consecutive generations), on searching time. The three-way interaction of experimental replication, parasitoid population and plant species tests for possible changes in searching responses over the generations tested. For means comparisons in this experiment, $P = 0.05(3)/6 = 0.025$.

Searching response of Upper Marlboro and Wye *C. congregata* to nicotine

In this experiment, we determined whether females of *C. congregata* can detect nicotine and tested the hypothesis that females from the Upper Marlboro and Wye strains differ in their searching responses to different concentrations of nicotine solution applied to tobacco leaf. The range of nicotine concentrations tested (0, 0.001, 0.01, 0.1, or 1.0%) was chosen to include that found in cultivated tobacco varieties (Sisson and Saunders 1983). Nicotine solutions were prepared by making serial dilutions of a stock 10% nicotine (Kodak): acetone solution. Fresh solutions were prepared each day and applied to tobacco leaf with a cotton swab. Nicotine is excreted by trichomes (Thurston et al. 1966) and thus is one of several cuticular compounds of tobacco leaf, but its concentration on the cuticle may not reflect the within-leaf nicotine concentration (Jackson and Severson 1987; D.M. Jackson, personal communication). Therefore, we removed nicotine (and other acetone-soluble cuticular compounds) by swabbing the upper leaf surface with acetone to create a 0% nicotine treatment.

Immediately before each assay, we excised the third fully-expanded leaf from a single greenhouse-grown tobacco plant ("NC95") with a scalpel and trimmed off the outer area of the leaf outside the lateral vein network (c. 1.25–2.5 cm), as well as the base and terminal portions of the leaf, so that 6–7 lateral leaf veins remained intact. Then we removed the mid-vein and each remaining lateral vein to retain 10–12 sections from the central area of the leaf. This eliminated areas of leaf with higher nicotine concentration (terminal portion and edges) or lower nicotine concentration (basal portion), reserving the area with similar (c. 0.01%) nicotine concentration (Kester et al., unpublished). Leaf disks (0.8 cm) were then cut from each remaining section with a cork borer and randomly assigned to one of six glass petri dishes. Each petri dish was randomly assigned to one of six treatment groups: untreated control, 0% nicotine (acetone only), 0.001% nicotine:acetone, 0.01% nicotine:acetone, 0.1% nicotine:acetone, or 1.0% nicotine:acetone.

This experiment was designed as a two-way ANOVA to test the main effects of parasitoid strain (Upper Marlboro or Wye) and nicotine concentration [whole leaf control, 0% (solvent control), 0.001%, 0.01%, 0.1% or 1.0% nicotine], as blocked by experiment replication (six replications), on searching time. The experiment was blocked to adjust for slight fluctuations in abiotic factors that can affect searching responses, e.g., barometric pressure (Kester, unpublished). Treatment means were compared using the PDIFF function of SAS GLM and Bonferroni's method for adjusting the P -value: $P = 0.05$ (number of possible independent comparisons)/number of comparisons made. For this experiment, $P = 0.05$ (11 possible independent comparisons)/15 comparisons made) = 0.0367.

Searching responses of F_1 crosses of Upper Marlboro and Wye *C. congregata* to tobacco or tomato

In this experiment, we evaluated the heritability of differential searching responses and tested the hypothesis that these searching responses differed for F_1 hybrid females resulting from reciprocal crosses between the Upper Marlboro and Wye strains of *C. congregata*. Methods were the same as described for the two strains above. This experiment was designed as a two-way ANOVA to test the main and interactive effects of hybrid cross (Upper Marlboro females \times Wye males or Wye females \times Upper Marlboro males) and plant species (tobacco or tomato), on searching time. Means were compared as described in the preceding section [$P = 0.05(3)/6 = 0.025$].

Results

Field parasitism preferences

Early in the season, the two populations of *C. congregata* differed in their parasitism preferences for *M. sexta* on tobacco or tomato (Table 1, Fig. 1A). Parasitoids at Upper Marlboro ("tobacco population") showed equal preferences for hornworms on the two plants ($P = 0.7161$), whereas parasitoids at Wye ("tomato population") preferred to parasitize hornworms on tomato rather than on tobacco ($P = 0.0028$). Mean percent parasitism of horn-

Table 1 Results of analysis of variance performed to test the effects of parasitoid population (Upper Marlboro or Wye) and plant species (tobacco or tomato) on the number of hornworms (*Manduca sexta* L.) parasitized per patch by *Cotesia congregata* (Say). Experiment was conducted to July 14–16, 1987 at Upper Marlboro ("tobacco site") and July 15–17, 1987 at Wye ("tomato site"). Analysis was performed using SAS GLM, SS3, (SAS Institute 1985). Prior to analysis, data were transformed by \sqrt{x} (no. hornworms parasitized per patch + 0.5) to correct for normality of residuals; all other assumptions for ANOVA were met. "Number of Hornworms Recovered per Patch" was entered as a covariate to adjust for potential host density effects

Source of variation	df	F-value	P
Number of hornworm recovered per patch	1	8.09	0.0062
Parasitoid population	1	7.83	0.0070
Plant species	1	3.68	0.0607
Parasitoid population \times plant species	1	5.77	0.0196
Error	61		

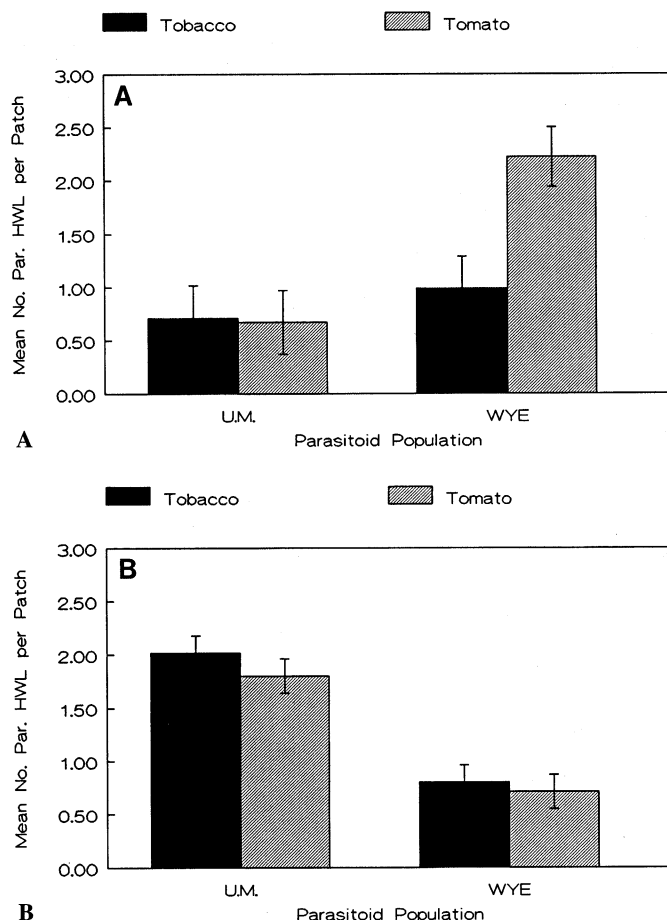


Fig. 1 A,B Parasitism of hornworm (*Manduca sexta* L.) larvae on tobacco (solid bars) or tomato (shaded bars) by two populations of *Cotesia congregata* (Say); Upper Marlboro (U.M.) = "tobacco population", Wye = "tomato population". **A** July 1987. Each bar represents the least squares mean (LSM) \pm SE response for 15–16 patches. U.M. tobacco vs U.M. tomato, $P = 0.7161$; Wye tobacco vs Wye tomato, $P = 0.0028$. **B** 1–4 August 1987. Each bar represents the LSM \pm SE response for 16 patches. U.M. tobacco vs U.M. tomato, $P = 0.9829$; Wye tobacco vs Wye tomato, $P = 0.9727$.

worms by *C. congregata* at Upper Marlboro was $11.7 \pm 3.4\%$ per patch ($12.9 \pm 4.6\%$ on tobacco and $10.5 \pm 4.9\%$ on tomato). Mean percent parasitism of hornworms by *C. congregata* at Wye was $30.0 \pm 3.2\%$ per patch ($17.0 \pm 4.6\%$ on tobacco and $43.1 \pm 4.6\%$ on tomato). Over the 48-h test period, the day/night temperature averaged $29 \pm 3/14 \pm 1^\circ\text{C}$ at Upper Marlboro, and $27 \pm 2/17 \pm 2^\circ\text{C}$ at Wye; 0.13 cm of rain was recorded during the first night at Wye.

Later in the season, the two populations of *C. congregata* did not differ in their parasitism preferences for *M. sexta* on tobacco or tomato and both populations (pooled) showed equal preferences for hornworms on tobacco or tomato, as indicated by the insignificant effects of parasitoid population and plant species, and of plant species (Table 2, Fig. 1B). Both parasitoid populations showed equal parasitism preferences for *M. sexta* on tobacco or tomato, regardless of the pre-release diet on which these hornworms had fed, as indicated by the in-

Table 2 Results of analysis of variance performed to test the effects of parasitoid population (Upper Marlboro or Wye), plant species (tobacco or tomato) and pre-release diet (tobacco or tomato foliage), on the number of hornworms (*Manduca sexta* L.) parasitized per patch. Experiment was conducted August 3–5, 1987 at Wye ("tomato site") and August 4–6, 1987 at Upper Marlboro ("tobacco site"). Analysis was performed using SAS GLM, SS3, (SAS Institute, 1985). Prior to analysis, data were transformed by $\sqrt{\text{no. hornworms parasitized per patch} + 0.5}$ to correct for normality of residuals; all other assumption for ANOVA were met. "Number of Hornworms Recovered per Patch" was entered as a covariate to adjust for potential host density effects. Main effect of "Plant Species" was tested over full error term since Plant Species \times Replicates effect was not significant

Source of variation	df	F-value	P
Number of hornworms recovered per patch	1	40.98	0.0001
Parasitoid population	1	58.71	0.0001
Plant species	1	0.03	0.8545
Plant species \times replicates	30	1.24	0.2162
Pre-release diet	1	1.09	0.2983
Parasitoid population \times plant species	1	0.04	0.8343
Parasitoid population \times pre-release diet	1	0.18	0.6737
Plant species \times pre-release diet	1	0.04	0.8341
Parasitoid population \times plant species \times pre-release diet	1	2.33	0.1307
Error	127		

significant effects of parasitoid population, plant species and pre-release diet, of plant species and pre-release diet, and of plant species. Rates of parasitism were best explained by differences in overall parasitism rates between the populations ($56.1 \pm 3.6\%$ per patch at Upper Marlboro and $22.2 \pm 3.7\%$ per patch at Wye). At Upper Marlboro, mean percent parasitism of *M. sexta* ranged from $44.5 \pm 7.3\%$ per patch for hornworms fed on tomato and released onto tobacco, to $65.1 \pm 7.3\%$ per patch for hornworms fed on tobacco and released onto tobacco. At Wye, mean percent parasitism of *M. sexta* ranged from $22.0 \pm 7.3\%$ per patch for hornworms fed on tomato and released onto tomato, to $22.4 \pm 7.6\%$ for hornworms fed on tobacco and released onto tomato. Over the 48-h test period, the day/night temperature averaged $34 \pm 2/22 \pm 1^\circ\text{C}$ at Upper Marlboro and $31 \pm 1/23 \pm 1^\circ\text{C}$ at Wye; no precipitation was recorded at either site during the test period.

Behavioral responses to tobacco and tomato

Females from the Upper Marlboro and Wye strains of *C. congregata* did not differ in their landing preferences for tobacco or tomato, and neither Upper Marlboro nor Wye females showed any preference for landing on one plant over the other ($P \geq 0.05$, generalized Fisher's exact test), as shown in Fig. 2A. However, Upper Marlboro ("tobac-

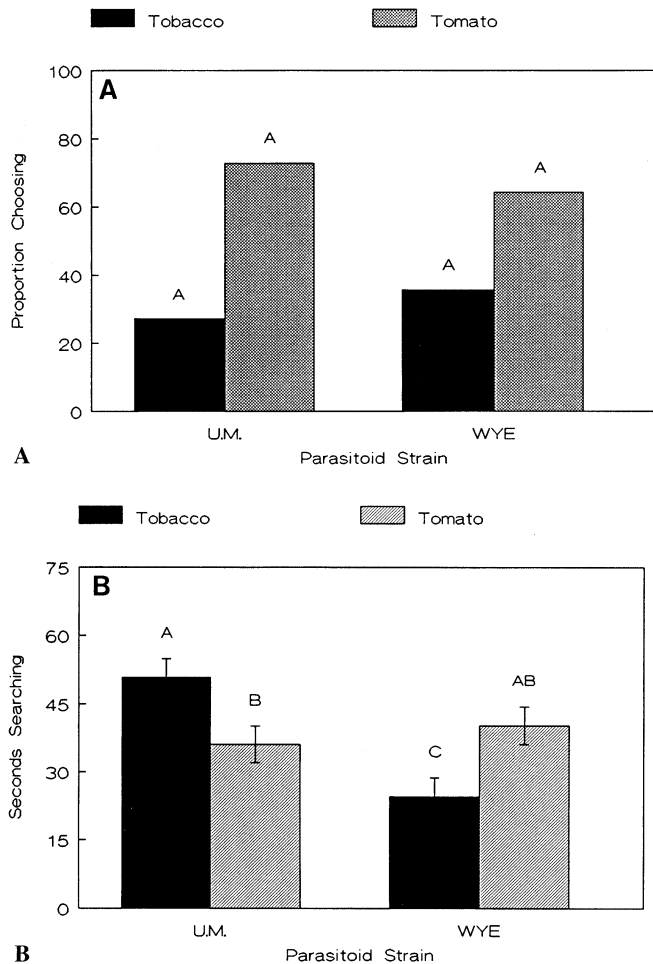


Fig. 2 A,B Behavioral responses of two strains of *Cotesia congregata* (Say) to tobacco (solid bars) or tomato (hatched bars); Upper Marlboro (U.M.) = "tobacco strain", Wye = "tomato strain". **A** Landing preferences in choice trials. Strains (U.M., $n = 22$; Wye, $n = 14$) did not differ in their responses and neither strain preferred to land on one plant over the other ($P > 0.05$), as determined by a generalized Fisher's exact test (Mehta and Patel 1983). **B** Searching responses in no-choice trials. Each bar represents the $LSM \pm SE$ response of 35 females over 4 generations. Means with different letters differ significantly ($P \leq 0.0117$)

co strain") females searched longer on tobacco than on tomato and Wye ("tomato strain") females searched longer on tomato than on tobacco, as shown in Fig. 2B. These searching responses did not change over the four generations tested, as indicated by the insignificant three-way interaction of experimental replication, parasitoid strain and plant species (Table 3).

Searching responses to nicotine

The two parasitoid strains differed in their responses to the concentration of nicotine solution applied to tobacco leaf, as indicated by the significant two-way interaction of parasitoid strain and nicotine concentration ($P = 0.0001$, 311 *df*; Fig. 3). Main effects of experimental replication, parasitoid strain, and nicotine concentration

Table 3 Results of analysis of variance to test the effects of experimental replication (over four consecutive generations), parasitoid strain (Upper Marlboro or Wye), and plant species (tobacco or tomato), on searching time of *Cotesia congregata* (Say). Analysis was performed using SAS GLM, SS3 (SAS Institute 1985). Prior to analysis, data were transformed by $\sqrt{\text{secs. searching} + 0.5}$ to correct for normality of residuals; all other assumption for ANOVA were met

Source of variation	df	F-value	P
Experimental replication	3	11.81	0.0001
Parasitoid strain	1	6.85	0.0100
Plant species	1	0.39	0.5350
Experimental replication	3	0.46	0.7096
xparasitoid strain			
Experimental replication	3	0.56	0.6430
xplant species			
Parasitoid strain	1	17.46	0.0001
xplant species			
Experimental replication	3	0.43	0.7289
xparasitoid strain			
xplant species			
Error	140		

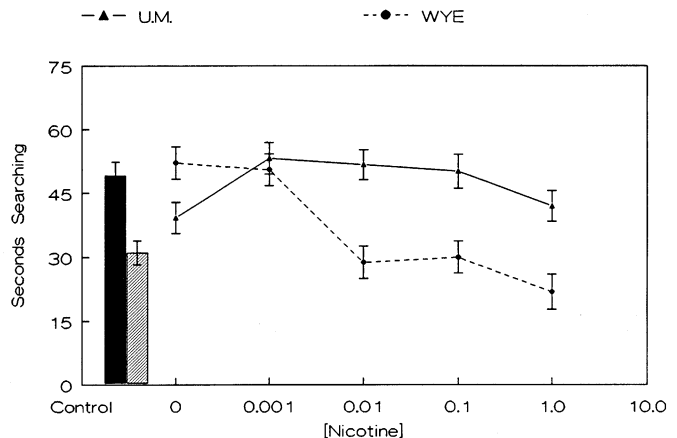


Fig. 3 Searching response of two strains of *Cotesia congregata* (Say) to nicotine applied to tobacco leaf; Upper Marlboro = "tobacco strain" (solid line, triangles); Wye = "tomato strain" (dashed line, dots). Each bar or point represents the $LSM \pm SE$ response of 26 females. For control (tobacco leaf control), solid bar = U.M. strain, shaded bar = Wye strain. Strains differed in their responses to nicotine concentration ($P = 0.0001$). See text for further explanation

were also significant (P Symbol $> £0.0001$). Overall, the Upper Marlboro ("tobacco strain") females responded to nicotine as a stimulant, in that they searched longer on the control (tobacco leaf with no added acetone or nicotine solution) than on tobacco leaf with 0% nicotine (surface nicotine removed by swabbing with acetone) ($P = 0.0292$), and searched as long on tobacco leaf with added nicotine (0.001, 0.01 or 0.1% nicotine/acetone solutions) as they did on the control leaf disk ($P = 0.2227$). In contrast, Wye ("tomato strain") females responded to nicotine as a deterrent, in that they searched longer on tobacco leaf with 0% nicotine or with 0.001% nicotine than they did on the whole-leaf control ($P = 0.0001$), and searched as long on tobacco leaf with 0.01, 0.1 or 1.0%

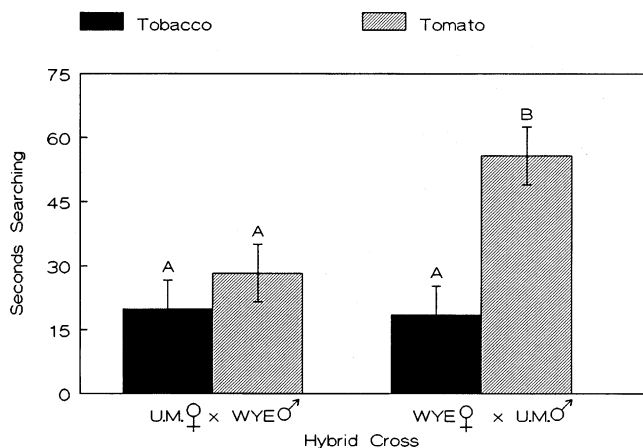


Fig. 4 Searching responses of F₁ hybrid females from reciprocal crosses between two strains of *Cotesia congregata* (Say); Upper Marlboro (U.M.) = “tobacco strain”, Wye = “tomato strain”. Each bar represents the LSM±SE response of 10 females (solid bars tobacco, shaded bars tomato). Strains differed in their responses to plant species ($P = 0.0397$). Means with different letters differ significantly ($P \leq 0.0066$)

nicotine as they did on the control leaf disk ($P = 0.0639$). Overall, these results demonstrate that both strains detect nicotine and that Upper Marlboro females may perceive lower concentrations of nicotine than the Wye females. These results also suggest that differential searching responses of the two strains to tobacco reflect differential searching responses to nicotine.

Searching responses of F₁ hybrids to tobacco and tomato

Females resulting from reciprocal crosses between the Upper Marlboro and Wye strains of *C. congregata* differed in their searching responses to tobacco and tomato, as indicated by the significant two-way interaction of hybrid cross and plant species ($P = 0.0397$, 39 *df*; main effects: hybrid cross ($P = 0.0602$) and plant species ($P = 0.0017$; Fig. 4). Progeny of Upper Marlboro (“tobacco strain”) females and Wye (“tomato strain”) males searched as long on tobacco as they did on tomato ($P = 0.3824$), whereas progeny of Wye females and Upper Marlboro males searched longer on tomato than on tobacco ($P = 0.0066$). These results suggest that searching responses to tobacco and to nicotine exhibited by Upper Marlboro females (Figs. 2B, 3) are determined by the genetic contribution of both parents.

Discussion

Our results demonstrate that the Upper Marlboro and Wye strains of *C. congregata* differed in their searching responses to tobacco and that their responses may be related to differential responses to nicotine (Figs. 2B, 3). Further, these responses are consistent with the differen-

tial survival responses of these strains (Kester 1991; Kester and Barbosa 1991a) and their historical association with tobacco. Upper Marlboro females, originating from an area of intense tobacco cultivation, searched longer on tobacco than on tomato, and searched longer on tobacco leaf with added (0.001–1.0%) nicotine than on tobacco leaf with 0% nicotine (acetone treatment). In contrast, Wye females, recovered from an area where tobacco cultivation is rare, searched longer on tomato than on tobacco, and searched longer on tobacco leaf with 0% nicotine (acetone treatment) than on tobacco leaf with added (0.01–1.0%) nicotine. Since both strains were reared and tested under similar conditions at the same time, and their searching responses to tobacco and tomato remained consistent over four generations, we conclude that these differences in searching responses are genetically based. The preliminary crossing experiment suggests that the “positive” searching response to tobacco of Upper Marlboro females requires both a maternal and paternal contribution, since females resulting from crosses between Upper Marlboro females and Wye males did not search longer on tobacco than on tomato, and females resulting from crosses between Wye females and Upper Marlboro males searched longer on tomato than tobacco (Fig. 4). Such genetically based differences in searching responses of *C. congregata* may be facilitated by post-emergence learning (Kester and Barbosa 1991b).

Although the two strains differed in their searching responses to tobacco and tomato, they did not differ in their landing responses to these plants; neither plant was preferred for landing over the other (Fig. 2A). In related studies, we have found that searching responses and landing preferences of *C. congregata* are often disparate (Kester and Barbosa 1992). Similar disparities in pre-alighting and post-alighting responses to plants have been reported for herbivores (Rauscher et al. 1981; Stanton 1982; Papaj and Rauscher 1983). At least in some cases, since butterflies will alight on more plants than they will oviposit on, population differences in ovipositional preferences for specific plants may be reflected in differential post-alighting responses (MacKay 1985). In combination, the similarity of landing preferences and differential searching responses of the Upper Marlboro and Wye strains of *C. congregata* may explain the differential ovipositional preferences of the Upper Marlboro and Wye field populations detected in our first field experiment, i.e., hosts on tobacco or tomato were equally acceptable to Upper Marlboro parasitoids, but hosts on tomato were preferred over hosts on tobacco by Wye parasitoids (Fig. 1A). However, as indicated by the absence of parasitism preferences found for both populations of *C. congregata* later in the season (Fig. 1B), factors other than inherent behavioral responses to plants are obviously important. Alternatively, the design of our study plots may have obscured possible landing preferences for either of the plant species due to the relative proximity of treatment plots, i.e., more widely separated treatment plots might have permitted finer pre-landing discrimination of plant species by parasitoid females, possibly re-

sulting in differential parasitism rates. However, as discussed above, differential parasitism rates of *C. congregata* most likely reflect differential post-alighting responses to plants that would not be affected by the relative distance of treatment plots.

The disappearance of parasitism preferences we observed as the season progressed has important implications for how our findings are viewed. In particular, near the end of the growing season, inherent behavioral preferences exerted negligible influence on patterns of parasitism under field conditions. However, early in the season, when host and parasitoid populations were small, inherent preferences produced marked non-random effects. These early season circumstances correspond to those conditions under which many biocontrol projects are initiated, which suggests that an understanding of inherent responses to plant cues could be crucial to the success of biocontrol projects involving parasitoids, especially in the early stages of establishment. Most likely, inherent tendencies were less predictive in the late than in the early season due to the overriding influences of learning and host experience that accumulate over time. The interplay of inherent tendencies and learning needs to be better understood if we are to genuinely predict the response of parasitoids to complex environments.

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