Effects of Coloration on Parasitism, Predation, and Field Survivorship of the Tobacco Hornworm, *Manduca sexta* (L.)[1]

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ABSTRACT Studies were conducted to determine the effect of pigmentation on the field survivorship of larvae of the tobacco hornworm, *Manduca sexta* (L.). Larvae of the white-mutant tobacco hornworm were found to have a lower mean survival rate as compared with black-mutant and wild-type strains. Tests in the greenhouse demonstrated the larvae of the white-mutant were able to establish on the tobacco plants as well as the black-mutant and wild-type strains. Differential predation seems to be an important factor in the low recovery of the white tobacco hornworm in the field. Parasitism rates by *Cotesia congregata* (Say) were low for all three strains.

KEY WORDS Insecta, *Manduca sexta,* insecticyanin, *Cotesia congregata,* predation, camouflage.

The tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), is a pest of many solanaceous crops (Davidson and Lyon 1979). The normal green coloration provides a protective camouflage to this phytophagous insect (Dahlman 1969, Kawooya et al. 1985). A mutant of the tobacco hornworm has previously been found to form black melanized cuticle in the fourth and fifth larval instars (Safranek and Riddiford 1975). However, in the first to the third instars, the black mutant has the same apparent coloration as the wild-type larvae. The black phenotype has been attributed to low juvenile hormone levels and is caused by a single sex-linked, recessive gene whose expression can be changed by one or more modifier gene. Addition of juvenile hormones to the head capsule of molting penultimate instars produced terminal instar larvae with normal cuticle coloration (Safranek and Riddiford 1975). Dahlman (1969) reported the occasional presence of a light yellow or white color form of the tobacco hornworm in burley tobacco in Kentucky. He examined the relative concentrations of insecticyanin in plant-reared wild-type, diet-reared wild-type, and white mutant tobacco hornworm larvae. The genetics and field ecology of this white mutant were not studied as the colony was difficult to maintain. In 1988, a white mutant of the tobacco hornworm was observed in the laboratory

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colony maintained by the Department of Entomology, North Carolina State University in Raleigh. The white mutant was isolated and a laboratory colony was established. Genetic analysis by Lampert et al. (1990) revealed that the white larval phenotype was controlled by a single recessive gene.

Camouflage seems to play a major role of survival of tobacco hornworms in the field. Thurston and Prachuabmoh (1971) reported heavy predation by the common grackle, *Quisculus quiscula* (L.), on wild-type tobacco hornworms in the fields of Kentucky. In North Carolina, Rabb and Lawson (1957) reported that *Polistes* sp. wasps were the major predators of hornworms, while Lawson (1959) ranked *Cotesia congregata* (Say) (= *Apantales congregatus*) as the major parasitoid of tobacco hornworm. Rabb and Thurston (1969) reported that *C. congregata* seemed to have an important effect on the mortality of the third and fourth generations of tobacco hornworm in the field. In the late season, predation by *Polistes* was negligible, but parasitoids reduced the numbers of tobacco hornworm population significantly.

The objective of the work reported here was to determine the field survivorship of white tobacco hornworm as compared with black-mutant and wild-type larvae, especially with regard to the effect of pigmentation on natural predation and parasitism.

Materials and Methods

Determination of appropriate number of neonates on tobacco plants. A study was conducted in the greenhouse to determine the number of neonates that could be released on a tobacco plant without reducing survival. Twelve potted tobacco plants (*Nicotiana tabacum* (L.) var. 'McNair 373') with approximately 10 leaves per plant were selected and treated with Safer Insecticidal Soap (Safer Inc., Newton, Mass.) to remove any aphids or whiteflies. Two days later, these plants were infested with either 3 or 10 wild-type neonates (one-day-old larvae) per plant in a randomized complete block design with 6 blocks. The neonates were placed individually in the bud of each tobacco plant using a camel-hair brush. This study was conducted in the greenhouse under natural light conditions with a temperature of 24 ± 5°C. Two and four days after infestation, the number of surviving larvae per plant was determined, the proportion of larvae that established was calculated, and an analysis of variance (ANOVA) was performed after transforming the data by taking the arcsine (square root (proportion)) (SAS Institute 1982).

Establishment of neonates on tobacco plants in the greenhouse. A study was conducted to determine the percent establishment, under greenhouse conditions, of wild-type larvae and white and black mutants. This study was conducted in the absence of parasites and predators using plants grown in the greenhouse under natural light conditions with a temperature of 24 ± 5°C. Twelve potted tobacco plants (prepared as above) were selected and three neonates (one-day-old larvae) belonging to the same strain were placed individually into the bud of each plant using a camel-hair brush. Neonates were assigned to the plants in a randomized complete block design with 4 blocks. Two and four days after release, the number of surviving larvae was recorded. An ANOVA (SAS Institute 1982) was performed after transforming the data to square root (counts + 0.05).

Field survival of neonates on tobacco plants. Field experiments were conducted at the Central Crops Research Station, Clayton, N. C. during the summers of 1989 and 1990 to examine the survival of the white-mutant, wild-type, and black-mutant strains of tobacco hornworm under field conditions. The day before the test, tobacco plants in the plots were carefully examined and cleaned of all naturally occurring tobacco hornworm eggs and larvae. Twenty healthy tobacco plants (var. 'McNair 373') were selected and tagged with colored vinyl ribbons for each block. Three different strains of the tobacco hornworm were assigned to each block according to a randomized complete block design. The number of replicates varied from 3-6 during the course of the field studies, depending on the availability of plants.

The preliminary test was conducted from 9 to 12 September 1989 on "cutback" tobacco plants with approximately 10 leaves per plant. "Cutback" tobacco is flowering tobacco plants that were cut at approximately 3-5 leaf nodes above the ground to promote secondary growth. Each plot was 4 rows by 13.7 m with 20 plants per row. The preliminary test had 3 replicates (60 neonates released per plot). The second and third tests were conducted from the 20 to 23 June 1990 and 30 July to 2 August 1990, respectively, on tobacco plants with approximately 15-20 leaves per plant. These tests had 6 replicates each (60 neonates released per plot). The fourth and final test was conducted from 1 to 4 September 1990 on "cut-back" tobacco plants with approximately 10 leaves per plant. The last test had 5 replicates (60 neonates released per plot). On the morning of each test, 3 neonate tobacco hornworms of the same strain were placed individually into the bud of each tobacco plant with a camel hair brush. On flowering plants, which lack a terminal bud, larvae were placed on the apical leaves near the flower of the tobacco plant. Two and four days after release, the number of larvae surviving on each plant was recorded. Any neonates resulting from the immigration of ovipositing females could be detected based on the size of the larvae and were discarded when observed. While visually monitoring hornworm larvae on tagged plants, the presence of parasitoids, natural predators, and signs of attack by predators were noted and recorded. Typical signs of attack by predators included scars on the integument of hornworm larvae or signs of struggle on the leaves, including stains on the leaf surface from stomach contents or other body fluids and body fragments found frequently in association with torn or damaged leaves. After four days, all surviving larvae were collected, returned to the laboratory (16L: 8D, 26°C) and reared on artificial diet (Yamamoto 1969) until pupation. Parasitism by *Cotesia congregata* (Say), a braconid wasp which parasitizes tobacco hornworm larvae during earlier instars, was determined by direct observations of parasitoid cocoons on fifth instars by the presence of parasitoid eggs or larvae in dissected *M. sexta*. An ANOVA (SAS Institute 1982) was conducted on field survivorship after transforming the count data by square root (count + 0.05). A Waller-Duncan K-ratio t test was performed on the results to determine significant differences in the mean recovery from these three hornworm strains.

Results and Discussion

Determination of appropriate number of neonates to release on tobacco plants. At two and four days after the introduction of neonates to tobacco plants in the greenhouse, there was no significant difference in the percent recovery of tobacco hornworm larvae from plants initially infested with 3 neonates versus plants with 10 neonates (Table 1). Recovery rate was approximately 50% overall. Because the differences in establishment were not significant between initial infestation with 3 larvae per plant and 10 larvae per plant, it was decided to use 3 larvae per plant in future tests to reduce resources (larvae, diet, rearing containers, time to infect plants, etc.) needed for field experiments.

Table 1. Percent recovery of wild-type tobacco hornworm larvae 2 and 4 days after release on tobacco plants in the greenhouse.

	Plant recovery*	
Larvae per plant	2 days	4 days
3	55.6	66.7
10	48.3	46.7
F	0.33	0.44
df	1,5	1,5
$P > F$	0.591	0.540

* The percent recovery data was transformed into an arcsine (square root (percent/100)) before ANOVA was performed. Means of raw untransformed data are reported.

Field survivorship of white-mutant, wild-type, and black-mutant tobacco hornworm larvae. The mean number of tobacco hornworms and percent recovered 4 days after release from the 4 field tests are shown in Table 2. The mean number of white tobacco hornworm recovered was significantly lower than that of the wild-type and black-mutant tobacco hornworm larvae in all tests except 1 to 4 September 1990. In 3 of the 4 tests, similar numbers of wild-type and black-mutant hornworm larvae were recovered. The low recovery of white tobacco hornworms could be due to differences in establishment between strains, higher parasitism, or higher predation.

Note that in the September 1990 field test the mean number of white tobacco hornworm larvae recorded was statistically similar to the number of wild-type larvae recovered and significantly exceeded the number of black-mutant larvae recovered. These results were different from the earlier field tests; the reason for this is uncertain. The number of natural predators and parasitoids present in the field varied in composition and density among the four tests. This may partially explain the different patterns in recovery of the three strains in this test.

Table 2. Mean number of tobacco hornworm larvae of the three different strains recovered from flue-cured tobacco 4 days after release in field tests conducted during the summers of 1989 and 1990. Central Crops Research Station, Clayton, NC.*

Hornworm strain	9-12 September 1989	20-23 June 1990	30 July - 2 August 1990	1-4 September 1990
White	4.33 B (7.2%)	1.83 C (3.05%)	7.25 B (12.08%)	16.80 A (28.0%)
Wild	9.67 A (16.11%)	6.67 B (11.11%)	21.33 A (35.55%)	14.80 AB (24.66%)
Black	10.00 A (16.66%)	10.83 A (18.05%)	23.33 A (38.88%)	10.00 B (16.66%)
F	8.95	22.94	15.68	5.76
df	2,4	2,10	2,8	2,8
Pr > F	0.033	0.0002	0.002	0.028

* Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test, K = 100). Counts were transformed to square root (count + 0.05) before conducting the ANOVA. Means of raw (untransformed) counts are presented. Percent recovery per plot of the three different strains of neonates four days after release is given in parenthesis.

In addition, it has been shown that the rate of nicotine accumulation increases after plants are topped in the field by removing their flowering structures (Bush and Saunders 1977). Although tobacco hornworms can detoxify or excrete these deleterious tobacco constituents, these processes are energy consuming and significant negative correlations between nicotine concentrations in diet and growth (e. g., reduced larval weight, reduced pupal weight, and increased development time) have been observed (Barbosa 1988). These differences between the tobacco leaf chemistry of older plants in the field as compared with relatively younger plants in the greenhouse could have contributed to the poor establishment of the white-mutant tobacco hornworm in the field.

Establishment of the three hornworm strains under greenhouse conditions. There were no significant differences (F = 2.99; df = 2, 11; $P > F$ = 0.101) in the recovery of the 3 strains when reared for 4 days on tobacco plants grown in the greenhouse. Of the 3 larvae introduced per plant, the mean number of larvae recovered for the white-mutant, wild-type and black-mutant strains were 1.80 (SEM = 0.07, N = 4), 1.48 (SEM = 0.16, N = 4), and 1.80 (SEM = 0.07, N = 4), respectively. This suggests that the low recovery of the white mutant in the field was not due to the mutant's inability to feed and establish on tobacco plants.

Predation. The percent of known predation for white-mutant, wild-type, and black-mutant tobacco hornworm larvae were 1.55, 0, and 0%, respectively, in 1989; 1.1, 0.6, and 0%, respectively, in June 1990; 0.55, 0.27, and 0.27%, respectively, in August 1990; and 1, 1, and 0.33%, respectively, in September 1990. Due to extremely low predation, no statistical comparisons could be conducted. During the two seasons of field testing, the following predators were observed preying on hornworm larvae on one or more occasions: *Polistes* wasps

(Hymenoptera: Vespidae), green stink bug (*Acrosternum hilare* (Say) (Hemiptera: Pentatomidae)), stilt bug (*Jalysus wickhami* Van Duzee (Hemiptera: Berytidae)), and lady bird beetles (Coleoptera: Coccinellidae). Lawson (1959) conducted field studies to investigate the disappearance of first instar wild-type larvae and reported most of the predators found in our study actively preyed upon larvae in the field. In an earlier study, Rabb and Lawson (1957) reported that *Polistes* sp. wasps were the major predator of tobacco hornworms in North Carolina and that *Polistes* wasps mainly select third and fourth instar tobacco hornworm larvae to bring to their nest. However, these wasps were observed to kill and feed on smaller larvae as well. In a sample of 37 tobacco hornworm populations, Lawson (1959) found the average generation mortality of the tobacco hornworm was nearly 98%, with more than half of the mortality due to *Polistes* wasps. The higher predation of white larvae observed in our study could be due to the fact that predators are primarily visual hunters (Bishop and Cook 1975, Kettlewell 1959, Thurston and Prachuabmoh 1971), and the larvae of the black-mutant and wild-type tobacco hornworm are better camouflaged than the white larvae, which became yellow when feeding on tobacco plants.

Parasitism. Percent parasitism by *C. congregata* on tobacco hornworm larvae recovered from field tests was quite low, and results from all tests were pooled together. Parasitism of the white-mutant was 1.4% as compared with 2.2% and 1.7% of the wild and black strains. It appears as though all three strains of tobacco hornworm larvae have an equal level of parasitism by *C. congregata* in the field. However, parasitism rates were very low and no distinct preferences could be detected.

Parasitoids have been shown to be adapted to chemicals or kairomones rather than visual cues (Vinson 1975, Weseloh 1981), and the parasitoids still may have recognized the white mutant as a host. While occurrences of confirmed predation were low for all strains, it appears that differential predation is a likely factor accounting for increased mortality of white hornworm in the field. Most hornworm predators are visual hunters, e. g., birds, *Polistes* spp., and field spiders (Lawson 1959, Thurston and Prachuabmoh 1971). White hornworms sequester carotenoid pigments from the plant but lack insecticyanin (Cherbas 1973). Thus, they appear yellow on the tobacco plant and not as well camouflaged as the wild-type strain and black-mutant tobacco hornworm larvae. Other environmental factors acting in the field, but not in the greenhouse, cannot on the basis of this study be ruled out. However, collectively, these studies suggest that increased predation is an important factor in reduced survival of the insects.

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