GENETICS OF HORNWORM RESISTANCE IN MARYLAND TOBACCO[1]

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During outbreak years, the two species complex of tobacco hornworms, *Manduca sexta* (L.) and tomato hornworms, *M. quinquemaculata* (Haworth), may cause considerable economic loss to Maryland tobacco, *Nicotiana tabacum* (L.). This study was designed to aid breeding efforts by providing basic quantitative genetic information on the inheritance of host-plant resistance to hornworm damage in Maryland tobacco and to evaluate methods of rating this resistance in a breeding program.

A two-year study was conducted to evaluate 'MD 341' and 'MD 872' (two Maryland tobacco cultivars), I-35 (source of hornworm resistance), and the F1, F2, and both backcross populations (BC1 and BC2) derived from each cross of MD 341 × I-35 and MD 872 × I-35. Measures of resistance evaluated included the number of eggs and larvae found on each plant and visual damage ratings.

Low egg and larval counts and low visual damage ratings appear to be related to hornworm resistance. These measures of resistance indicate that host-plant resistance is a partially dominant trait in Maryland tobacco. Significant additive effects were observed for the egg and larval counts. This indicates that there are several genes acting in an additive fashion for resistance as measured by egg and larval counts and that accumulation of desirable factors through selection is possible. Visual damage ratings as utilized in this study would not be as useful as quantitative measures of insect activity. However, better separation into more distinct classes and ratings taken on pre-flowering plants could aid in increasing the precision of this method of rating resistance. A modified breeding program utilizing recurrent selection and/or backcross breeding would be most suitable to incorporate the resistance present in I-35 into the Maryland tobacco breeding population for development of improved cultivars.

Additional key words: Generation mean analysis, insect resistance, *Nicotiana tabacum* (L.), *Manduca sexta* (L.), *Manduca quinquemaculata* (Haworth).

INTRODUCTION

Tobacco hornworm, *Manduca sexta* (L.), and tomato hornworm, *M. quinquemaculata* (Haworth), are serious pests of commercial tobacco, *Nicotiana tabacum* (L.), and during the larval stage they can consume large quantities of leaf material. In a normal production schedule, hornworms, along with tobacco budworms *Heliothis virescens* (F.), and tobacco aphids *Myzus nicotianae* (Blackman), are controlled by insecticides and cultural practices. Host-plant resistance could be an important adjunct to these control measures to provide the greatest protection at the least cost to the grower.

Maryland tobacco cultivars typically show little or no resistance to foliar feeding damage by hornworms. Kolodny-Hirsch et al (11) reported that 'MD 872' exhibited several physiological compensatory processes following defoliation simulating hornworm damage. Burk and Stewart (4) reported resistance to feeding by hornworm larvae among *Nicotiana* species of sections Repandae and Bigelovianae, subgenus Petunioides. However, interspecific hybridization is often difficult due to incompatibility, sterility, chromosome losses, and seedling lethality as suggested by Apparao et al (1) and Chaplin and Burk (6). Resistance found within *N. tabacum* would therefore be preferable and more easily transferred to acceptable cultivars.

The ARS-USDA, Georgia Agricultural Experiment Station, and North Carolina Agricultural Research Service cooperatively developed and released I-35, a breeding line which carries resistance to hornworms, budworms, and tobacco aphids (12). This germplasm line is a doubled haploid derived via anther culture and chromosome doubling with colchicine from an F1 of Tobacco Introduction (T.I.) 1112 and 'Speight G-33', a multiple disease resistant flue-cured cultivar. The yield and grade index of I-35 were about one-half as much as flue-cured checks, and I-35 most closely resembled its primitive T.I. 1112 parent, having a profuse branching habit, fewer leaves, and simple (glandless) leaf surface trichomes.

Investigations of the utilization of hornworm resistance in T.I. 1112 have focused on genetic control of leaf surface trichomes and their exudates. Simple (glandless) trichomes or non-secreting glandular trichomes do not secrete diterpenes whereas glandular trichomes do secrete products that may be involved in host-plant:insect relationships. Burk et al (3) reported the fully glandless trichome condition to be controlled by three recessive genes in T.I. 1112 and I-35 germplasm. Johnson et al (10) suggested that due to high heritabilities and additive genetic effects, selections for glandular trichome density would be effective in a pedigree selection procedure. They further proposed a model of three loci with partially dominant alleles responsible for glandless trichomes in T.I. 1112 and that glandular trichomes are produced as a result of the presence of a recessive allele at any of these loci. Greer and Nielsen (8) investigated trichome traits with the aim of evaluating the use of these traits as selection criteria. Hornworm damage scores and trichome types and densities were investigated on field grown, artificially infested tobacco and, therefore, measured larval activity and antibiosis rather than ovipositional activity. Although genotypic differences were found for hornworm damage, they concluded there may be little relationship between damage and trichome density and/or large inherent environmental and sampling variation in the study. Miles et al (12) showed I-35 to be resistant to heavy natural infestations of hornworms as determined by leaf damage ratings in the presence of the insect pest. The nature of the hornworm resistance is believed to be ovipositional nonpreference. Thus, I-35 germplasm offers a source of hornworm resistance for developing an acceptable Maryland tobacco cultivar. Therefore, the objectives of this study were (a) to obtain basic quantitative genetic information on the inheritance of hornworm resistance in Maryland tobacco as measured with natural field infestations, and (b) to evaluate methods of rating this resistance in a breeding program.

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MATERIALS AND METHODS

To obtain the two families for this study, two commercial Maryland tobacco cultivars, 'MD 341' and 'MD 872', were each crossed with tobacco germplasm line I-35 (MD 341 × I-35 and MD 872 × I-35), producing the F_1, F_2, BC_1 (MD parent × F_1), and BC_2 (I-35 × F_1) populations as described below.

MD 341 (P_1) × I-35 (P_2)
↓
$BC_1 \leftarrow P_1 \times F_1 \times P_2 \rightarrow BC_2$
↓
F_2

MD 872 (P_1) × I-35 (P_2)
↓
$BC_1 \leftarrow P_1 \times F_1 \times P_2 \rightarrow BC_2$
↓
F_2

This study was conducted for two years (1984 and 1985) at the Southern Maryland Research and Education Facility (CMREC), University of Maryland, Upper Marlboro, Maryland. The six populations (Maryland parent, I-35, F_1, F_2, BC_1, and BC_2) from each of the two families were planted in a randomized complete block design with six replications. Within each replication, the non-segregating populations (Maryland parent, I-35, and F_1) were represented by one-row plots. Multi-row plots were used for the segregating populations. The F_2 population utilized three-row plots, and each backcross population, BC_1 and BC_2, was planted in two-row plots. In 1984, the BC_2 population was not available, therefore analysis combined over years does not include this population. There were 15 plants per row in the 1984 season, and 12 plants per row in the 1985 season. Rows were 91.4 cm apart and plants were spaced 61.0 cm apart within the row. In 1985, the six populations of both families were planted at two locations (sites) spaced approximately 450 m apart at the research facility on a Monmouth fine sandy loam soil.

Natural field infestations of tobacco and tomato hornworms were relied upon for this study. Weekly egg and larval counts were obtained over the five-week period 2 August through 31 August, 1984. In 1985, counts were taken from 25 July through 22 August at Location 1, and from 2 August through 30 August at Location 2. Blacklight trap collections of adult hornworms were used to determine the period to conduct egg and larval counts. Because Borth and Harrison (1984) reported a significant vertical preference of the egg and first two larval stages for the upper two thirds of Maryland tobacco plants on the distal half of the lower leaf surface, this area was the focus of egg and larval counts in this study.

Each week the numbers of hornworm eggs and first and second instar larvae were obtained in each plot. The total number of hornworm eggs and larvae per week should be indicative of the ovipositional activity occurring on that plot during the previous week. The seasonal total was obtained by adding the five weekly counts and dividing by the number of plants searched per plot. Counts taken in 1984 on the Maryland parent, I-35, and F_1 populations included 10-plants per plot: the F_2 included 30-plants per plot, and the BC_1 consisted of 20-plants per plot. In 1985, the Maryland parent, I-35, and the F_1 populations included 6-plants per plot: the F2 included 18-plants per plot, and both backcross populations consisted of 12-plants per plot. Due to a high degree of skewness and non-normality, count data were transformed by the square root method ($Y' = (Y + 0.5)^{1/2}$).

Seasonal totals of egg and larval counts were separated into total number of eggs per plant and total number of larvae per plant over the season. This should indicate whether resistance was due to ovipositional nonpreference.

Visual damage ratings were taken on the same plants that were searched for hornworm eggs and larvae. Ratings were taken on 20 and 21 September, 1984 and on 20 September, 1985. Each plant was rated on a scale of 1 to 5 with a rating of 1 indicating little or no visual damage on the leaves and 5 indicating all leaves were stripped to the midveins (15).

Generation mean analysis, as described by Gamble (7), was used to calculate gene effects on the data collected from the two locations in 1985.

RESULTS

Tables 1 and 2 present the combined data over both years for the analysis of variance mean squares and population means for egg and larval counts and visual damage ratings,

Table 1. Mean squares for seasonal egg and larval counts and visual damage ratings for MD 341 and MD 872 families evaluated in 1984 and 1985.

Source of variation	df	Seasonal total eggs & larvae/plant[a]		Total no. of eggs/plant[a]	
		MD 341	MD 872	MD 341	MD 872
Years (Y)	1	0.024	0.041	0.039	0.027
Reps/Y	10	0.024	0.021	0.013	0.018
Populations (P)	4	0.123**	0.190**	0.068**	0.090**
P × Y	4	0.015	0.003	0.015	0.009
Pooled error	40	0.018	0.022	0.012	0.014

Source of variation	df	Total no. of larvae/plant[a]		Visual damage ratings, 1–5	
		MD 341	MD 872	MD 341	MD 872
Years (Y)	1	0.000	0.005	2.499**	1.825**
Reps/Y	10	0.010	0.004	0.041	0.042
Populations (P)	4	0.022	0.046**	0.188**	0.157**
P × Y	4	0.002	0.004	0.011	0.046
Pooled error	40	0.009	0.012	0.029	0.019

[a] Data transformed by the square root method ($Y' = (Y + 0.5)^{1/2}$).
** Significant at the 0.01 level of probability.

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Table 2. Population means for seasonal egg and larval counts and visual damage ratings for MD 341 and MD 872 families evaluated in 1984 and 1985.

Family	Population					LSD
	MD Parent	I-35	F_1	F_2	BC_1	(0.05)[a]
			Seasonal total, eggs & larvae/plant[b]			
MD 341	1.19 (0.92)[c]	0.93 (0.36)	0.97 (0.44)	1.06 (0.62)	1.03 (0.56)	0.11
MD 872	1.24 (1.04)	0.95 (0.40)	0.93 (0.36)	1.06 (0.62)	1.11 (0.73)	0.12
			Total no. of eggs/plant[b]			
MD 341	1.03 (0.56)	0.84 (0.21)	0.86 (0.24)	0.94 (0.38)	0.92 (0.35)	0.09
MD 872	1.08 (0.67)	0.87 (0.26)	0.87 (0.26)	0.94 (0.38)	0.98 (0.46)	0.10
			Total no. of larvae/plant[b]			
MD 341	0.92 (0.35)	0.81 (0.16)	0.84 (0.21)	0.86 (0.24)	0.84 (0.21)	N.S.
MD 872	0.93 (0.36)	0.80 (0.14)	0.78 (0.11)	0.86 (0.24)	0.87 (0.26)	0.09
			Visual damage ratings, 1-5[d]			
MD 341	1.91	1.59	1.63	1.69	1.75	0.14
MD 872	1.96	1.68	1.72	1.71	1.76	0.11

[a] The values of least significant differences apply to the populations within each family only.
[b] Data transformed by the square root method ($Y' = (Y + 0.5)^{1/2}$).
[c] Original data before transformation.
[d] Ratings based on a scale of 1 to 5 with 1 indicating little or no visual damage and 5 indicating all leaves were stripped to the midveins.

respectively. Visual damage ratings will be discussed later in this section. Significant variation among the five populations (minus BC_2) was observed for seasonal total of eggs and larvae per plant and total number of eggs per plant for the MD 341 and MD 872 families. Significant variation among the populations for total number of larvae per plant was found only for the MD 872 family. For these significant comparisons, the Maryland parent had a higher count than the I-35 parent, and the F_1 population was not different from the I-35 parent (**Table 2**). In the MD 341 family, the seasonal total of eggs and larvae per plant ranged from 0.36 for I-35 to 0.92 for MD 341. The F_2 was not different from the F_1 or BC_1, and the BC_1 was not different from I-35, F_1, or F_2 populations. In the MD 872 family, the seasonal total ranged from 0.36 for the F_1 population to 1.04 for MD 872. The F_2 was not different from I-35 or BC_1, but BC_1 was different from I-35.

Gene effects by location and combined over the two locations in 1985 for egg and larval counts are shown in **Table 3.** Significant mean effects were found for both families, and additive gene effects were significant in the MD 341 family for Location 1 and the combined analysis. Also, the additive × additive epistatic component was significant in the MD 341 family at Location 1.

Tables 1 and 2 also contain the data for visual damage ratings combined over years. Significant year effects were observed in both families due to higher visual damage ratings in 1984 than in 1985. Significant variation among the five populations was found for both families (**Table 1**). The Maryland parents exhibited higher damage than the I-35 parent, and the F_1 rating did not differ from I-35 (**Table 2**). Although not significantly different from the F_1 population, the BC_1 population did show a shift in damage rating toward the Maryland parent in both families.

Table 4 presents the gene effects for visual damage ratings. When combined over locations, mean effects were significant for both families; however, the additive, dominance, and epistatic effects were not significant for either family. The gene effect estimates for individual locations were similar to estimates obtained over both locations with one exception: for Location 1 the MD 872 family showed a highly significant additive × dominance epistatic effect.

DISCUSSION

Seasonal totals of weekly hornworm eggs and first and second instar larvae data suggest that the resistance factor from I-35 is a partially dominant trait. The Maryland parents had consistently higher counts than the I-35 parent each year and combined over years, and the F_1 population was generally not different from I-35. Relative values for the segregating populations were similar for both families, and the backcross populations tended to have values closer to their respective parents.

Although this study was not designed to determine the mechanism of resistance, there is some evidence for ovipo-

Table 3. Gene effects for seasonal hornworm egg and larvae counts at two locations, 1985.[a]

Family	Gene effect[b] m	a	d	aa	ad	dd
			Location 1			
MD 341	61.17**	2.03*	-1.70	-1.96*	-0.53	1.07
MD 872	14.74**	1.71	-0 29	0.27	-0.28	-0.64
			Location 2			
MD 341	23.38**	1.53	-0.91	-0.77	0.90	1.48
MD 872	17.61**	0.68	1.09	0.97	0.98	-0.45
			Locations 1 and 2			
MD 341	39.24**	2.28*	-1.56	-1.50	0.32	1.69
MD 872	23.39**	1.39	0.60	0.90	0.66	-0.71

[a] Seasonal total used in calculations of gene effects. Data transformed by the square root method $(Y' = (Y + 0.5)^{1/2})$.

[b] m, mean; a, additive; d, dominance; aa, additive x additive; ad, additive x dominance; dd, dominance x dominance effects.

*,**Significant at the 0.05 and 0.01 level of probability, respectively.

Table 4. Gene effects for visual damage ratings at two locations, 1985.

Family	Gene effect[a] m	a	d	aa	ad	dd
			Location 1			
MD 341	26.00**	0.64	-0.87	-0.39	-0.97	0.42
MD 872	25.41**	-1.00	1.34	1.58	-2.66**	-0.15
			Location 2			
MD 341	31.56**	1.79	1.07	1.15	0.21	-0.83
MD 872	26.97**	1.86	0.26	-0.05	0.41	-0.27
			Locations 1 and 2			
MD 341	41.75**	1.79	0.15	0.55	-0.44	-0.35
MD 872	37.71**	0.64	1.10	1.04	-1.41	-0.95

[a] m, mean; a, additive; d, dominance; aa, additive x additive; ad, additive x dominance; dd, dominance x dominance effects.

** Significant at the 0.01 level of probability.

sitional nonpreference. Field studies by Greer and Nielsen (8) focused on larval antibiosis, but they suggested that ovipositional nonpreference or egg survival may also be important in hornworm resistance. Jackson et al. (9) confirmed ovipositional nonpreference as a mechanism of hornworm resistance in field tests of I-35 and T.I. 1112. Both entries received fewer hornworm eggs than flue-cured cultivars 'NC 2326' and 'Speight G-33'. In our study, differences were found among populations for egg counts and for total counts of eggs and larvae in both families. The higher counts found on the Maryland parents and lower counts on I-35 suggest that the resistance is due to ovipositional nonpreference.

Generation mean analysis of the egg and larval counts in 1985 indicates that additive gene effects were important in the inheritance of resistance. Additive × additive gene effects may be important in the MD 341 family since their effect was significant at one location. Estimates of additive and additive × additive epistatic effects at Location 1 were relatively small in magnitude compared to mean effects. With additive effects being important in the MD 341 family, a recurrent selection program is most suitable for this genetic material. The backcross method of breeding may also be effective if selections are made in the F_3 or F_4 populations following crossing and if progeny testing is practiced.

Mean separation of visual damage ratings suggests that the resistance of I-35 as measured by visual damage ratings is a partially dominant trait in Maryland tobacco. The Maryland parents had higher ratings than the I-35 parent, and the F_1 population was not different from I-35. Significant mean effects from the generation mean analysis suggest the presence of genetic variability for visual damage ratings; however, there were no significant gene effects for this trait except for the additive × dominance effect in the MD 872 family at Location 1 in 1985. Lack of additive gene effects are a cause for concern indicating that the trait may not be highly heritable. Inadequate insect pressure or plant-to-plant migration of fifth larval stages could have affected ratings. It is also possible that hornworm adults were attracted to developing inflorescences prior to topping and visual

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evaluation. Inflorescences are also a source of nectar for adult moths and possess glanded trichomes that secrete duvane diterpenes implicated in ovipositional attraction in studies with tobacco budworms (Jackson et al, 9). Use of this measure of resistance in a breeding program would not be very effective alone since epistasis might impair selection efficiency. Better separation into more distinct visual classes and ratings of preflowering plants would aid in increasing the usefulness of this measure of hornworm resistance.

Previous studies by Burk et al (3), Greer and Nielsen (8), Jackson et al (9), Johnson et al (10), and Nielsen et al (13) have focused on trichome characteristics and/or artificial infestations using T.I. 1112, I-35, and flue-cured and burley tobacco cultivars. Results from this field study of natural insect infestation and visual damage ratings indicate that I-35 could be used as a source of hornworm resistance for developing an acceptable Maryland cultivar. Significant additive effects contributing to the inheritance of hornworm resistance are a preliminary indication that selection for low hornworm damage as measured by egg and larval counts is possible. Correlation coefficients between weekly counts and seasonal totals indicate that fewer weekly counts are needed to evaluate levels of hornworm infestation. The highest correlations were found between the seasonal totals and a combination of a few of the last weekly counts. Visual damage ratings could be used in conjunction with egg and larval counts if more distinct classes are observed and ratings are taken earlier in the season. A recurrent selection and/or backcross breeding program would be most suitable to incorporate this resistance present in I-35 while reconstituting the desirable agronomic characteristics of the Maryland cultivar.

The results from this study do not indicate what level of hornworm resistance could be obtained from I-35 and also maintain an acceptable level of agronomic performance for Maryland tobacco. Immunity would be desirable, but probably not possible with the present gene pool. However, a medium level of resistance coupled with a good Integrated Pest Management Program could reduce the need for insecticides and reduce production costs for the producer.

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