Screening Peanut Germplasm for Resistance to Corn Earworm[1]

R. N. Holley, W. W. Weeks, J. C. Wynne* and W. V. Campbell[2]

ABSTRACT

The corn earworm (*Heliothis zea* Boddie) commonly causes damage to peanut (*Arachis hypogaea* L.) in North Carolina. Resistance to the corn earworm has been identified in peanut and correlated with resistances to tobacco thrips (*Frankliniella fusca* Hinds), potato leafhopper (*Empoasca fabae* Harris), and the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber). Development of corn earworm-resistant cultivars which might also assist in managing several insect pests could be aided by an understanding of the nature and inheritance of corn earworm resistance.

This study was conducted to develop a laboratory procedure to screen for corn earworm resistance, to determine the relationship between laboratory and field measurements of resistance, to determine the chemical basis of resistance, and to determine the inheritance of resistance.

Neither the laboratory measurement of larval growth after feeding of fresh leaf tissue nor after feeding on a synthetic diet containing ground freeze-dried leaf tissue consistently separated resistant and susceptible germplasms. Because of the inconsistency in laboratory tests, the relationship between lab and field results was inconsistent. Since these procedures did not always distinguish resistant and susceptible genotypes, these techniques cannot be used in lieu of field screening. However, these studies do suggest that antibiosis is a major component of corn earworm resistance. Chemical analyses indicate that a flavone glycoside is probably responsible for the antibiotic response. Neither field nor laboratory evaluations of progencies from the cross of NC 6 (resistant) and Florigiant line C (susceptible) could be used to separate plants into resistant and susceptible classes. Antibiosis did appear to be a dominant trait for this cross.

Key Words: Antibiosis, *Arachis hypogaea, Heliothis zea.*

Corn earworm (*Heliothis zea* Boddie), tobacco thrips (*Frankliniella fusca* Hinds), potato leafhopper (*Empoasca fabae* Harris), and southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) commonly cause damage to peanut (*Arachis hypogaea* L.) in North Carolina. An economical method to manage these pests would be the use of resistant cultivars. Low to moderate levels of resistance have been found to these pests among cultivated germplasm (3).

Development of resistant cultivars would be aided by an understanding of the nature and inheritance of insect resistance. Previous work has indicated antibiotic effects ranging from 0% survival of corn earworm larvae fed on leaves from a species of *Arachis* to a 50% reduction in weight gain of larvae fed on leaves of a resistant cultivar (4). The correlation of corn earworm resistance and resistance to the other foliage-feeding insects enhances the potential value of a laboratory method for screening germplasm for resistance to corn earworm (5). In addition, the identification of the chemicals involved in the observed antibiosis response of corn earworm to some peanut cultivars would permit the selection of lines based on the presence and/or concentration of a chemical such as gossypol in cotton (7) or dimboa (6) and mayasin (10, 11) in corn.

Therefore, objectives of this study were to (a) develop a laboratory procedure for screening peanut germplasm for corn earworm resistance, (b) determine relationships between laboratory and field resistance, (c) determine the chemical basis of resistance, and (d) elucidate the inheritance of resistance mechanisms.

Materials and Methods

Genotypes used in this study were the cultivars NC 6, NC 7, Florigiant, NC 2, NC 5; breeding lines NC 3033, GP-NC 343, NC 18222 (Shulamit//GP-NC 343/Va 61R), Robut 33-1, and a component line of the cultivar Florigiant designated line C. NC 6 and line C were used in most tests since they represent resistant and susceptible genotypes to the corn earworm, respectively.

Field Tests

Field tests were conducted in 1981 and 1982 at the Peanut Belt Research Station at Lewiston. Five genotypes - NC 6, Florigiant, NC 3033, NC 7 and line C - were grown in a randomized complete block design (RCBD) with four replications in 1981. In 1982 ten genotypes - NC 6, Florigiant, line C, NC 7, NC 3033, NC 2, NC 5, NC 18222, GP-NC 343 and Robut 33-1 - were evaluated using a RCBD with five replications. Also in 1982, 40 F2 families in F3 generation from the cross of NC 6 and line C were grown in a RCBD with two replications. Plots consisted of a single row of 25 plants spaced 25.4 cm apart with 91 cm between rows except for the F2 families which consisted of 10 plants per row. Recommended cultural practices were used during the growing season except no insecticides were applied. Plots were rated for percent damage by thrips, leafhoppers, and corn earworms, sequentially, at the time of peak infestation of the appropriate insect. The expanding young tetrafoliates from exposed terminals were collected from one replicate of the 1982 test involving the 10 genotypes. These samples were used in laboratory testing of freeze-dried tissue incorporated into the diet and fed to larvae of the corn earworm.

Laboratory Tests

Larval leaf-feeding tests. Feeding tests were conducted in a .75 x .75 x 1.2 m long wooden-frame plastic chamber in which a 14-hr. light period at 29.5 C, 75% relative humidity and a 10-hr. dark period at 22.8 C and 85% humidity were maintained. Larvae were reared in 4.5-cm deep x 4.0-cm plastic cups with perforated plastic lids covered by organdy cloth. The larvae, supplied by the N. C. State Entomology Department insect-rearing laboratory came from two populations, one local and one from Tifton, GA. Larvae, reared for 48 hrs. (first instar) on the Burton diet (2), were selected for uniform size, transferred to individual cups and fed peanut leaf tissue. The leaf tissue was selected from the appropriate genotype of 65- and 90-day-old plants grown in the greenhouse. Individual plants were used to feed one larvae, except in the testing of F2 plants where two larvae were fed from each plant. Plants were grown in wooden flats filled with a 2:2:1:1 mixture of sand, clay loam, peat, and perlite fertilized with a complete liquid (6-25-15) nutrient.

At the end of a 7- to 10-day feeding period in which leaf tissue was changed daily, the larvae were weighed to the nearest tenth of a milligram. In later tests, the larvae were dried at 43.4 C and weighed again. The laboratory tests included six evaluations of NC 6 and Florigiant line C, two with these two lines and their F1 hybrids, two with NC 6, line C, NC 7, NC 3033 and Florigiant, and two with NC 6, line C and individual F2 plants from the cross of NC 6 and line C. A randomized complete block design was used for all tests except those involving F2 plants where a completely randomized design was used. The number of replications varied from 5 to 10 except in the F2 tests where only two replications were possible.

[1]Paper No. 9394 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695. Part of a thesis submitted by the senior author as partial fulfillment of the requirements for the Master of Science degree.

[2]Graduate Research Assistant, Associate Professor and Professor, Department of Crop Science and Professor, Department of Entomology, North Carolina State University, Raleigh, NC 27695.

Freeze-dried tissue in diet. Three tests were conducted with leaf material that was collected from one replicate of the 1982 field test. Unexpanded terminal leaves were hand-picked in the field, transported on ice, freeze-dried, ground, and incorporated into a modified Burton diet (2). The tests were designed to reduce any variation in tissue selection on larval growth. The tests were conducted similarly to the leaf-feeding tests. One test of eight genotypes from the 1982 field study (excluding NC 6 and line C) and two tests of NC 6 and line C were conducted using freeze-dried tissue.

Chemical Analysis

A series of analyses were performed to determine if a difference in chemical composition of selected leaf tissue of NC 6 and line C of Florigiant could be detected. Analyses which focused on polar constituents of the leaf commonly associated with insect resistance (9) included colorimetric determinations of total phenols, thin-layer chromatography, column chromatography, and extraction of highly polar compounds. All procedures were followed by spectrophotometric scanning from 190 to 550 nm.

Phenol content. Ground freeze-dried terminal leaf samples from the field and greenhouse were prepared in 100 mL of a 70:30 solution of methanol and water as the samples were homogenized in a Polytron Kinemitica sonic extractor. Nonpolar and chlorophyll fractions were removed from the extracts by a standardized preparatory procedure before the poplar fraction was determined (1) using a Gilford Model II Stasar spectrophotometer at 725 mu.

Thin-layer chromatography. Two grams of leaf material from the field for each of the two genotypes were extracted with ethanol (100 mL), filtered and concentrated (10 mL) using a Buchi rotary evaporator. The remaining solution was applied to a silica gel chromatographic plate. Plates were run through a dual solvent system with the first solvent containing an apolar solvent of benzene and ethylacetate (9:1) and the second solvent containing a polar solution of chloroform, methanol, and water (65:25:4 in early tests and 65:40:15 in later tests). Plates were compared under ultra-violet, infrared, and visible light. Areas appearing different were eluted and dissolved in methanol and water.

Column chromatography. Columns were prepared using 40 g of silica acid 100:120 mesh that was slurried in HPLC hexane and poured into a 2.5 x 31 cm glass column. Hexane (100 mL) was eluted through the column under 5 psi nitrogen gas. A 1.27 cm bed of glass beads 80:100 mesh was poured on the silica acid. Two sets of 3 g freeze-dried leaf material were extracted with 95% ethanol (50 mL). The homogenate was filtered, the tissue rinsed with extracting solvent and concentrated to dryness under vacuum at 25 C. Extracts were redissolved in ethanol (2 mL) and 1 mL was loaded on the columns. Following the first washing a methanol gradient was prepared by increasing the methanol concentration 2% every 100 mL until the solution was saturated with methanol. The eluted solutions were collected and assayed spectrophotometrically. Solutions were combined into four fractions, concentrated to 10 mL and assayed again. A final set of samples were extracted similarly to the first two sets except they were selectively prepared by partitioning in a 500-mL separatory funnel. The aqueous ethanolic extracts were saturated with salt and hexane to remove chlorophyll and apolar constituents. Three extractions with methylene chloride was followed by drying with sodium sulfate. The extracts were then dissolved in 2 mL of methylene chloride and chromatographed similarly to the first group.

High polarity compound isolation. Two g samples of leaf material were treated for 3 days with 300 mL of acetone that was shaken for 1 hr. and replaced with fresh acetone each day. The leaf material was then dried and placed in a flask with 70 mL of methanol and shaken for 20 minutes. The material was then filtered with 30 mL of water and extracted twice with hexane (50 mL). This procedure was similar to that used by Waiss *et al.* (10) to isolate the flavone glycoside "maysin" from corn silks resistant to the corn earworm.

Results and Discussion

Field Tests

Significant differences among genotypes for percent damaged leaves from the corn earworm, tobacco thrips and potato leafhopper were detected in both growing seasons (Table 1). NC 6 had significantly less corn earworm damage than Florigiant, line C for both years.

Table 1. Means for percent damaged leaves by corn earworm, thrips, and leafhoppers in field tests, Lewiston, NC.

Genotype	% Damaged leaves					
	1981			1982		
	Corn earworm	Thrips	Leaf-hopper	Corn earworm	Thrips	Leaf-hopper
NC 6	10.2	22.5	9.0	1.4	60.0	30.6
NC 7	12.2	35.5	34.2	3.0	75.0	44.0
NC 3033	18.7	52.5	65.0	9.4	84.0	64.0
Florigiant	17.5	42.5	18.7	9.2	79.0	57.0
Florigiant, line C	17.7	42.5	16.7	9.8	80.0	69.0
NC 2	--	--	--	6.2	52.0	54.6
GP-NC 343	--	--	--	2.4	58.0	32.0
NC 5	--	--	--	2.4	66.0	46.0
Robut 33-1	--	--	--	3.6	78.0	72.0
NC 18222	--	--	--	2.0	61.0	45.0
LSD (.05)	3.4	12.5	16.4	2.1	8.9	6.8

The ranking of the five genotypes evaluated both years were similar over years for corn earworm and thrips damage, although there was greater damage from corn earworm in 1981 and thrips in 1982. Two genotypes, Florigiant and Florigiant, line C were not consistent over both seasons for leafhopper damage.

The three traits measured in 1982 were all significantly correlated ($p = 0.01$) with the correlation coefficients for thrips damage with potato leafhopper and corn earworm damage being .40 and .50, respectively. The correlation coefficient for corn earworm and potato leafhopper damage was .61.

However, no significant differences in insect damage were detected among the F2 families in F3 generation from the cross of NC 6 and line C. The lack of significance which may have resulted from too few replications, and small plot size made it impossible to determine the inheritance of insect resistance in the field for this cross.

Laboratory Tests

Larval leaf-feeding tests. The field resistance to corn earworm of NC 6 compared to line C can be partially attributed to antibiosis as evidenced by the reduced corn earworm larvae fresh weight on NC 6 (Table 2). Results from four tests were similar to test a; however, results were not always significant as indicated by test b which consisted of 10 replications. Using NC 6, line C, and three other lines (Florigiant, NC 3033 and NC 7), corn earworm larval fresh weight after 1 week of feeding on leaf tissue separated the lines into groups corresponding to corn earworm resistance in the field, with NC 6 and NC 7 showing significant antibiotic effects compared to the other three lines. However, 2 weeks later a repeat of the test gave no significant differences among the lines due to extremely high larval variability.

Table 2. Mean fresh weight of corn earworm larvae fed leaf tissue of two genotypes.

Genotype	Mean larval fresh weight (mg)	
	Test a	Test b
NC 6	34.1a*	16.5a
Florigiant, line C	71.1b	48.8a

*Means within columns with similar letters not significantly different ($p = 0.05$) according to F-protected LSD.

Differences were detected among genotypes when percent dry matter of the corn earworm larvae (larval dry weight/fresh weight x 100) was measured (Table 3). In the test of NC 6 and line C and their F_1 hybrids, there was no significant difference among the genotypes for fresh or dry larval weight but percent dry matter of the larvae significantly separated the susceptible parent and the resistant parent. The mean for percent dry matter for the F_1 hybrids was equal to the mean for NC 6, the resistant parent, suggesting that the resistant antibiotic response is dominant. No significant difference for antibiotic effects among the F_2 plants was detected in the laboratory tests, making it impossible to determine the inheritance of antibiosis for the NC 6 x line C cross.

Table 3. Means for antibiosis rating of resistant and susceptible peanut lines and their F_1 hybrids to corn earworm larvae.

Genotype	Larval fresh weight (mg)	Larval dry weight (mg)	Dry matter
NC 6	12.6a*	1.8a	15.0a
Florigiant, line C	12.1a	2.1a	19.0b
F_1	19.2a	2.7a	14.2a

*Means within columns with different letters are significantly different (p = 0.05) according to F-protected least significant difference.

Freeze-dried tissue in diet. Tests using a diet containing freeze-dried leaf material from various genotypes also produce inconsistent results. Test 1 involving NC 6, line C and the standard diet as a control showed significant differences among the three treatments for fresh weight of larvae (Table 4). However, a second test did not show significant differences between NC 6 and line C for larval fresh or dry weight. In addition, a third test using freeze-dried leaf material from the field gave significant differences for the eight genotypes tested on the basis of fresh weight of larvae,

Table 4. Means for weight of corn earworm larvae with freeze-dried leaf tissue incorporated in the diet.

Treatment	Test 1	Test 2		
	Larval fresh weight (mg)	Larval fresh weight (mg)	Larval dry weight (mg)	% Dry matter
NC 6	4.9c*	50.8a	6.1a	11.5b
Florigiant, line C	11.7b	71.5a	9.4a	12.8a
Control (diet)	28.3a	--	--	--

*Means within columns with different letters are significantly different (p = 0.05) according to F-protected least significant difference.

but the results were not correlated with corn earworm resistance for the eight genotypes in the field.

Chemical Analysis

The chemical composition of leaf tissue of corn earworm-resistant and susceptible genotypes did not identify differences that could be used to select resistant lines. Initial scanning of methanol and water extracts of leaf tissue identified a consistent quantitative difference between NC 6 and Florigiant, line C in the 200 nm region. Since few components known to be associated with insect resistance absorb in this area, these observations were assumed to be unrelated to antibiosis.

Significant differences for total phenols were found for NC 6 and line C (Table 5). However, results were not repeatable by subsequent work using freeze-dried samples from the field. Two-dimensional thin-layer chromatography identified a difference in a nonpolar compound(s) with an RF value of around 0.8. The compound appeared yellow under visible and ultraviolet light. Elution of this compound into methanol and water (70:30), followed by scanning, indicated that NC 6 contained higher levels of this compound which was absorbed at 420 to 490 nm. Column chromatography was used to concentrate this compound. The first set of columns produced a yellow band that produced two absorbance peaks in the 435 to 475 nm region. This difference between NC 6 and line C was not observed in the second set of columns because chlorophyll masked the compound. A third column set was designed to selectively remove chlorophyll. However, this technique also removed the yellow band which was also apolar.

Table 5. Phenolic content of leaf tissue extract of NC 6 and Florigiant, line C.

Genotype	Total Folin's reagent reactive material (chlorogenic acid equivalents)	Percent of total representing true phenolic compounds (%)
NC 6	66.0a*	37.6a
Florigiant, line C	49.1b	30.6b

*Means with different letters are significantly different (p = 0.05) according to F-protected least significant difference.

The high polarity compound isolation technique was similar to that used by Waiss *et al.* (10) to identify "maysin," a flavone glycoside which was involved with corn earworm resistance in corn. A scan of the prepared extract gave an absorption band at 291 and 322 nm, similar to the spectra of several flavone glycosides identified by Mabry *et al.* (8). This compound was more concentrated in the extract from NC 6, suggesting that this compound may be involved in corn earworm resistance in peanuts. A refinement of this isolation technique in conjunction with the use of synthetic larval diets containing different chromatographic fractions similar to the procedures of Waiss *et al.* (10) could lead to the identification of the compound involved in antibiosis to the corn earworm in peanut.

One of the objectives of this study was to develop a laboratory procedure for screening peanut germplasm for resistance to the corn earworm which would relate to expressions of field resistance. Measurement of antibiosis for resistant and susceptible genotypes in the laboratory by determining larval growth after feeding on fresh leaf tissue or after feeding on a synthetic diet con-

taining ground freeze-dried leaf tissue correlated with genotypic response to corn earworm damage in the field. However, results from the laboratory studies were not always repeatable. Resistant and susceptible genotypes were most often separated by expressing larval weight as percent of dry matter, but even this measurement did not always separate NC 6 and Florigiant line C. Because the laboratory procedures used in this study were time-consuming and did not always distinguish resistant and susceptible genotypes, these techniques cannot be used in lieu of field screening.

The inconsistency of results from the laboratory tests and the large amount of leaf tissue required to feed larvae for a 10-day period made it impossible to determine the inheritance of corn earworm antibiotic effects in the cross of the resistant NC 6 and the susceptible Florigiant line C. The antibiotic response of NC 6 did appear to be dominant to the lack of response of Florigiant line C. Antibiosis could be under the control of one or a few genes which would differ from previous results for the inheritance of corn earworm resistance (5). The inheritance of resistance will probably have to be determined by growing large replicated progeny rows from F_2 plants in the field with good infestations of the corn earworm.

These studies suggest that antibiosis is a major component of the resistance of NC 6 (3, 4). Refinement of the techniques for isolation of the chemicals involved in antibiosis could lead to the identification of the compounds involved in resistance. Chemical analysis suggests that a flavone glycoside may be responsible for the antibiosis of NC 6. Determination of the chemical basis for resistance could aid in the development of better screening techniques for corn earworm resistance.

Literature Cited

1. Anderson, R. A. and J. R. Todd. 1968. Estimation of total tobacco plant phenols by their bonding to polyvinylpyrrolidone. Tob. Sci. XII:107-111.
2. Burton, R. L. 1970. A low cost artificial diet for the corn earworm. J. Econ. Ent. 63:1969-1970.
3. Campbell, W. V. and J. C. Wynne. 1980. Resistance of groundnuts to insects and mites. Proc. Int. Workshop on Groundnuts, ICRISAT, Hyderabad, India. pp. 149-157.
4. Campbell, W. V., and J. C. Wynne, and H. T. Stalker. 1982.- Screening groundnut for *Heliothis zea* resistance. Proc. Internat. Workshop on *Heliothis* Management. ICRISAT, Hyderabad, India. pp. 267-276.
5. Holley, R. N., J. C. Wynne, W. V. Campbell, and T. G. Isleib. 1985. Combining ability for insect resistance in peanuts. Oleágineux (In press).
6. Klun, J. A. and J. F. Robinson. 1969. Concentration of two 1,4-benzo-oxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. J. Econ. Ent. 62:214-220.
7. Lukefahr, M. J., J. E. Houghtaling, and D. G. Cruhm. 1975. Suppression of *Heliothis* spp. with cottons containing combinations of resistant characters. J. Econ. Ent. 68:743-746.
8. Mabry, T. J., K. R. Markham, and M. B. Thomas. 1970. Systematic Identification of Flavonoids. Springer-Verlag, New York. 350 pp.
9. Norris, D. M. and M. Kogan. 1980. Biochemical and morphological bases of resistance, p. 23-61. *In* F. G. Maxwell and P. R. Jennings (eds.), Breeding Plants Resistant to Insects. John Wiley and Sons, New York.
10. Waiss, A. C., Jr., B. G. Chan, C. A. Elliger, B. R. Wiseman, W. W. McMillian, N. W. Widstrom, M. S. Zuber, and A. J. Keaster. 1979. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. J. Econ. Ent. 72:256-258.
11. Widstrom, N. W., A. C. Waiss, Jr., W. W. McMillian, B. R. Wiseman, C. A. Elliger, M. S. Zuber, R. W. Straub, J. L. Brewbaker, L. L. Darrah, A. R. Henson, J. M. Arnold, and J. L. Overman. 1982. Maysin content of silks of nine maize genotypes grown in diverse environments. Crop Sci. 22:953-955.

Accepted December 4, 1984