Laboratory and Field Evaluations of Virginia-Type Peanut Genotypes for Resistance to *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae)

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ABSTRACT

The southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) is the primary soil insect pest of peanut (*Arachis hypogaea* L.) in Virginia and North Carolina. The majority of acreage is planted to recently released cultivars which have not been extensively screened for rootworm resistance. The objectives of this study were to evaluate five virginia-type cultivars (NC-V 11, VA-C 92R, VA 93B, NC 10C, and AgraTech VC-1) and 19 breeding lines for resistance to southern corn rootworm and develop efficient resistance laboratory and field screening techniques for peanut. Laboratory-grown seedlings and pegs, and immature and mature pods taken from field-grown plants were used in bioassays. Neonates from a laboratory colony were reared to the adult stage on plant tissues and evaluated for mortality. Pod damage was determined from field studies where plants were exposed to natural southern corn rootworm infestations. With seedling feeding, NC 6 resulted in significantly more rootworm mortality as compared to NC 7, NC 9, NC 10C, NC-V11, N90013E, PI 121067, AgraTech VC-1, VA 93B, VA 861101, and VA 9211290. With feeding on field-grown peg and immature pod tissues, NC 6 also resulted in significantly more rootworm mortality compared with NC 7, AgraTech VC-1, VA 93B, and VA 861101. In field studies, NC 6 also incurred significantly less total pod damage than NC 7. Results indicate that NC 6 is still the only cultivar currently planted that demonstrates significant resistance to southern corn rootworm.

Key Words: *Arachis hypogaea* L., groundnut, integrated pest management, insect resistance.

The southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, long has been considered a major pest of peanuts (*Arachis hypogaea* L.) grown in Virginia and North Carolina (Herbert *et al.*, 1992), and is now considered a major soil pest in some areas of South Carolina, Georgia, Alabama, and Texas. Current management is based solely on preventive applications of soil-incorporated insecticides (Herbert, 1995). In 1990, approximately 90.4 and 50.6% of the total peanut acreage in Virginia and North Carolina, respectively, was preventively treated with soil-incorporated insecticides to control southern corn rootworm. These preventive treatments represented 158 t of active ingredient at a cost of over $3 million (Phipps *et al.*, 1992; Toth *et al.*, 1994). Larvae injure peanuts by feeding on developing pods and cause either direct yield loss or indirect yield loss by allowing entry of secondary pathogenic microorganisms (Grayson and Poos, 1947). Larvae also feed on pegs, which prevents pod development. In addition, even superficial external scarring can reduce the value of the crop since the virginia-type peanuts grown in this area are primarily for the in-shell market (Brandenburg and Herbert, 1991). Most feeding occurs in late July to early August when many pods are immature and susceptible.

One of the most effective and economic means of control would be to develop a peanut cultivar resistant to southern corn rootworm (Campbell and Wynne, 1985). NC 6 was introduced as a rootworm-resistant virginia-type peanut cultivar in 1977 (Campbell *et al.*, 1977; Wynne *et al.*, 1977). Although NC 6 appears to confer resistance to rootworm and was grown on as much as 23% of Virginia acreage in 1990 (USDA and Virginia Dept. of Agric. and Consumer Serv., 1994), it is not grown as widely now because it does not compete well with newer cultivars that either have higher yield potentials or earlier maturity. Reports of relative resistance levels of the more recently released cultivars for rootworm resistance, which are planted on the majority of acreage, have not been published. Thus, these cultivars must be treated as if they have no resistance in order to ensure minimal losses and judicious use of pesticides or other appropriate pest management alternatives is not possible. The objectives of this study were to compare resistance to southern corn rootworm of newer peanut cultivars and breeding lines to that of NC 6, and develop efficient resistance laboratory and field screening techniques for peanut.

Materials and Methods

Seedling Bioassay. Laboratory bioassays were conducted in 1994 and 1995 at the Virginia Polytech. Inst. & State Univ. (VPI&SU), Tidewater Agric. Res. and Ext. Ctr. (AREC), Suffolk, VA, to determine the effect of seedling feeding on five virginia-type peanut cultivars (NC-V 11, VA-C 92R, VA 93B, NC 10C, and AgraTech VC-1) and 19 breeding lines (N90013E, VA 861101, VA 9211920, VA 9211289, VA 891438, VA 901072, VA 9010343, VA 8911115, VA 9109213, VA 9109235, VA 9109237, VA 9111309, N93007L, N92066L, N92074L, PI 121067, GP-NC 343, N92064L, and N93003L) on mortality of southern corn rootworm. The breeding lines were selected because they were believed to have some insect resistance, were soon to

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be released, or had never been extensively evaluated for southern corn rootworm resistance. The cultivars were tested because they were planted on approximately 58% of the total peanut acreage in Virginia in 1994 (USDA and Virginia Dept. of Agric. and Consumer Serv., 1994), but also have never been extensively evaluated for rootworm resistance. NC 6 was used as a resistant check (Campbell *et al.*, 1977; Wynne *et al.*, 1977), and NC 7 (Wynne *et al.*, 1979) and NC 9 (Wynne *et al.*, 1986) as susceptible checks.

All laboratory procedures were modified from methods described by Chalfant and Mitchell (1967). Test peanut seed was treated with Vitavax (Gustafson Seed Tech., McKinney, TX) seed protectant to prevent any seed decay or seedling disease and placed on and covered with moist paper towels to induce germination. When primary roots on seedlings were 3.5 to 4.0 cm long, then three seedlings of each genotype were placed in moist vermiculite within a 411-mL clear plastic cup. Ten neonate southern corn rootworms were put into each cup and allowed to feed on roots.

Insects for all bioassays were obtained from the VPI&SU laboratory colony. The colony was established from eggs (French Agric. Research, Lamberton, MN) and augmented with field-collected adults. Eggs were allowed to hatch in petri dishes and neonates transferred to bioassay arenas or placed into the laboratory colony. Larval diet in the laboratory colony consisted of corn seed (Pioneer Hybrid 3140) spread evenly on the bottom of a plastic shoe box (9.4 × 8.2 × 33.6 cm) and covered with a 2-cm thick layer of horticultural vermiculite (Palmetto Vermiculite Co., Arcadia, LA). Adults that emerged from the boxes were stored in wooden-framed, aluminum-screened cages with a 45 × 45-cm base and a 60-cm height for egg production.

Insects were monitored for mortality during development from the neonate to the adult stage. Mortality was recorded for 10 d beginning after a 35-d development period. Intermediate life stages were not monitored because of the high probability of physical damage to larvae or pupae. Percentage rootworm mortality was determined for each genotype. Each replicate consisted of the 27 genotypes placed into a growth chamber at 25 ± 1C and at 50-80% relative humidity. The experiment was replicated 10 times in a completely randomized design. A photoperiod of 10:14 hr (light:dark) was used. Data were analyzed by analysis of variance (PROC ANOVA) and means separated by Tukey's t-test (SAS, 1985).

Field-Grown Peg and Pod Tissue Bioassays. A combined laboratory and field study was conducted in 1994 and 1995 at the VPI&SU Tidewater AREC, Suffolk, VA to determine the effect of feeding on mortality of southern corn rootworm for field-grown pegs and pods of four released virginia-type peanut cultivars (NC-V 11, VA-C 92R, VA 93B, and AgraTech VC-1) and an advanced breeding line (VA 861101). NC 6 was used as a resistant check, and NC 7 and NC 9 as susceptible checks. Each genotype was planted on 16 May 1994 and 6 May 1995 in plots with two 11-m rows to provide sufficient quantities of host tissue for bioassays. All field plots were replicated eight times in a randomized complete block design.

Peg or pod tissue was bioassayed during the growing season when phenology had proceeded as follows: (a) when peanut plants were pegging, (b) when immature pods were developing, and (c) when mature pods were produced. Tissue was removed by lifting the vines of the plants and removing desired quantities of tissue from all entries. After tissues were removed from the plant, soil and debris were removed by washing, and peg or pod tissues were surface sterilized by soaking in a beaker with a 1% sodium hypochlorite solution for 5 min. Plant tissue and 10 neonates were placed into 411-mL clear plastic cups. They were placed into the growth chamber as described in the seedling bioassay. Comparable quantities of tissue for each genotype were determined by weighing samples to the nearest 0.1 g, using 3-5 g of peg tissue (8-15 pegs), 7-10 g of immature pod tissue (3 pods), and 12-15 g of mature pod tissue (3 pods). Pods were considered mature when pronounced veination was evident on the exterior pod wall. Both the larvae and tissue were covered with vermiculite to more closely simulate a natural larval feeding environment. Each tissue type was replaced three times (with weights described above) during the course of the bioassay to provide larvae with sufficient quantities of fresh food and to prevent mold from developing on peanut tissues. Genotypes were bioassayed for each tissue type using eight replicates arranged in a completely randomized design within a growth chamber. The same environmental conditions, methods to monitor mortality, and data analyses were used as in the seedling study.

Field Studies. Experiments were conducted at the VPI&SU Tidewater AREC, Suffolk, VA and at a grower's field in Greensville County, VA in 1994 and 1995 to determine the effect of peanut genotypes on pod damage from natural populations of southern corn rootworm. NC 6, NC 7, VA 861101, and AgraTech VC-1 were planted in two fields that had a history of southern corn rootworm infestation. Each genotype was planted in four 11.5-m rows with a 0.91-m row spacing. A randomized complete block design was used with four replicates. Soil types were a Dendron loamy sand in Suffolk and a Varina fine sandy loam in Greensville.

Five plants of each genotype were selected randomly from each replicate prior to harvest on 20 Sept. 1994 and 10 Oct. 1995. Damage to 100 randomly selected pods from each five-plant sample was used to compare genotype susceptibility to southern corn rootworm. Pods which were punctured or with external scarification were scored as damaged. Data were analyzed by analysis of variance (PROC ANOVA) and means separated by Tukey's t-test (SAS, 1985).

Results

Seedling Bioassay. In 1994, significant differences occurred among genotypes with respect to mortality of southern corn rootworm resulting from feeding on seedlings during development from the neonate to the adult stage (Table 1). Feeding on NC 6 resulted in significantly more mortality ($P \leq 0.01$) as compared to NC 10C, N90013E, NC 9, VA 93B, and VA 921190. In 1995, NC 6 resulted in significantly more mortality ($P \leq 0.01$) as compared to NC-V11, VA 861101, NC 7, NC 9, AgraTech VC-1, PI 121067, and VA 93B (Table 2).

Field-Grown Peg and Pod Tissue Bioassay. In 1994, feeding on peg tissue of NC 9 and NC 6 resulted in significantly higher mortality ($P \leq 0.01$) during development from the neonate to the adult stage as compared to VA 93B. In 1995, NC 6 resulted in more mortality and significantly more ($P \leq 0.01$) as compared with NC 7, VA

Table 1. Mean percent mortality of southern corn rootworm during development from the neonate to the adult stage reared on seedlings of different peanut genotypes in 1994.

Entry	Mortality[a] (neonate-adult)
	%
NC 6	40.0 a
VA 9010343	37.0 ab
VA-C 92R	34.0 ab
VA 861101	32.0 ab
AgraTech VC-1	31.0 ab
VA 9109213	28.0 ab
NC-V11	25.0 ab
NC 7	25.0 ab
VA 9111309	24.0 ab
VA 901072	23.0 ab
VA 891438	23.0 ab
VA 9109235	22.0 ab
VA 9211289	21.0 ab
VA 8911115	21.0 ab
VA 9109237	19.0 ab
NC 10C	17.0 b
N90013E	17.0 b
NC 9	16.0 b
VA 93B	14.0 b
VA 9211290	14.0 b

[a]Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's t-test.

Table 2. Mean percent mortality of southern corn rootworm during development from the neonate to the adult stage reared on seedlings of different peanut genotypes in 1995.

Entry	Mortality[a] (neonate-adult)
	%
NC 6	81.0 a
GP-NC 343	69.0 ab
N92074L	68.0 ab
N93003L	68.0 ab
N93007L	66.0 abc
N92064L	61.0 abc
N92066L	59.0 abc
VA-C 92R	59.0 abc
NC-V11	43.0 bcd
VA 861101	41.0 bcd
NC 7	38.0 bcd
NC 9	36.0 bcd
AgraTech VC-1	36.0 bcd
PI 121067	32.0 cd
VA 93B	21.0 d

[a]Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's t-test.

93B, VA 861101, and AgraTech VC-1 (Table 3). In both 1994 and 1995, feeding on immature pods of NC 6 resulted in the highest mortality during development

Table 3. Mean percent mortality of southern corn rootworm during development from the neonate to the adult stage on peg tissue of eight different peanut genotypes in 1994 and 1995.

Entry	Mortality[a] (neonate-adult)	
	1994	1995
	%	%
NC 9	96.2 a	70.0 ab
NC 6	93.7 a	88.7 a
VA 861101	88.7 ab	52.5 b
NC 7	87.5 ab	60.0 b
NC -V11	83.7 ab	63.7 ab
AgraTechVC-1	75.0 ab	56.2 b
VA-C 92R	73.7 ab	67.5 ab
VA 93B	67.5 b	56.2 b

[a]Means in the same columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's t-test.

from the neonate to adult stage; and, in 1995, significantly more mortality occurred on NC 6 as compared to NC 7, VA 93B, VA 861101, and AgraTech VC-1 ($P \leq 0.01$) (Table 4). In 1994, 100% mortality resulted before the pupal stage when neonates were subjected to mature pods for all genotypes. Tests were not conducted on mature pods in 1995 since no feeding appeared to occur when peanut pods reached this stage.

Field Study. In 1994, significant differences were found among genotypes in the percentage total pod damage caused by natural southern corn rootworm infestations. NC 6 sustained the least amount of pod damage (5.0%) and significantly less ($P \leq 0.01$) than NC 7 (27.0%) (Table 5). In 1995, NC 6 again sustained the least amount of pod damage from natural infestations at both the Tidewater AREC and Greensville sites. At the Tidewater AREC site, NC 6 (5.5%) incurred significantly less ($P \leq 0.05$) pod damage as compared to NC 7 (18.0%). At the Greensville site, NC 6 (6.0%) incurred

Table 4. Mean percent mortality of southern corn rootworm during development from the neonate to the adult stage on immature pod tissue of eight different peanut genotypes in 1994 and 1995.

Entry	Mortality[a] (neonate-adult)	
	1994	1995
	%	%
NC 6	90.0 a	88.7 a
AgraTechVC-1	80.0 a	56.2 b
VA 861101	78.7 a	52.5 b
VA-C 92R	82.5 a	67.5 ab
NC -V11	82.5 a	63.7 ab
NC 9	81.2 a	70.0 ab
VA 93B	78.7 a	56.2 b
NC 7	78.7 a	60.0 b

[a]Means in the same columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's t-test.

Table 5. Mean percentages of total pod damage by southern corn rootworm on four peanut genotypes in natural infestations of southern corn rootworm (1994-1995).

Entry	Total pod damage[a]		
	1994	1995	
	Tidewater AREC	Tidewater AREC	Greensville Co.
	%	%	
NC 6	5.0 a	5.5 a	6.0 a
VA 861101	15.3 ab	9.0 ab	10.2 a
AgraTech VC-1	15.6 ab	11.0 ab	13.2 a
NC 7	27.0 b	18.0 b	24.5 b

[a]Means in the same columns followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's t-test.

significantly less ($P \leq 0.01$) pod damage as compared to NC 7 (24.5%) (Table 5).

Discussion

NC 6 was the only genotype evaluated that exhibited consistent and significant resistance to southern corn rootworm. NC 6 showed resistance both in laboratory seedling and field-grown pod tissue bioassays, and against natural rootworm infestations in the field. Feeding on NC 6 resulted in substantial larval mortality, and plants suffered significantly less pod damage when grown in fields with high rootworm populations. This corresponds to conclusions by Wynne *et al.* (1977) who registered NC 6 as a rootworm resistant variety and demonstrates that resistance is still present after nearly 20 yr. Because NC 6 provided the greatest level of rootworm resistance, it should be maintained as a viable cultivar even though it lacks the yield and early maturity characteristics of some of the more recently released genotypes. Emphasis of the North Carolina-Virginia peanut breeding program should be placed on using NC 6 as a parent to develop rootworm resistant cultivars with more competitive yields. In the interim, NC 6 should continue to have appeal in areas with consistently high levels of southern corn rootworm damage and should reduce or eliminate some preventive insecticide applications.

The cultivars AgraTech VC-1, NC 7, and VA 93B appear to be highly susceptible to southern corn rootworm and should not be planted in potential problem fields unless soils are treated with soil-incorporated insecticides. These cultivars are at high risk to significant yield loss, especially if other factors such as high loam content and/or and soil moisture in late July and August favor rootworm development.

Of the bioassays using seedlings, field-grown pegs, and immature and mature pods, exposure to seedlings and field-grown immature pods, in conjunction with field studies involving natural rootworm infestations, appears to be the most efficient way to screen peanut genotypes for resistance to southern corn rootworm. Seedling bioassays, which are relatively easy to complete and can be done anytime during the season, can give an early indication of possible resistance. With field-grown tissue bioassays, mature pods have proven unacceptable, as larvae are unable to feed and develop in the absence of other food sources. Mortality trends with pegs and immature pods were similar; however, immature pods were much less time-consuming to collect and did not degenerate as quickly or need replacement as often as pegs in the bioassay cup-vermiculite arena.

When evaluating genotypes in the laboratory alone, potential resistance may be overlooked because of reasons related to survival of larvae in the field and environmental conditions affecting plant maturity. Amount of pod damage may be influenced by factors other than those associated with true resistance, such as genotype differences in rate of maturity, time of or level of infestation in relation to plant maturity, and soil conditions affecting rootworm survival. Although exposure to field-grown tissue may provide a good indication of resistance, it will not provide the necessary field exposure to natural rootworm populations which is necessary for developing confidence in resistance data.

Genotypes that have a high percentage of pegs at the time of peak rootworm infestation could yield less due to peg damage. Also, any genotype that matures earlier or later than surrounding peanuts possibly could avoid infestation, depending on coincidence of immature pods (the most susceptible plant stage) with time of peak infestation. Field evaluations, although labor-intensive and subject to the many biotic and abiotic variances that affect rootworm and peanut pod damage, are the final test of cultivar resistance. Because rootworm infestations are not uniformly distributed within fields, evaluations should have as many replicates as resources allow. Evaluations also should be conducted over years and encompass ranges in factors that influence rootworm damage, such as soil type and planting date.

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