

# Screening for Cucumber Antibiosis to Pickleworm

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**Abstract.** A detached-leaf test was used to screen a cucumber (*Cucumis sativus* L.) germplasm collection for antibiosis to the pickleworm (*Diaphania nitidalis* Stoll.). Data were collected on 1160 lines planted in the field in 1981. The 36 most resistant and 36 most susceptible lines were retested with improved methods, reducing the number of lines to 8 and finally to 6 based on leaf damage by pickleworm larvae. Selections were made within those lines to stabilize the resistant or susceptible reaction of each line in our test. In a final test, no significant differences were found among the selections, which included the most resistant and most susceptible lines identified in all studies. A heritability study was run on a population produced by intercrossing the 5 most resistant and 3 most susceptible lines identified in the initial field screening for 3 generations. Parent-offspring regression was used to estimate a narrow-sense heritability of 0.03. Thus, there was little or no genetic variation in cucumber for antibiosis to pickleworm larvae, and other methods of control should be used.

Pickleworm is one of the 2 most important insect pests of cucumber in the southern United States (G.R. Hughes, N.C. State Univ., personal communication). Pickleworms can be controlled with insecticides, but it would be more economical and safer if genetic resistance could be identified and incorporated into adapted cultivars. Even if resistance alone would not provide complete control, its use in combination with insecticides might prove more effective than resistance or insecticides alone (1).

Research has been done on genetic resistance of cucumber to pickleworm using field screening tests in naturally infested areas (5, 7, 8, 9, 11). Field screening techniques have been developed (6), most using some index of fruit damage caused by the feeding of pickleworm larvae. However, after screening 500 cultivars and plant introduction lines, no useful levels of resistance were identified (9). Differences in field resistance to pickleworm feeding, ranging from 51% to 100% infested fruit were measured for 10 lines of muskmelon (*Cucumis melo* L.) (2). However, it was not known what the inheritance

pattern was, nor whether the resistance was high enough to be useful.

Resistance to oviposition by adult females is conferred by the single-gene controlled glabrous mutant (10) in both muskmelon and cucumber (2, 3, 8, 11). Apparently, resistance is due to the lack of plant hairs, since the tests were run on glabrous plants that were near-isogenic with adapted cultivars (3). However, glabrous plants lack vigor and are as susceptible to feeding by pickleworm larvae as normal plants (3). Therefore, that

source of resistance probably would not be horticulturally useful.

Control of pickleworm is best done before the larvae become established in the fruit. Genetic resistance, therefore, would be most effective if it prevented oviposition by adult females, or feeding by newly hatched larvae. We decided that antibiosis to larvae would be the easier trait for which to test and select. The objective of this study was to develop a test for leaf antibiosis of cucumber leaves to pickleworm larvae, screen the cucumber germplasm collection for resistance, and measure the heritability of that resistance.

Preliminary tests were run to determine optimum conditions for testing detached cucumber leaves for antibiosis to first instar pickleworm larvae. These tests were followed by a screening of the U.S. collection of cucumber germplasm for gross differences in resistance, a retest of the most different lines, and a refining of the screening method. Finally, a heritability study was run using a population developed by intercrossing the 8 most diverse lines from the initial screening of germplasm.

**Pickleworm rearing.** All studies were run using newly hatched (first instar) pickleworm larvae. The larvae were obtained from a laboratory-reared colony established in 1976 from moths developed from larvae collected from cucumber production areas near Charleston, S.C. Moths were induced to lay eggs on fiberglass insulation by spraying the insulation with an ethanol extract of squash (*Cucurbita pepo* L.) leaves. The eggs then were sent to Raleigh, N.C. (where most of the tests were run) and allowed to hatch. Preliminary studies indicated that the laboratory population simulated closely the results obtained in natural field infestations.

**Preliminary tests.** Several preliminary tests were run using a number of different cultivars to determine the best way to evaluate detached leaves of cucumber for antibiosis

Table 1. Performance of 8 selected cucumber lines in 2 leaf antibiosis tests and in 1 preference test run on a collection of 1194 lines in 1981.

Cultivar or line	Seed source	Detached-leaf test			Preference test <sup>x</sup>
		Clinton score <sup>z</sup>	Charleston Score <sup>z</sup>	Larva No. <sup>y</sup>	
<i>Resistant</i>					
C541C2	Joseph Harris	3.0	3.1	1.1	54
Femscore	Vanderploeg	3.0	3.2	4.1	37
PI 205996	Sweden	3.0	3.2	3.9	55
RS 79031	Royal Sluis	3.0	3.3	2.0	53
Earlipik 14	Northrup-King	3.5	1.5	0.5	56
<i>Susceptible</i>					
MSU 581H	Michigan State U.	5.5	5.0	4.1	40
PI 263079	U.S.S.R.	6.5	4.3	5.0	42
VDP 328	Vanderploeg	6.5	4.5	3.2	39
LSD (5%)		2.4	1.2	1.4	19
CV (%)		24	40	55	46
<i>r</i> (Clinton vs. Charleston score)		0.72*			
<i>r</i> (Charleston score vs. larva no.)				0.72*	
<i>r</i> (Charleston score vs. preference)					-0.70*

<sup>z</sup>Leaf damage scored 1 to 9 (1 = no damage, 9 = leaf tissue between veins completely eaten).

<sup>y</sup>Number of larvae on leaf at test completion (out of 5 at the start).

<sup>x</sup>Percentage of larvae on test line (remainder are on check cultivar, Columbia).

\*Significant at the 5% and 10% levels, respectively.

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Table 2. Performance of 7 lines tested for antibiosis to pickleworm larvae in the greenhouse in 1982 using 2 growth stages (3- and 7-week-old plants), and in 1983 using leaves from 2 nodes per plant.

Cultivar or line	Seed source	1982 Greenhouse test <sup>a</sup>		1983 Greenhouse test <sup>b</sup>	
		3-week-old plants	7-week-old plants	Node 1	Node 2
C541C2	Joseph Harris	46	24	28	29
PI 205996	Sweden	30	25	24	29
Femscore	Vanderploeg	40	27	24	26
PI 263079	USSR	35	30	42	26
Earlipik 14	Northrup-King	45	38	---	---
RS 79031	Royal Sluis	46	40	23	23
VDP 328	Vanderploeg	72	49	22	26
X		45	33	28	26
LSD (5%)		17	15	8	NS
CV(%)		46	52	34	32
r (3- vs. 7-week damage)		0.31**			
r (node 1 vs. node 2)				0.38**	

<sup>a</sup>Means of 6 single-plant replications and 2 nodes per line.

<sup>b</sup>Means of 10 single-plant replications and 2 stages per line.

\*\*Correlation significant at the 1% level.

to first instar pickleworm larvae. Leaves were harvested from the top 3 nodes of field-grown plants, placed in plastic bags, and brought to the laboratory where they were folded with the abaxial (lower) side of the leaf facing in. Pickleworm larvae were placed inside the fold of the leaf, and the leaf was put into a 100-mm diameter Petri plate. Deionized water (2, 3, or 4 ml) was added to 2 layers of filter paper in the bottom of the Petri plate, and the plates were kept closed 4 to 6 days. The plates were kept in the laboratory in stacks in plastic bags or left uncovered. The data collected were the amount of feeding damage to the leaf caused by the pickleworm larvae and the amount of rotting of the leaf that occurred (both characters scored 1 to 9, with 1 = no damage, 9 = completely damaged). We were looking for a treatment where there was little leaf rot (rot occurred occasionally when field-grown leaves were used, obscuring the pickleworm damage to the leaves) and an intermediate level of feeding damage, permitting the identification of lines with higher or lower levels of resistance than the lines used in the preliminary tests.

The best screening method (data not shown) was to use 2 ml of water on 2 pieces of filter paper in 100 mm diameter Petri plates, with 1 leaf per plate, 5 pickleworm larvae per leaf, and plates in stacks on the laboratory bench without plastic bags for cover. The test was read 4 to 6 days after inoculation, depending on how much damage had occurred to the leaves by the pickleworms and secondary rot organisms.

**Field test.** Cucumbers were obtained from as many sources as possible to make a col-

lection of 1160 cultivars and lines of pickling and fresh-market type (including the USDA plant introduction collection). The lines were planted 27 May 1981 at the Horticultural Crops Research Station near Clinton, N.C. Harvests were made 23 June and 14 July for the 2 replications, respectively. The youngest fully expanded leaf was harvested from one plant in each plot, placed in a plastic bag, and stored in a cooler in the field until the harvest was completed. The following day, leaves were placed in Petri plates with 5 neonate pickleworm larvae as described previously. After 6 days at room temperature, the leaves were removed from the plates and scored 1 to 9.

**Laboratory tests.** Of the 1160 cultivars and lines tested, the 18 most resistant and 18 most susceptible were selected and tested at Charleston, S.C., using 2 laboratory methods. Those were run in the fall of 1981 and consisted of a detached-leaf test as described above and a preference test.

The preference test was run by placing 28 mm diameter leaf disks of each of the lines on moist filter paper in a Petri plate next to a similar piece of leaf from the cultivar Columbia (a standard check used frequently for insect studies at the Charleston laboratory). The leaf disks were 12 mm apart and in that space were placed 5 newly hatched larvae. The number of larvae on each disk was counted after 2 hr to determine whether the test line was preferred over 'Columbia'. The experiment was a randomized complete block with 10 replications and 36 lines. Data were analyzed using a  $\chi^2$  test for a 50:50 distribution of larvae between the test and check

lines. The 5 most resistant and 3 most susceptible lines were selected for further testing.

**Methods development and retests.** The number of lines was reduced to 7 and finally to 6 through the 2 additional tests described here. The tests were used to select the most resistant and most susceptible lines and also to refine further the test method. The first of the methods tests used 7 lines and 2 plant growth stages, 3- and 7-week-old plants. The test was run in 1982 and used the same methods as before, except that the plants were greenhouse-grown and a completely random experimental design with 6 replications was used. Also, the 1 to 9 scoring system was replaced by a more accurate system where the percentage of damage to the leaf was estimated by comparison with a series of photos (representing categories from 0% to 100%). The percentage of damage in the photos had been measured using an area meter. This system of scoring was used for all subsequent tests.

The 2nd methods test was run in 1983 using selections that had been made from 6 of the lines with the same methods as before, except that leaves were harvested from the 2 youngest nodes where the leaves were fully expanded. A completely random experimental design with 10 replications was used.

**Heritability study.** To determine heritability of antibiosis of cucumber to pickleworm larvae, a population was developed by intercrossing the 5 most resistant and 3 most susceptible lines from the field screening test (Table 1). The progeny was intercrossed at random 2 more generations before evaluating as a parent-offspring regression study.

Full-sib families were produced from the population by crossing 124 plants in random pairs. The 2 parents and the offspring of each of the 124 families were tested using the same method as described for the above experiments. Leaves were harvested from the 2 youngest nodes with fully-expanded leaves, and the experiment was run with a single plant of each parent and 4 offspring per family. Parents were tested in 1983 and offspring in 1984, and the mean of the offspring was regressed on the values for the female and male parents separately. Narrow-sense heritability ( $h^2_N$ ) was estimated as twice the regression of offspring on female ( $b_{O,F}$ ) or male parent ( $b_{O,M}$ ) as described by Hallauer and Miranda (4). Correlations of offspring with female ( $r_{O,F}$ ) and male parent ( $r_{O,M}$ ) also were calculated.

Before beginning this study, we thought that resistance, if found, would not be inherited simply, because research in this area had been done before without success. Thus, we took the approach of looking for multi-genetic control of resistance which would have to be increased to useful levels by recurrent selection. However, we were disappointed to find very low levels of leaf antibiosis among the 1160 cucumber lines tested. It became apparent as we proceeded through the steps of refining the screening method that previous variation was primarily caused by environmental effects, but it was not until the

Table 3. Heritability analysis of the North Carolina Pickleworm Antibiosis population using 124 parent-offspring families tested in 1983 and 1984.

Variable <sup>a</sup>	Definition	Value
X <sub>L</sub>	Lowest (% leaf damage)	13
X <sub>H</sub>	Highest (% leaf damage)	28
b <sub>O,F</sub>	Offspring - female parent regression	0.014
b <sub>O,M</sub>	Offspring - male parent regression	0.011
h <sup>2</sup> <sub>N</sub>	Narrow-sense heritability	0.03
r <sub>O,F</sub>	Offspring - female parent correlation	0.05
r <sub>O,M</sub>	Offspring - male parent correlation	0.04

last 2 experiments that this was demonstrated conclusively.

**Screening lines for antibiosis.** The feeding damage observed for the 1160 lines for which data were collected ranged from 2 (mean of 2 replications) to 7 in our scoring system of 1 to 9 (data not shown). In this study, it appeared that the differences were genetic and were of a useful level. However, most of the apparently resistant lines proved to be misclassified when tested more carefully in the detached-leaf test and the preference test at Charleston, S.C. Only 8 lines gave results which were consistent with their previous classification as resistant or susceptible (Table 1). Data from the preference test, however, were correlated negatively (significant at the 10% level) with the detached-leaf test, which measured consumption in the absence of choice. That phenomenon has been observed in other crops, and may be due to a 2nd mechanism of resistance.

Upon retest of the 7 most consistent of the lines using a more refined test and more replications, it became apparent that the resistance was not consistent (Table 2). The data from plants tested at 3 weeks were correlated slightly (low but significant) with data from the same plants at 7 weeks. Further, some of the lines appeared to change from resistant to susceptible in their response (for example, RS 79031) in this test compared with previous tests. The correlation between nodes 1 and 2 of each plant ( $r = 0.38$ ) was highly significant, but the value was low enough to be of questionable use in substituting data from one node for another. Finally, when selections were made within each of the lines, there was no significant correlation between the performance of the selections and their  $S_1$  progeny (data not shown).

**Heritability study.** The population developed by intercrossing the most resistant and most susceptible lines from the original screening was diverse in morphological appearance, including pickling and fresh-market fruit types. However, the range in leaf damage of the 124 progeny tested was only from 13% to 24% (Table 3). That range was not very wide and did not include what we considered to be useful levels of resistance. The heritability estimate was 0.03 and was so low as to be indistinguishable from environmental variation.

**Conclusions.** The screening of an extensive collection of lines and recovery of little useful variation indicates that genetic differences for leaf antibiosis of cucumber to pickleworm larvae are essentially nonexistent in the cucumber germplasm collection. The heritability estimate confirms that conclusion. The results are disappointing because previous studies show little genetic variation for resistance to fruit infestation in the field as well (9). We concluded, therefore, that control of pickleworm in cucumbers will have to be through the use of cultural practices and chemical means, and not by resistant cultivars.

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