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A Laboratory Assay for Resistance to the Tobacco Hornworm in *Lycopersicon* and *Solanum* spp.[1]

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Abstract. **A laboratory procedure for assaying *Lycopersicon* spp. for resistance to the tobacco hornworm *Manduca sexta* (L.) is described. Results from this procedure, which employed excised foliage from greenhouse grown plants and first instar hornworm larvae from a colony maintained in the laboratory on artificial diet, were similar to those obtained with intact plants, with field collected hornworm larvae, and with field grown resistant plants. The *Lycopersicon hirsutum* f. *glabratum* accessions PI 134417 and LA 407 were highly resistant to *M. sexta.* Resistance was manifest as a significant reduction in larval survival and in weight gained by survivors over a 72 hour period. The interplant variation in larval weight gains within accessions was highly significant, suggesting that most of the accessions tested were segregating for resistance to *M. sexta.***

The tobacco hornworm commonly attacks tomato, *Lycopersicon esculentum* Mill., feeding on both the foliage and fruit. In commercial tomato plantings it is usually kept under control by the insecticide programs directed against the tomato fruitworm, *Heliothis zea* (Boddie). In home gardens, where insecticide usage on tomatoes is less intense, the tobacco hornworm can be a serious pest. The availability of tomato cultivars highly resistant to the tobacco hornworm would eliminate this widespread problem in home garden tomatoes.

Resistance to a number of important insect pests of tomato has been reported in *Lycopersicon hirsutum* Humb. and Bonpl., *L. hirsutum* f. *glabratum* C. H. Mull and *Solanum pennellii* Correll (5, 6, 7, 8, 9, 10, 11, 12). The present study was undertaken to determine if resistance to *M. sexta* is present in these species and to develop a laboratory procedure to assay for this resistance.

Materials and Methods

Unless otherwise stated, *M. sexta* larvae were obtained from a stock colony which had been maintained on artificial diet for 10 years. All experiments employed newly-hatched, first instar larvae. Plants were grown in a greenhouse in 15.2 cm clay pots containing Promix B soil mixture and were fertilized every 3 weeks to maintain vigorous growth.

With the exception of the initial screening for resistance, all experiments comparing resistance in *L. hirsutum* f. *glabratum* PI 134417 and 'Stakeless' tomato employed a clone of PI 134417 propagated by cuttings from a single plant selected as highly resistant to *M. sexta* in the initial screening experiment. All laboratory experiments employed a completely randomized experimental design and were conducted in a walk-in, insect-rearing room under conditions of 27°C and 50% relative humidity with a 16 hr photophase and an 8 hr scotophase.

Screening for resistance. Accessions of the following species were evaluated for resistance to *M. sexta* using excised foliage: *L. hirsutum,* PI 127826; *L. hirsutum* f. *glabratum,* LA 1266, LA 407, PI 134417, and PI 199381; *Solanum pennellii,* PI 365972; and *L. esculentum* cv. 'Stakeless' (susceptible standard). All plants were evaluated for resistance by confining 3 first instar hornworm larvae on a fully expanded leaflet held in a snap-cap plastic vial (diam 37mm × ht 62 mm). A damp strip of paper towel was included to provide moisture. Leaflets were replaced after 48 hr and the survivors were counted and weighed after 72 hr. There were 4 replicates for each plant evaluated; the no. of plants evaluated for each accession is given in Table 1.

Data on larval survival from all plants of each accession were pooled for each replicate to obtain the mean percentage survival. These values were transformed to arcsin $\sqrt{\%}$ and subjected to analysis of variance with mean separation by Duncan's multiple range test at $P \leq 5\%$. Data on larval wt were analyzed using a nested analysis of variance to determine if both the weight differences observed between accessions and those observed between plants within accessions were significant.

Field collected vs. laboratory reared hornworms. To verify that the plants found to be resistant in the screening study were also resistant to natural populations of *M. sexta,* an experiment was conducted to compare the survival and weight gain of field collected and laboratory reared hornworms on excised foliage of *L. hirsutum* f. *glabratum* (PI 134417, identified as resistant in the preceding experiment) and 'Stakeless' tomato. Hornworm eggs were collected from a tobacco field at Clayton, N.C., removed from the foliage, placed in a Petri dish with a plug of agar + sucrose diet and held at 27°C until hatching. Newly hatched larvae from the field and laboratory were weighed prior to confining them as described previously at a density of 3 per leaflet on excised foliage from resistant and susceptible plants. After 72 hr survivors were weighed, counted and weight gain by the survivors determined. The entire experiment was replicated 10 times.

Survival data from all replicates were pooled and analyzed using the Chi square test for independence. Weight data were

Table 1. Survival of *M. sexta* larvae after 72 hr on excised foliage of *Lycopersicon* and *Solanum* spp.

Entry	No. plants	Mean % larval survival
L. esculentum cv. Stakeless	26	89a[z]
L. hirsutum, PI 127826	21	79a
L. hirsutum f. *glabratum,* LA 1226	10	89 a
L. hirsutum f. *glabratum,* PI 199381	29	81 a
L. hirsutum f. *glabratum,* PI 134417	30	56 b
L. hirsutum f. *glabratum,* LA 407	3	19 c
S. pennellii, PI 365972	18	77 a

[z]Mean separation by Duncan's multiple range test using transformed data (Arcsin $\sqrt{\%}$); $P \leq 5\%$.

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Table 2. Mean wt of *M. sexta* larvae after 72 hr on excised foliage of *Lycopersicon* and *Solanum* spp.

Entry	Plants	Mean wt (mg)/ larvae (+/– SD)	Range	CV (%)
L. esculentum				
cv. Stakeless	26	9.9 (± 1.58)	7.0-12.3	15.96
L. hirsutum				
PI 127826	21	6.2 (± 1.48)	3.3-8.3	24.06
L. hirsutum f. *glabratum*				
LA 1266	10	8.8 (± 1.87)	6.3-11.3	21.38
PI 199381	29	6.4 (± 1.52)	4.3-9.7	23.78
PI 134417	30	4.0 (± 0.95)	3.0-6.7	23.52
LA 407	1	5.3	–	–
S. pennellii				
PI 365972	18	4.4 (± 1.08)	2.8-6.5	24.94

not pooled, but were subjected to analysis of variance with mean separation by Duncan's multiple range test at $P \leqslant 5\%$.

Field vs. greenhouse grown plants. To insure that resistance observed in greenhouse grown plants was also present in those grown in the field, hornworm larvae from the laboratory colony were reared on excised foliage from greenhouse and field grown plants of PI 134417 and greenhouse grown 'Stakeless plants.' Newly-hatched first instar larvae were placed in lots of 7 in rearing vessels which consisted of plastic Petri dishes (150 mm diam × 25 mm ht) containing the appropriate excised foliage on a piece of moist filter paper. Fresh foliage was supplied every second day for the first 6 days and daily thereafter. Larval survival was recorded after 5 and 10 days; those surviving for 10 days were weighed. The experiment was replicated 6 times.

Intact plants vs. excised foliage. To verify that the use of excised leaves provided a reliable index of resistance in intact plants and excised leaves of PI 134417 and 'Stakeless,' 6 larvae were confined to each intact plant using glass lamp chimneys which were closed at the top with a piece of cotton organdy. These plants were about 15 cm tall at the start of the experiment. Larvae were confined in lots of 6 to excised foliage using the rearing vessels described previously. Larval mortality was recorded after 4 days. The experiment was replicated 5 times.

Results and Discussion

Screening for resistance. High levels of resistance to *M. sexta* were apparent in *L. hirsutum* f. *glabratum,* PI 134417 and LA 407. Larval mortality on these entries was greater than on all other entries (Table 1). Larval development among the survivors on PI 134417 and LA 407 was apparently retarded, since the mean wt of survivors on the former was only 40% and on the latter only 54% of that of survivors on 'Stakeless' (Table 2). Although mortality on *S. pennillii,* PI 365972, during the 72 hr experiment did not differ from that on 'Stakeless,' larvae on *S. pennillii* attained only 44% of the final wt of those on 'Stakeless.'

Analysis of variance indicated the difference in larval wt observed between accessions was highly significant as were the differences between plants within accessions. The differences in larval wt on different plants of the same accession are in agreement with differences in larval survival observed between plants of certain entries (e. g. PI 134417 and LA 407), and suggest that most of the accessions tested are still segregating for resistance to *M. sexta.* The coefficient of variation for larval wt is less for 'Stakeless' than for the other accessions (Table 2). Segregation for insect resistance within PI's is not unique since Stoner (11) observed segregation with *Lycopersicon* spp. for carmine spider mite resistance.

Field collected vs. laboratory reared hornworms. Resistance of PI 134417 first observed in assays with *M. sexta* larvae from a laboratory colony was confirmed by assays using larvae from field collected eggs (Table 3). Both percent survival and wt gained by survivors were less on PI 134417 than on 'Stakeless' for both field collected and laboratory reared larvae. While there was no difference in wt gained by larvae from the field or laboratory on PI 134417, mortality among field collected larvae was significantly greater than among those from the colony. These data indicate that *M. sexta* larvae from a colony maintained on artificial diet for 10 years can be used effectively to assay for the resistance found in PI 134417. This situation is unlike that of the European corn borer which could not be used to study resistance in corn after it had been reared on artificial diet for 34 generations (4).

Field vs. greenhouse grown plants. Both field and greenhouse grown plants PI 134417 were resistant to *M. sexta* (Table 4). However, plants raised in the greenhouse were more resistant than those from the field since mortality after 5 days was greater on the former. After 10 days, there was no difference between survival on greenhouse and field grown PI 134417. The few larvae that survived for 10 days on the field grown plants were extremely small, compared to those reared on 'Stakeless,' and died before reaching the pupal stage.

From a practical perspective field grown plants of PI 134417 are still highly resistant to *M. sexta.* The difference in the level of resistance of greenhouse and field grown plants may be related to the amount of glandular hair exudate present on the leaf surface. Such exudate from other *L. hirsutum* accessions has been shown to be important in resistance to spider mites (1, 8). Plants raised in the greenhouse had a much greater abundance of this exudate on their leaf surfaces than did those raised in the field.

Excised vs. intact foliage. Survival of *M. sexta* larvae after

Table 3. Response of field and laboratory populations of *M. sexta* after 72 hr on excised foliage of resistant and susceptable *Lycopersicon* spp.

Entry	Mean % larval survival	Mean larval wt gain (mg)
L. esculentum cv. Stakeless		
Lab	100	19.9 a[z]
Field	100	24.2 a
	Chi Square = 0 (NS)	
L. hirsutum f. *glabratum,* PI 134417		
Lab	40	3.3 b
Field	23	1.9 b
	Chi Square = 9.09 ($P \leqslant 0.05$)	
Stakeless vs. PI 134417		
	Chi Square = 26.67 ($P \leqslant 0.001$)	

[z]Mean separation by Duncan's multiple range test; $P \leqslant 5\%$.

Table 4. Survival and wt of *M. sexta* larvae reared on excised foliage of field and greenhouse grown plants of *L. hirsutum* f. *glabratum*, PI 134417 and *L. esculentum*, cv. Stakeless.[z]

Entry	Mean % larval survival		Mean wt of 10 day old survivors (g)
	5 day	10 day	
L. esculentum, Stakeless			
Greenhouse	88 a	76 a	1.269 a
L. hirsutum f. *glabratum*, PI 134417			
Greenhouse	0 b	0 b	–
Field	24 c	5 b	0.053 b

[z]Mean separation in columns by Duncan's multiple range tests; $P \leq 5\%$.

7 days on excised foliage and intact plants of PI 134417 was 50% and 27%, respectively. These values were not different ($P > 5\%$), but both were less than the survival on excised foliage and intact plants of 'Stakeless' tomato, the susceptible standard. Survival on these latter averaged 90% and 93%, respectively.

Although the resistance of PI 134417 may have been slightly reduced in excised foliage, these data indicate that excised foliage can be used in assays for resistance to *M. sexta* derived from this accession.

The resistance of *L. hirsutum* f. *glabratum* to *M. sexta* is not surprising since numerous accessions have been previously reported to be resistant to various insect pests of tomato. Of the accessions included in the present study, both *L. hirsutum* PI 127826 which was susceptible and *L. hirsutum* f. *glabratum* PI 134417 which was resistant to *M. sexta*, have been reported as having resistance to tomato fruitworm (5), tobacco flea beetle (6), the carmine and twospotted spider mites (8, 11), leafminer (12) and the tomato pinworm (10). PI 134417 was reported resistant and PI 127826 susceptible to the Colorado potato beetle (9) while the former was reported susceptible and the latter resistant to the greenhouse whitefly (7). Presently, it is not known what relationship, if any, exists between resistance to hornworm and resistance to these other pests.

Since *L. hirsutum* f. *glabratum* can be used as a pollen parent in crosses with *L. esculentum*, PI 134417 and LA 407 should be of value in breeding tomato cultivars resistant to the tobacco hornworm. PI 134417 should be of value as a source of resistance to various important insect pests as well as diseases (2, 3) of tomato. Progress in breeding for hornworm resistance can be facilitated by assaying for resistance under laboratory conditions using excised foliage.

Literature Cited

1. Aina, O. J., J. G. Rodriguez, and D. E. Knavel. 1972. Characterizing resistance to *Tetranychus urticae* in tomato. *J. Econ. Entomol.* 65:641-3.
2. Alexander, L. J. 1953. Progress report of national screening committee for disease resistance in the tomato for 1952. *Plant Dis. Rptr.* 37:317-24.
3. Bashi, E., M. Pilowsky, and J. Rotem. 1973. Resistance in tomatoes to *Stemphylium floridanum* and S. *botryosum* f. sp. *lycopersici*. *Phytopathology* 63:1542-4.
4. Brindley, T. A., A. N. Sparks, W. B. Showers, and W. D. Guthrie. 1975. Recent research advances on the European corn borer in North America. *Annu. Rev. Entomol.* 20:221-23.
5. Fery, R. L. and F. P. Cuthbert, Jr. 1975. Antibiosis in *Lycopersicon* to the tomato fruitworm (*Heliothis zea*). *J. Amer. Soc. Hort. Sci.* 10:276-8.
6. Gentile, A. G. and A. K. Stoner. 1968. Resistance in *Lycopersicon* spp. to the tobacco flea beetle. *J. Econ. Entomol.* 61:1347-9.
7. _____, R. E. Webb, and A. K. Stoner. 1968. Resistance in *Lycopersicon* and *Solanum* to greenhouse whiteflies. *J. Econ. Entomol.* 61:1355-7.
8. _____, _____, and _____. 1969. *Lycopersicon* and *Solanum* spp. resistant to the carmine and the two-spotted spider mite. *J. Econ. Entomol.* 62:834-6.
9. Schalk, J. M. and A. K. Stoner. 1976. A bioassay differentiates resistance to the Colorado potato beetle on tomatoes. *J. Amer. Soc. Hort. Sci.* 101:74-6.
10. Schuster, D. J. 1977. Resistance in tomato accessions to the tomato pinworm. *J. Econ. Entomol.* 70:434-6.
11. Stoner, A. K. 1968. Resistance in *Lycopersicon* species to the carmine spider mite. U.S. Dept. Agr. Prod. Res. Rpt. 102.
12. Webb, R. E., A. K. Stoner, and A. G. Gentile. 1971. Resistance to leafminers in *Lycopersicon* accessions. *J. Amer. Soc. Hort. Sci.* 96:65-7.