Evaluating Resistance of Apple Fruits to Four Insect Pests[1]

Hilary F. Goonewardene[2], W. F. Kwolek[3], R. E. Dolphin[4], and E. B. Williams[5,6]
U.S. Department of Agriculture, West Lafayette, Indiana

Abstract. **Methods of evaluating resistance of apple fruit to 4 insect pests were established by modifying existing rearing procedures. When 'Jonathan' was used as the check cultivar and an adjustment was made for variation between trays among checks, it was possible to separate selections that were significantly more resistant from randomly selected samples of apple genotypes. We found 9.7 22.9, 32.3, and 17.0% of the selections tested against the codling moth, *Laspeyresia pomonella* (L.), plum curculio, *Conotrachelus nenuphar* (Herbst), apple maggot, *Rhagoletis pomonella* (Walsh), and redbanded leafroller, *Argyrotaenia velutinana* (Walker), respectively, had significantly less damage than the check.**

Resistance to such fruit attacking insects as codling moth (CM), plum curculio (PC), apple maggot (AM), and redbanded leafroller (RBLR), would be desirable for genetic improvement of the apple, *Malus domestica* Borkh. Our objective was to develop screening procedures for the identification of resistant selections from the breeding program for disease resistance developed cooperatively by the Agricultural Experiment Stations of Indiana, Illinois, and New Jersey. These selections could then be used as parents in breeding for resistance to the above-mentioned aple pests.

Test procedures. The insect species used in the study are maintained in continuous cultures at the USDA, ARS, Midwest Deciduous Fruit Insect Laboratory, Vincennes, IN. Methods of rearing AM, PC, and CM on thinning ("June drop") apples (2.5-3.8 cm diam) have been reported (2, 4, 6). Although several cultivars of thinning apples (1, 2, 5, 6) were used in the procedures, the choice was based on storage quality at 0-1°C. The rearing procedures cited above for CM, AM, and PC were modified in our tests so that we could evaluate resistance of apple selections. We used the criterion of damage (entry and exit holes) to measure resistance of these selections to each species. Preliminary studies indicated that self-adhesive color coding dots were suitable for identifying selections. We also found that saran wrap was suitable as a cover for AM but that PC did better when plastic screen (20 mesh Chicopee Manufacturing Co., Cornelia, Ga.) instead of saran wrap was used as a cover. Our unpublished data confirmed that saran wrap could also be used as a cover for trays of apples infested with CM or RBLR. We selected 'Jonathan' as our check cultivar, after a preliminary trial, for all tests because it gave as good hatch (86%) of AM eggs as 'Golden Delicious'.

One hundred hand-harvested apples (diam 3.2-4.4 cm) were placed in each waxed cardboard tray (35.5 × 61.0 × 10.2 cm) lined with aluminum foil (ca. 0.002 cm thick). Each tray contained 10 apples from each of 7 selections, plus 30 check apples. This procedure follows the experimental design recommendation that controls be assigned more units for increased precision (3). Most test apples, except those used for checks, were from 6-year-old trees grown at the Purdue University Horticulture Farm, West Lafayette, IN. Apples were harvested in 2 batches from June 24 to 26 and from July 8 to 9. Apples were selected for uniformity of size. The dates of harvests generally coincided with periods of insect activity from mid-June through July. Apples were held at 0-1°C until used. All tests were set up within 2 days after harvest. Selections were color coded and randomly mixed in the tray. Laboratory reared adult insects or insect stages were used. Infested trays were held in illuminated rearing rooms at 25-26°C and 70% RH. In tests with AM and PC, all apples were confined in trays by a cardboard strip until infestation was completed to prevent injury to the insects by rolling apples.

The no. of insects selected to infest a tray was estimated to provide measurable differences in damage. Damage counts from each study (i.e., for each insect species) were transformed to log (X+1) where X = the damage (no. of entry holes, exit holes, oviposition stings, feeding scars) to each apple of the selection, and analyses of variance were computed. The geometric means (= antilog of the log means) were computed for the check apples in each tray and for the 10 test apples from each selection per tray. The deviation of the log mean for each selection from the mean of the check from the same tray was calculated. This difference was then divided by the standard error of the difference to obtain a t value (7). This procedure allowed adjustment for variation between trays in the check results.

An entry hole indicated that the apple presented no deterrent to entry by the insect, and an exit hole indicated that the apple supported some or all of the larval development of the insect; hence, either 1 or both criteria were selected from all other criteria of damage that were measured and analyzed to determine the degree of resistance of the selection. The t values >2 in absolute value for the respective damage measurements were significant at the 95% level of probability. Only those selections having a significantly lower damage count relative to the check are reported.

Screening procedures and results.

Codling Moth: Twenty-five pairs of newly emerged adult CM were allowed to oviposit for 5 days in each cylindrical quart fiberboard container lined with wax paper; a sugar solution was provided as food. The moths were removed, and the containers were held for another 12 days. Then the wax paper with eggs was removed, cut into several pieces, and distributed evenly over the apples in the tray. The trays were then covered with saran wrap and the edges were taped to the sides. The top of the saran wrap was perforated for air exchange. We estimated that each female laid between 70-138 eggs. Therefore, there were 1750-3450 eggs

[1]Received for publication April 10, 1975. Approved for publication as Journal Article No. 5853, Purdue University Agricultural Experiment Station, W. Lafayette, IN 47907. Mention of a commercial or proprietory product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.

[2]Research Entomologist, Agr. Res. Serv., U.S. Department of Agriculture and Adjunct Assoc. Professor, Department of Entomology, Purdue University, W. Lafayette, IN 47907.

[3]Biometrician, Agr. Res. Serv., U.S. Department of Agriculture, NCR, 1815 North University St., Peoria, IL 61604.

[4]Research Entomologist, Agr. Res. Serv., U.S. Department of Agriculture and Adjunct Asst. Professor, Department of Entomology, Purdue University, P.O. Box 944, 1118 Chestnut St., Vincennes, IN 47591.

[5]Professor, Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907.

[6]We wish to thank T. Mouzin, Ruby Radwanski, H. Candler, J. Burnside, and Sonja Myer of the USDA Laboratory, Vincennes, IN for providing us with the insects used in our tests; Rosemary Lemons and D. Helderman, summer help, for their assistance in the program at Vincennes; and C. Hendricks and K. Glover, summer help, for their assistance at W. Lafayette. Our thanks also to the following cooperators: D. F. Dayton and J. B. Mowry, Department of Horticulture, University of Illinois; and L. F. Hough and Catherine Bailey, Department of Horticulture and Forestry, Rutgers University, for the use of cultivars developed by them.

Table 1. Comparison of mean (geometric) damage counts to some of the more resistant selections of apple fruit and to the check ('Jonathan') by codling moth, plum curculio, apple maggot, and redbanded leafroller in the laboratory.

Selection no.	Geometric mean[z] (damage per apple): Codling moth, Entry holes	Codling moth, Exit holes	Plum curculio, Feeding scars	Plum curculio, Exit holes	Apple maggot, Oviposition stings	Apple maggot, Exit holes	RBLR, Feeding scars
54-50-13					2.27	.85	.15
1653-1							.0
1653-2					1.74	.37	.0
1660-3					.91	.60	
1660-4					1.89	1.31	
1660-5			1.45	1.50			
1677-2							.07
1695-10					2.69	.41	
1698			1.00	2.89			
1698-1					3.01	1.18	.0
1743-2			1.26	3.40	3.32	.94	
1754-1			1.35	2.86			.15
1754-2					1.46	1.31	
1757-3					.97	.58	
674-103					.81	.34	
674-104			1.00	1.62	.74	.37	
841-102			1.17	1.22			
882-100					.93	.53	
1236					2.27	.20	
1557-1					1.53	.45	
1563-1					.95	.73	
1563-2					2.25	.58	
1576-1					1.15	.30	
1592-1	2.20	1.00					
1054-1	1.65	.94					
1094-4			1.35	2.84			
1228-3	1.59	1.58					
1235-2	1.56	1.26					.15
1255-3	1.62	1.55					
1290-2			1.26	4.62			
1294-2			1.17	3.84	.53	.52	
1279-7	1.29	.74					
1587-1	.12	1.42			1.73	.20	
Overall GM/for check variety	2.87	1.93	1.38	7.17	5.47	2.40	.33

[z]All geometric means of damage counts (except plum curculio feeding scars) reported above were significantly lower than the check at the 95% level of probability.

[y]Selection no. refers to the cooperative breeding program of the Indiana, Illinois, and New Jersey Agricultural Experiment Stations.

per paper with an expected hatch of 40-60%, ca. 700-2070 larvae per tray. Two days after infestation, the entry holes in the apples were counted. Four weeks later, exit holes were counted. We tested 93 selections and chose 9 that showed significant reduction in damage for both criteria of CM damage we measured. There were 38 selections that showed significantly lower counts for either entry or exit holes.

Plum Curculio: The rim of each tray was lined with self-adhesive plastic foam tape (0.48 x 0.94 cm). Then 50 unsexed mature adult PC (5-10 days old) were placed in each tray containing a small dish of water. Screen covers were placed over the trays and secured with tape and rubber bands. After 7 days, the adults were removed, and the apples were examined for feeding scars and rated (none, low, medium, high = 0, 1-5, 6-10, and >10 feeding scars, respectively). The covers were replaced and the trays were returned to the rearing room for another 14 days; then the trays were brought out for counting exit holes. We evaluated 74 selections by this procedure. Because of PC's indiscriminate feeding behavior, we found no significant differences between selections based on feeding scars; therefore, we based our selections on the significantly lower number of larval exit holes.

Apple Maggot: Twenty-five pairs of mature (8+ day old) CO_2-anaesthetized AM flies were confined in each tray with apples. The flies were provided with laboratory diet and water. Saran wrap was stretched over the tray, taped to the sides, and perforated for air exchange. All flies were removed 24 hr later. The trays were placed in saran bags and left in the rearing room for 7 days. There the apples were examined for oviposition stings and egg hatch before being returned to the rearing room for another 20 days after which they were checked for exit holes. This procedure was used to evaluate 65 selections. A t value >2 for oviposition stings (entry holes) and exit holes was used for separating differences between selections. Although 39 selections showed significantly lower counts for one or the other criteria, we selected 21 that gave this t value for both criteria.

Redbanded Leafroller: This insect generally feeds on apple leaves; it moves to the fruit only in the later instars for surface feeding. Fifty, 4th instar larvae (reared for ca. 14 days on laboratory diet of supplemented alfalfa meal) were placed in each tray with apples. Saran wrap was used to cover the trays and after air holes were punched in the saran wrap, trays were placed in the rearing room. The apples were periodically examined, but after ca. 10 days, final counts of feeding damage and pupation were recorded. We evaluated 47 selections and have reported only selections that gave significantly lower feeding scars.

Although we found that 9.7, 22.9, 32.3, and 17.0% of selections exposed to the CM, PC, AM and RBLR, respectively, had significantly less damage than the check at the 95% level of probability, Table 1 shows only those selections that showed most resistance based on criteria of damage measured.

Our data illustrates that identifiable sources of insects resistance are available which can possibly be used in a breeding program. If insect and disease resistance are combined through breeding the current heavy pesticide requirement for apple production could be considerably reduced.

Literature Cited

1. Boulanger, L. W., G. E. Stanton, and A. L. Padula. 1969. Continuous rearing of the apple maggot. *J. Econ. Entomol.* 58:1056-1057.
2. Dickerson, R. C., M. M. Barnes, and C. L. Turzan. 1952. Continuous rearing of the codling moth. *J. Econ. Entomol.* 45:66-68.
3. Finney, D. J. 1952. Statistical method in biological assay. Hafner Publishing Co., New York.
4. Garman, P. 1937. Notes on breeding the apple maggot, *Rhagoletis pomonella.* p. 436-437. *In* J. G. Needham (ed.) Culture methods for invertebrate animals. Comstock Publ. Co., Ithaca, N.Y.
5. Nielson, W. T. A. 1965. Culturing apple maggot, *Rhagoletis pomonella. J. Econ. Entomol.* 58:1056-1057.
6. Smith, E. H. 1957. A method for rearing the plum curculio under laboratory conditions including some biological observations. *J. Econ. Entomol.* 50:187-190.
7. Snedecor, G. W. and W. G. Cochran. 1968. Statistical methods. 6th ed., Iowa State University Press, Ames, Iowa.