J. Amer. Soc. Hort. Sci. 107(5):737–740. 1982.

# Differentiation of *Malus* Clones into Resistance Classes by their Effects on the Biology of *Eriosoma lanigerum* Hausmn.[1]

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*Additional index words.* rootstock breeding, woolly apple aphids, resistance breeding, antibiosis

***Abstract.*** **Degrees of resistance of 4 *Malus* clones to woolly apple aphid (WAA) were determined by the survival, development and behavior of nymphs caged on the plants. Nymphal survival rates after 2 weeks on 'Empire', 'Northern Spy', and 'Robusta 5' were 78%, 30%, and 0.8% respectively. After 4 weeks, only the aphids on 'Empire' were at reproductive age. *M. baccata mandshurica* (Maxim.) Schneid. PI 322713 'Manchurian Crab' expressed partial resistance compared to 'Empire' on the basis of insect developmental times, even though insect survival after 2 weeks was unaffected. First instar developmental times on 'Manchurian Crab' and 'Empire' were 14 and 5 days respectively; days to reproductive maturity was greater than 30 days for 'Manchurian Crab' compared to 13 for 'Empire'. Different feeding behavior was expressed by the proportion of aphids in motion at the times of observation and proportion of aphids feeding at the same location for 12-hr periods. The data suggest that for comparing quantitative differences in resistances of clones, determining developmental times is a more sensitive technique than measuring nymphal survival.**

The woolly apple aphid *(Eriosoma lanigerum)* (WAA) colonizes both the roots and branches of apple trees (*Malus domestica* Borkh.) and may cause serious economic damage (1, 5, 6, 13, 15). Although 'Northern Spy' has been the most widely used source of resistance for many years (9), since 1965, new WAA biotypes have been reported to overcome this "Spy-type" resistance in North Carolina, South Africa, and Australia (6, 14, 16). The widely planted Malling-Merton rootstocks, derived from 'Northern Spy', are apparently susceptible to the new WAA biotypes. This recent development has stimulated interest in finding new sources of resistance (3, 4).

Success in breeding for host plant resistance to WAA depends on rapid estimation of plant injury or of insect infestation on thousands of seedlings. The simplest method to screen for resistance is observation under natural infestation. While the use of a selective insecticide makes this method more effective (3, 4), artificial infestation is still needed to differentiate susceptible escapes from truly resistant *Malus* clones. Methods for artificial infestation include placing aphids onto the base or roots of the plant, placing aphids on the stem with a fine brush, and tying infested cuttings to the plant (2, 4, 6, 9, 12, 17, 18).

All these methods involve assessment of levels of infestation achieved after many insect generations. Such methods do not reveal mechanisms of resistance and may indeed result in masking of partial resistances. In the Geneva apple rootstock breeding program (3), we required a technique which would facilitate both evaluation of potential parents and study of inheritance of resistance.

## Materials and Methods

To provide continuing opportunity to observe behavior of individual insects, miniature cages were made by cutting plastic drinking straws into sections about 1 cm long. Cages were trimmed on one side to fit the curvature of the stem (Fig. 1) and then fastened into place with parafilm strips. Caps were made from straws with slightly larger diam than the cage so that the cap would fit over the open end of the cage. The caps were covered with fine dacron mesh sealed in place by melting the plastic.

Four *Malus* clones were studied: *M. domestica* 'Empire', known to be highly susceptible to WAA; 'Manchurian Crab' on which WAA colonization usually has been limited; *M. domestica* 'Northern Spy', which is resistant to WAA; and *M.* X *robusta* (Carr.) Rehd. 'Robusta 5' which appears to be immune.

*Nymph Survival.* Survival of WAA nymphs was determined on 'Robusta 5', 'Northern Spy', and 'Empire'. Scions of these cultivars were grafted onto WAA-resistant MM 111 rootstocks. Trees were trained to 2 shoots in the greenhouse, and WAA tests were conducted in a controlled-environment chamber maintained at 24°C with a 16-hr photoperiod. Six cages were attached to each of the plants, 3 to each shoot; cages were evenly distributed along the shoot and each cage was positioned directly above a leaf axil. The natural pubescence of the stem was removed before attaching the cages.

First instar nymphs were used in the cage experiments because this stage is the primary dispersal form that establishes new colonies (8). Two gravid females were placed in each cage for 24 hr to deposit nymphs. The adults were then removed and the number of nymphs was adjusted to 8 per cage. Surviving aphids in each cage were counted weekly.

*Development and Behavior.* The developmental times, survival and feeding behavior were determined on 'Robusta 5', 'Empire', and 'Manchurian Crab'. A dead, dry apple shoot was included as a control to determine behavior and mortality when no feeding was possible. The trees were grafted and handled as in the Nymph Survival experiment. When the trees were 10

[1]Received for publication Dec. 11, 1981. Approved by the director of the New York State Agricultural Experiment Station as Journal Paper 3403.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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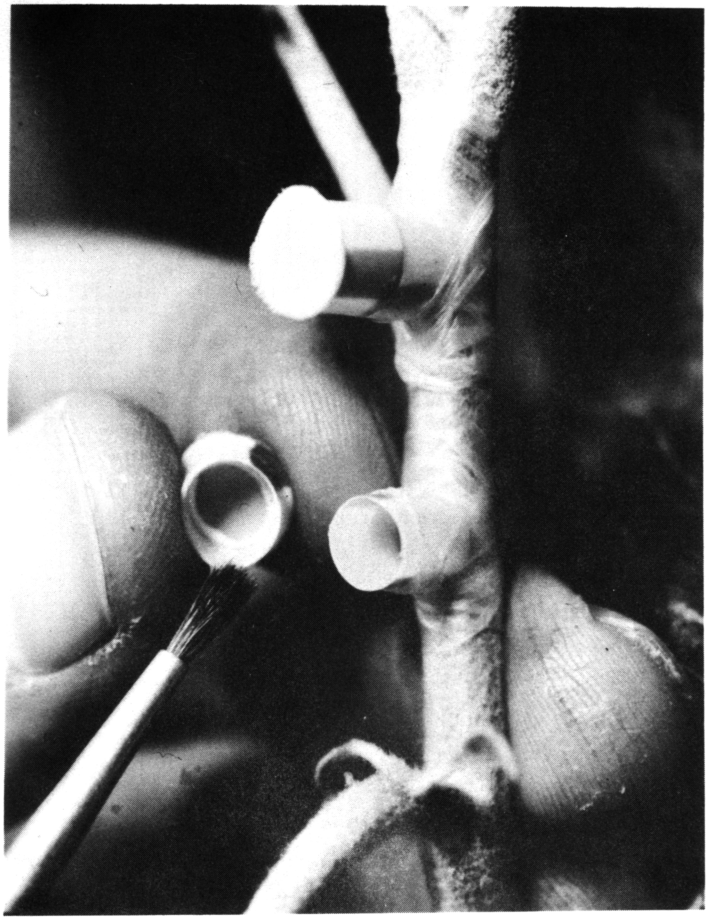

Fig. 1. Miniature cages constructed of drinking straws, dacron mesh, and parafilm, used for confinement of woolly apple aphid nymphs on apple stems (× 2).

weeks old, 4 cages were mounted on each cultivar and each was infested with 5 first instar nymphs. The nymphs were obtained by holding adults in vials for 18 hr to deposit nymphs; these were transferred to the cages with a camel's hair brush. This procedure allowed all nymphs to begin feeding at the same time. Survival and presence of any exuviae (cast skins) were recorded at 12-hr intervals following infestation. (WAA has 4 nymphal instars before reaching reproductive maturity; the exuviae are covered with white wax and thus may be readily found.)

The trial was repeated on a smaller scale when the trees were 4 months old, with 2 nymphs per cage. Every 12 hr, records were made of the number of nymphs alive, the number settled (motionless), and the number that had moved to new feeding sites since the previous observation.

Table 1. Survival of first instar nymphs confined on resistant and susceptible *Malus* cultivars in the growth chamber.[z]

| Cultivar | Shoot age (mo.) | Initial no. of aphids | Survival (%) 1 wk | 2 wk | 3 wk | 4 wk |
|---|---|---|---|---|---|---|
| Robusta 5 | 3 | 48 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 4 | 48 | 2.1 | 2.1 | 0.0 | 0.0 |
| | 5 | 48 | 0.0 | 0.0 | 0.0 | 0.0 |
| Avg. | | | 0.7 a[y] | 0.7 a | 0.0 a | 0.0 a |
| Northern Spy | 3 | 48 | 53.8 | 41.3 | 8.8 | 8.8 |
| | 4 | 48 | 43.8 | 27.5 | 3.8 | 0 |
| | 5 | 48 | 31.3 | 21.3 | 10.0 | 8.3 |
| Avg. | | | 42.5 b | 30.0 b | 8.5 b | 5.7 b |
| Empire | 3 | 48 | 87.5 | 93.8 | >2000 | >2000 |
| | 4 | 48 | 65.0 | 65.0 | >2000 | >2000 |
| | 5 | 48 | 77.5 | 77.5 | >2000 | >2000 |
| Avg. | | | 76.3 c | 78.8 c | >2000 c | >2000 c |

[z]16-hr photoperiod was maintained at a constant 24°C.
[y]Significantly different from 'Empire' at the 95% level based on analysis of variance calculated from the number of surviving aphids in each cage.

## Results

*Nymph Survival.* Survival of 144 nymphs caged on susceptible 'Empire' was 76% after 1 week and about the same after the second week (Table 1). Before the third week, aphids on 'Empire' reached reproductive maturity and the total number was estimated as more than a twentyfold increase. Survival on 'Northern Spy' was 43% after 1 week, 30% after 2 weeks, 8% after 3 weeks, and 6% after 4 weeks. None of the aphids surviving on 'Northern Spy' after the fourth week had reached reproductive maturity. On 'Robusta 5', only 1 aphid was alive after 1 week (2% survival), and it died by the third week.

The difference in survival rate between 'Northern Spy' and 'Empire', and the effect of shoot ages within cultivars, were analyzed using a nested classification. Differences between the 2 cultivars were significant at the 95% level at both 1 and 2 weeks. There were no significant differences attributable to age or tree. Nymph survival on a shoot was not affected by the growth stage of the shoot tip.

*Development and Behavior.* Both on 'Empire', the susceptible cultivar, and on 'Manchurian Crab', nymphal survival was 85% after 2 weeks (Fig. 2). However, the nymphs on 'Empire' reached second instar in 5 days and reproductive maturity within 13 days, compared to nymphs on 'Manchurian Crab' that required 14

Table 2. Settling behavior of woolly apple aphid nymphs on 3 *Malus* clones or on dead apple wood; observations were made every 12 hours for 5 days.

| Cultivar | Initial no. of aphids | Survival after 5 days (%) | Total observ.[z] | Aphids motionless when observed (%) | Aphids settled at same location as recorded 12 hr earlier (%) |
|---|---|---|---|---|---|
| Dead shoot | 4 | 0 | 10 | 10 | 0 |
| Robusta 5 | 8 | 13 | 43 | 61 | 31 |
| Manchurian Crab | 4 | 100 | 40 | 83 | 53 |
| Empire | 8 | 88 | 71 | 100 | 95 |

[z]No. surviving aphids at each observation summed for 10 observations.

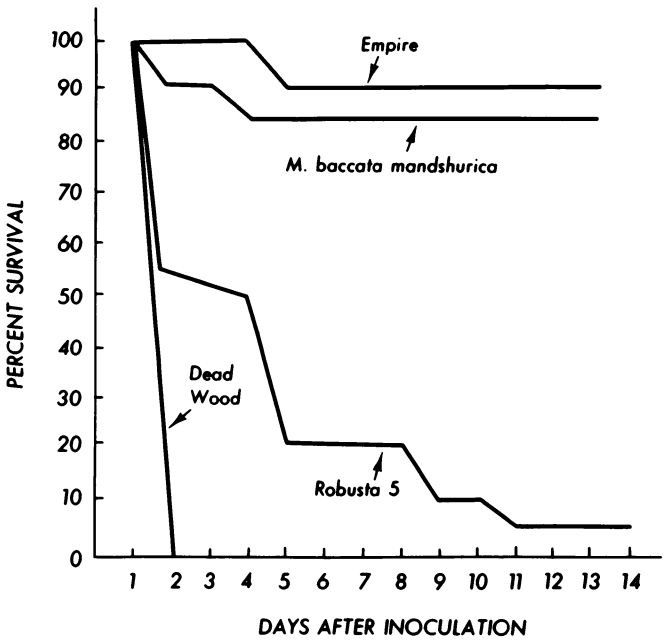


Fig. 2. Survival days of first instar woolly apple aphid nymphs confined in minicages on resistant and susceptible apple clones and on dead wood.

days to second instar and had not reached reproductive maturity after 4 weeks. Mortality on the highly resistant, perhaps immune, 'Robusta 5' was about 50% within 48 hr and increased to 80% after 1 week; a single aphid of the original 20 survived for 4 weeks and then molted to the second instar, but did not resettle after molting. This individual was the only nymph observed to advance past the first instar on 'Robusta 5' in any experiment. Nymphs caged on the dead wood check were all dead within 48 hr.

Settling behavior of nymphs caged on 'Robusta 5' and other cultivars was observed at 12-hr intervals (Table 2). Seven of the 8 nymphs caged on 'Empire' were alive after 5 days. Every surviving aphid was apparently settled (motionless) at the time of each observation. Diagrammatic records of positions of nymphs on 'Empire' showed that the survivors all settled within 12 hr after infestation and remained at the same feeding site until the first molt and sometimes longer. Seven nymphs caged on 'Robusta 5' were dead by the 5th day. Unsettled nymphs were observed 16 times during the experiment. Even when nymphs were observed to be motionless, it was uncommon for them to be in the same position 12 hr later; 36 hr was the longest time a nymph remained settled in the same place on 'Robusta 5' in this trial, compared to a maximum time of 108 hr on 'Empire'. Although nymphal survival was greater on 'Manchurian Crab' at 5 days than on 'Empire' (100% vs. 88%), the nymphs on the former were less settled at the times of observation (83% vs. 100%). Four nymphs confined on dead wood were almost always in motion; only once was one observed motionless, and all were dead by the second day.

## Discussion

'Northern Spy' and 'Robusta 5' were shown to be resistant to WAA by the greater mortality of caged nymphs compared to the susceptible 'Empire' check (Table 1). Reproductive maturity was not attained on 'Northern Spy' or 'Robusta 5' by 30 days, but nymphs matured in 13 days on 'Empire'; 14 to 17 days has been reported as the normal life cycle (14). The much greater mortality on 'Robusta 5' than on 'Northern Spy' clearly separated degrees of resistance. These results are consistent with field observations that 'Northern Spy' may sometimes be slightly colonized in the orchard, while 'Robusta 5' is not (3, 4).

That some nymphs survived longer than 7 days on'Robusta 5' indicates that some sustenance was obtained from this clone. Nevertheless, that 50% of the nymphs were dead within 48 hr emphasizes the very high level of resistance of 'Robusta 5'. This early mortality and the failure of WAA nymphs to molt suggest that the very high resistance of 'Robusta 5' may be attributed to antibiosis.

Although 'Manchurian Crab' seemed fully susceptible on the basis of nymphal survival, the developmental time for the first instar was more than 3 times that on fully susceptible 'Empire' (Fig. 2). We interpret this failure to develop normally as an expression of partial resistance; this may have real value in the field by increasing the period nymphs are exposed to environmental hazards. For comparing quantitative difference in resistances of 2 clones, measurement of nymphal development times is more sensitive than measuring nymphal survival. While measurement of aphid development required 2 weeks of testing, partial resistance was clearly demonstrated on the basis of behavioral differences in 5 days (Fig. 2). Difference was made evident by percent of aphids in motion at times of observation and by percent of aphids feeding at the same locations as 12 hr earlier. These observations suggested that resistance to WAA is expressed very rapidly; careful observation would probably permit detection of behavioral differences within 24 hr.

The caging technique may be useful in evaluating progeny as well as parents in a breeding program. For seedlings, grafting is not necessary, and several small cages may be used on each plant. The results suggested that for genetic studies, a replicated cage test would be sensitive enough to measure quantitative inheritance while open infestations in the greenhouse would not.

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*J. Amer. Soc. Hort. Sci.* 107(5):740–744. 1982.

# Effect of Soil Acidity and Nitrogen on Yield and Elemental Concentration of Bush Bean, Carrot, and Lettuce[1]

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*Additional index words. Phaseolus vulgaris, Daucus carota, Lactuca sativa,* P, Ca, Zn, Mn

***Abstract.*** **Bush beans (*Phaseolus vulgaris* L.), carrots (*Daucus carota* L.), and lettuce (*Lactuca sativa* L.) were grown for 3 years on soils amended with S or lime and N fertilizer. Yields of all crops increased with lime application but response to N varied among crops and years. Lettuce head weight tended to increase with N application at pH greater than 6.0, but it decreased with N application at lower pH levels. A soil pH of 5.6 to 6.4 was optimal for carrots and beans, and of 6.1 to 6.6 for lettuce. Plant tissue K and Mg concentrations were not affected by soil pH or N rate. Phosphorus and Ca concentration of plant tissue generally increased with lime application. Plant tissue Zn and Mn concentration usually decreased with increasing soil pH between pH 5.1 and 6.4. The reduction in bean and lettuce leaf Mn concentration between pH 5.1 and 5.7 ranged from 30 to 71%. Low bean yields at pH 5.1 were possibly caused by a combination of Mn toxicity and P deficiency. Failure of lettuce to head at low pH may have been caused by Mn toxicity.**

Soil acidity effects on plant growth are complex, and may be influenced by differences in sensitivity of species and cultivars, soil microorganisms, soil types, and nutrient availability. Hydrogen ion toxicity is seldom a factor in poor plant growth except at pH below 4.5 (3). Calcium (14), P (18), and Mo (2) deficiencies have been implicated in poor growth of plants on acid soils. Manganese (4, 6, 9) and Al (12, 18) toxicity have also been cited as being responsible for poor plant growth on acid soils. Some investigators (10, 11) have concluded that the primary benefit of liming acid soils is the reduced concentration of Mn and Al in the soil solution.

The use of soil pH alone as an indicator for predicting crop response to lime application on acid soils has been only partially successful. In addition to differences in species and cultivar response, the maximum pH at which a crop may respond to lime application can vary by as much as 1 unit depending on the organic matter content, type of clay, and P levels present in a soil (1).

The soils of western Oregon are moderately to strongly acidic and most crops respond favorably to application of lime. The following experiments were conducted to determine the response to lime and N fertilizer of 3 vegetable crops, and to identify soil pH levels and plant tissue elemental concentrations which would be useful in predicting crop response to lime application.

## Materials and Methods

Elemental S at 2.25 Mt/ha and agricultural limestone flour (95% $CaCO_3$ equivalent, less than 0.7% $MgCO_3$) at 0, 9.0, and 18.0 MT/ha were applied to 188 $m^2$ plots of Willamette silt loam (Pachic Ultic Argixeroll, fine-silty, mixed, mesic) 2 years prior to planting the first crop. Resulting soil pH averaged 5.1, 5.7, 6.4, and 6.6, respectively, at planting time. These plots were used in 1977 and 1978.

Agricultural limestone flour at 0, 4.5, 9.0 and 13.5 MT/ha was applied to 213 $m^2$ plots of Willamette silt loam 6 months prior to planting in 1979. Resulting soil pH averaged 5.3, 5.6, 5.9, and 6.1, respectively. In both cases, lime treatments were in randomized block design with 4 replications.

In 1977, the pH level main plots were randomly split into 4 subplots with N rates of 0, 56, 112, and 168 kg N/ha, as $NH_4NO_3$.

[1]Received for publication Dec. 10, 1981. Oregon State University Agricultural Experiment Station Technical Paper No. 6180.

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