Methods of Evaluating Pecan Cultivars for Tolerance to Feeding Damage Caused by the Black Pecan Aphid, *Melanocallis caryaefoliae* (Davis)[1]

T. C. Hale[2] and P. M. Estes

Department of Entomology
Auburn University
Auburn, Alabama 36849-5630 U.S.A.

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ABSTRACT Two aphid rearing methods were evaluated for their suitability of making biological observations on the black pecan aphid, *Melanocallis caryaefoliae* (Davis), and as rapid assessments of pecan cultivars for tolerance to aphid feeding damage. There were no significant differences in the biological and feeding damage data between the two experiments. In comparison, the agar method produced less aphid mortality, was easier to maintain, utilized less space, and would allow fewer samples to provide accurate results. 'Melrose' was the most tolerant of the nine cultivars evaluated for tolerance to feeding damage by the black pecan aphid using water agar plates. 'Jubilee', 'Suprize', and '82-17-680' (Wichita open-pollinated) exhibited intermediate tolerance to feeding damage while 'Salado', '82-17-1316' (Wichita open-pollinated), 'Schley', 'Oconee', and seedlings expressed little tolerance to damage caused by feeding. It has yet to be seen if there is a correlation between tolerance to aphid feeding defined by this experiment and actual resistance to the black pecan aphid in the field.

Key Words Black pecan aphid, *Melanocallis caryafoliae*, host plant resistance, pecans

The aphid complex infesting pecan, *Carya illinoensis* Koch, in the southeastern U.S. includes *Monellia caryella* (Fitch), *Monelliopsis pecanis* Bissell, and *Melanocallis caryaefoliae* (Davis). The black pecan aphid, *M. caryaefoliae* (Davis), is the most destructive of the three because its feeding can cause defoliation (Payne et al. 1979). The first sign of damage is light-yellow areas of foliage near aphid feeding sites; the damage increases in brilliance as time progresses. These areas then turn brown, and defoliation occurs (Tedders 1978). Premature leaf drop reduces tree vigor (Dutcher 1985) and carbohydrate reserve, preventing proper nut filling (Tedders and Wood 1985, Tedders et al. 1982), and adversely affects the following year's bloom (Reilly and Tedders 1990, Dutcher et al. 1984). Infestations typically occur between July and harvest,

[1] Received 29 March 1996; Accepted for publication 29 October 1996.
[2] Current address: 4511 Carter Creek #2, Bryan, TX 77801.

often leading to severe injury in untreated trees (Dutcher 1983, Tedders 1978). Pesticide applications can cause aphid resurgence and alter the seasonal patterns of infestation by the aphid complex (Dutcher 1983). Indiscriminate pesticide use has led to chemical resistance in the aphid complex, causing complications in their control (Reilly and Tedders 1990). As a result, reliance on many insecticides applied on a calendar spray schedule has been replaced with the selective use of a few materials on an as-needed basis along with the incorporation of tolerant cultivars. Nakayama (Carpenter et al. 1979) suggested that it was possible to breed pecan cultivars with a high level of tolerance to the black pecan aphid. Tedders (1978) found that in the spring 'Schley' showed more black aphid damage to leaves than did 'Stuart', and some seedlings showed tolerance to foliar damage.

Experiments were conducted to determine development time for each instar of the black pecan aphid on selected pecan cultivars. The time required for appearance of aphid feeding damage also was observed. Two excised leaf rearing methods were evaluated as screening procedures for cultivar tolerance to black pecan aphid feeding.

Materials and Methods

Stock aphid culture. Adult females were reared on container-grown seedling pecans in an insectary and a greenhouse. Adult aphids were aspirated from stock leaves and placed on floating leaf discs and agar plates to obtain first-instar nymphs for observation. Neonates were removed hourly, during the inoculation period, with an ultrafine camel's hair brush, and placed singly on individual floating discs and in prepared agar plates. After the samples were prepared, the floating leaf discs and agar plates were placed in two Percival® (Boone, IA) growth chambers maintained at 30° C with a 16:8 light:dark photoperiod. Samples were switched between growth chambers on a daily basis. The agar plates were inverted to create a more suitable habitat for the aphids (Reilly and Tedders 1990).

Nutrient solution cup method. The solution cup trial utilized a method described by Lakin (1972). About 8 ml of the nutrient solution were added to 30-ml clear plastic creamer cups. Nutrient solution was replaced in each cup as it evaporated. Fully-expanded mature leaves were excised from 'Cheyenne,' 'Pawnee', and seedlings and stored in a cooler before use. Each replication consisted of 100 leaf discs (16 mm diam) excised from each cultivar using a number 9 cork borer. The leaf discs were floated on top of the solution with the lower epidermis in contact with the solution.

Water agar plate method. The agar plate trial and evaluation of cultivars utilized a detached-leaf method described by Reilly and Tedders (1990). Water agar plates were prepared using tap water and 1% agar (Difco). After autoclaving 50 ml of agar were dispensed into 150-mm Petri dishes for the agar plate trial and 30 ml of agar were dispensed into 60-mm Petri dishes for the cultivar trial. Mature pecan leaves were collected in the field and stored in a cooler before use. Leaflets were washed for 5 min in running tap water, then cut into 2 × 2 cm squares. These squares were washed in a 1:3 chlorox:water solution for 2 min, then rinsed four times with sterile water. The leaf sections

were dried on sterile paper towels and then placed with the adaxial surface in contact with the agar.

Experimental design. A randomized complete block experimental design was used for all experiments. The solution cup trial tested 3 cultivars (n = 100) with 3 replications. The agar plate trial tested 3 cultivars (n = 100) with 5 replications. The cultivar evaluation tested 9 cultivars (n = 25) with 7 replications. All of the cultivars were not included in each replication of the experiments.

Sampling procedure. Each sample was checked daily in the solution cup trial and agar plate trial to record the number of hours required for completion of each developmental stage, the time when feeding damage was first evident on the leaf, and aphid mortality. Each sample was checked daily in the cultivar evaluation to record the number of hours required for feeding damage to become evident and to record aphid mortality. The various stages of aphid development can be differentiated by various morphological characteristics (Tedders 1978), varying sizes, and by the presence of cast skins. Leaf damage ratings were assigned based on presence or absence of chlorosis at each sample date. Aphid mortality was recorded when nymphs were determined to be dead or could not be located. For the purposes of these experiments any aphid that escaped was considered dead. Individual observations ceased when aphids were dead or reached the adult stage. Means were compared using Student-Newman-Keuls mean separation test (SAS® GLM $\alpha = 0.05$) (SAS Institute 1988).

Results and Discussion

Solution cup trial. Neonates feeding on 'Pawnee' leaves took longer to complete the first instar compared to aphids feeding on seedling and 'Cheyenne' leaves (Table 1). There were no differences in the mean number of hours required to complete the second instar among cultivars. Aphids feeding on seedling leaves required fewer hours to complete the third and fourth instars compared to aphids feeding on 'Pawnee' and 'Cheyenne'. The black pecan aphid took longer to develop from neonate to adult on 'Pawnee' leaves when compared to 'Cheyenne' and seedlings. There were no significant differences in aphid mortality among cultivars (Table 1). Seedling leaves exhibited feeding damage nearly 24 h before 'Pawnee' or 'Cheyenne'.

Agar plate trial. Aphids feeding on 'Cheyenne' took fewer hours to complete the first instar compared to 'Pawnee' and seedlings. The second instar was completed more rapidly on seedling leaves compared to 'Cheyenne'. The third instar was completed in the shortest amount of time on 'Cheyenne' leaves followed by seedlings and then 'Pawnee'. There were no differences among cultivars for the completion of the fourth instar. On average, aphids on 'Pawnee' leaves took longer to complete development than on 'Cheyenne' (Table 1). Developmental time on seedlings, 'Pawnee', and 'Cheyenne' were similar. There were no differences in aphid mortality among cultivars (Table 1). Aphid feeding damage took longest to express on 'Pawnee' followed by 'Cheyenne' and then seedlings. (Table 1).

Table 1. Mean developmental times for each instar of the black pecan aphid, first appearance of damage on selected cultivars, and aphid mortality.

Cultivar	Mean Nymphal Instar Developmental Time (h)	Mean number of hours until feeding damage was evident	% Mortality
	Nutrient Solution Cup Trial		
'Pawnee'	42.8 a	108.2 a	81.3 a
'Cheyenne'	38.9 b	102.0 a	89.7 a
seedling	36.4 c	73.7 b	73.0 a
	Water Agar Plate Trial		
'Pawnee'	39.6 a	98.8 a	38.8 a
'Cheyenne'	38.0 b	84.6 b	32.0 a
seedling	38.9 ab	76.9 c	23.8 a

Means followed by the same letter, within a trial and column, are not significantly different ($P > 0.05$, SNK) (SAS Institute 1988).

Comparison of solution cup trial and agar plate trial. When data for both experiments were combined there were no differences in mean developmental hours from neonate to adult or for individual instars between the solution cup trial and agar plate trial (Table 1). Nor were there any differences in mean number of hours before feeding damage was evident. The only differences between the two experiments were in the category of aphid mortality. The solution cup trial had a mean mortality (±SEM) of 82.3% ± 9.7% while the agar plate trial had 31.5% ± 8.1% mortality. This difference may be attributed to two factors. First, the agar plate trial offered a more suitable environment because black pecan aphids are usually located on the underside of the leaf (Tedders 1978). Second, aphids that wandered into the solution drowned, while aphids that wandered onto the agar had a better chance of survival. Because mortality was reduced in the agar plate trial, fewer observations had to be made to attain meaningful results. Based on these findings, the agar plate trial provided a better methodology of studying pecan cultivars and their responses to feeding by the black pecan aphid.

Cultivar trial. Nine pecan cultivars were evaluated for aphid mortality and tolerance to foliar damage caused by black pecan aphid feeding. There were no significant ($P = 0.05$) differences in aphid mortality among cultivars (Table 2). 'Melrose' exhibited the most tolerance to feeding damage with a mean of 100 h before damage could be detected. 'Jubilee', 'Suprize', and '82-17-680' (Wichita open-pollinated) showed a good tolerance ranging from 95.3 to 90.5 h before damage could be detected. The remaining cultivars, 'Salado', '82-17-1316'

Table 2. Mean number of hours until first appearance of damage and aphid mortality.

Cultivar	Damage (hours)	Mean Aphid Mortality (%)
'Melrose'	110.1 a	8.2 a
'Jubilee'	95.3 b	4.7 a
'Suprize'	92.1 b	4.0 a
82-17-680	90.5 b	6.5 a
'Salado'	83.7 c	4.5 a
82-17-1316	82.4 c	4.5 a
'Schley'	80.7 c	3.3 a
'Oconee'	78.3 cd	3.3 a
seedling	73.4 d	2.9 a

Means followed by the same letter, within a column, are not significantly different ($P > 0.05$, SNK) (SAS Institute 1988).

(Wichita open-pollinated), 'Schley', 'Oconee', and seedlings, expressed poor tolerance ranging from 83.4 to 73.4 h before feeding damage was evident. This experiment provides a quick method for screening a large base of cultivars for tolerance to feeding damage caused by the black pecan aphid. Based on unpublished field data we have collected, it is probable that cultivars exhibiting tolerance to feeding damage under the constraints of this experiment will be tolerant to aphid feeding damage in the field.

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