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## Relative Susceptibility of Pecan Germplasm to Blackmargined Aphid

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**Abstract.** The blackmargined aphid, *Monellia caryella* (Fitch), is an important phytophage in the pecan, *Carya illinoinensis* (Wangenh.) K. Koch, agroecosystem where it often is treated with insecticide. Pecan cultivars released by the USDA Pecan Breeding Program vary in susceptibility and risk of damage from the blackmargined aphid. We evaluated a new technique that measures honeydew deposition and found relative differences in susceptibility of a segregating pecan population were identifiable during the course of an outbreak of blackmargined aphids. This provided an efficient method for the simultaneous evaluation of hundreds of segregating pecan trees. Use of this new tool will also aid studies of inheritance, horticultural compatibility, and in determining the relative permanence of this character in pecan improvement.

### Introduction

The pecan, *Carya illinoinensis* (Wangenh.) K. Koch, is one of the most important crops native to the United States (Harris et al. 1986). Its native range extends from Illinois south into Mexico and from Texas east to Alabama (Grauke et al. 2011). The blackmargined aphid, *Monellia caryella* (Fitch), is autochthonous with pecan and typically outbreaks once in late spring to early summer each year in Texas (Liao 1984). Honeydew glistening from the exterior pecan canopy is often the trigger for pesticide application in commercial orchards (Harris 1983) even when the risk of economic damage is small (Harris et al. 1992). Action levels of ~30 aphids per leaf are recommended before applying insecticide. Outbreaks typically peak at 10-20 aphids per leaf during a week or two, and then natural enemies and intrinsic characteristics of the pecan tree combine to reduce the number of aphids to lower levels (Bumroongsook and Harris 1992). Outbreaks can be exacerbated if abundance of natural enemies is reduced by earlier season treatments and/or unusually aphid-susceptible pecan cultivars like 'Cheyenne' are grown. Similarly, outbreaks can be mitigated if less aphid-susceptible cultivars like 'Pawnee' are grown (Thompson and Grauke 2000).

Cheyenne, after evaluation in small plots focused on precocity and production potential for abundant, high-quality nuts, was named and released by the USDA Pecan Breeding Program (Madden 1970). Widespread planting in commercial orchards followed, and by the mid-1980s, reports of susceptibility to the

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aphid began. By the end of the decade this was a flaw in an otherwise excellent cultivar. Pawnee was named and released in 1984 (Thompson and Hunter 1985) and subsequently has been shown to be among the least susceptible to blackmargined aphid of all named cultivars (Thompson and Grauke 2000). Concurrently with these events, Cheyenne and Pawnee have been used together and independently as parents in the breeding program. Their progeny are part of the germplasm being evaluated for future release as new cultivars. The variation in aphid susceptibility between these two parents is significant and suggests deliberate selection for aphid susceptibility may be useful in the breeding program.

These studies were undertaken to: 1) refine evaluation methods to identify the relative amount of aphid susceptibility among cultivars, and 2) produce, if possible, a reliable, efficient protocol for evaluating hundreds of pecan cultivars to characterize their relative susceptibility to the blackmargined aphid.

## Materials and Methods

Investigations at sites in Burleson County and Brown County, Texas, were in cooperation with the USDA-ARS Pecan Breeding Program and focused on the evaluation of cultivars of known parentage developed in that program. Pawnee was chosen because of its resistance to aphids, and progeny that derived from Cheyenne as a parent were included because of the susceptibility of Cheyenne to aphids. All trees were part of randomized block tests planted as part of the National Pecan Advanced Clone Testing System (NPACTS) (Table 1).

Tree canopies were inspected for honeydew beginning in May, and specific sampling was initiated shortly after honeydew was detected. Fifty-four trees (six trees from nine cultivars) at the USDA ARS NPACTS orchard in Burleson County, TX, were evaluated on 15, 22, and 29 July 2008 and 21 July and 3 and 7 August 2009. The cultivars were Pawnee, 75-08-0005, 77-02-0008, 78-01-0023, 79-07-0044, 79-09-0006, 79-09-0038, 81-01-0007, and 82-15-0021 (Table 1). Sixty-six trees (six trees from 11 cultivars) at the USDA ARS NPACTS orchard in Brownwood, TX, were sampled on 12, 13, 19, 20, 27, and 28 August 2009. The cultivars were Pawnee, Wichita, Mandan, 86-2-1803, 86-2-2899, 86-3-0006, 86-3-0356, 86-2-2645, 86-3-0624, 86-2-0063, and 86-3-0008. Trees were monitored and when honeydew was first observed, the test was initiated. Four water-sensitive cards (2.6 X 7.6 cm) were deployed on a stand about 70 cm from the trunk beneath the canopy of each tree. Cards were left for 2 hours, with retrieval carefully timed in relation to deployment. Honeydew falling from the canopy registered on the card as a blue spot against the yellow background of the card. Cards were taken to a laboratory, scanned, and analyzed using Droplet Scan software DropletScan™ (WRK of Oklahoma, Stillwater, OK), which provided data that included calculating the liters of honeydew produced per acre during the 2-hour exposure. A few cards displaced by wind or adulterated with sweat were excluded from the analysis. Analysis of variance (ANOVA) using PASW Version 18 (PASW Statistics 2009) was used to test for differences in amounts of honeydew among cultivars. Means were separated using Tukey's HSD (Honest Significant Difference) test at $P < 0.05$ using PASW Version 18. Finally, a nonparametric chi-square test using PASW Version 18 provided analytical validity even when the data were not normally distributed.

Table 1. Pecan Selections in Tests in Burleson County (CSC) and Brown County (BWC), Texas, with All Known Parentage

| Plant ID | Female parent | Male parent | Orchard |
|---|---|---|---|
| Pawnee | Mohawk | Starking Hardy Giant | CSC, BWC |
| 75-08-0005 | Osage | Creek | CSC |
| 77-02-0008 | Cheyenne | 55-17-3 | CSC |
| 78-01-0023 | Cheyenne | Choctaw | CSC |
| 79-07-0044 | 53-09-0100 | 61-1-6 | CSC |
| 79-09-0006, 0038 | 61-01-0006 | Cheyenne | CSC |
| 81-01-0007 | Cherokee | Wichita | CSC |
| 82-15-0021 | Wichita | 53-9-1 | CSC |
| Wichita | Halbert | Mahan | BWC |
| Mandan | BW1 | Osage | BWC |
| 86-02-0063, 1803, 2645, 2899 | Wichita | Pawnee | BWC |
| 86-03-0006, 0008, 0356 | Cheyenne | Pawnee | BWC |
| Lipan | Cheyenne | Pawnee | BWC |
| Mohawk | Success | Mahan | Parent |
| Starking Hardy Giant | Missouri native | Open-pollinated | Parent |
| Osage | Major | Evers | Parent |
| Cheyenne | Clark | Odom | Parent |
| Creek | Mohawk | Western | Parent |
| 55-17-0003 | Oklahoma | 44-12-86 | Parent |
| 44-12-0086 | Moore | Nugget | Parent |
| Choctaw | Success | Mahan | Parent |
| 53-09-0001, 0100 | Mahan | Odom | Parent |
| 61-01-0006 | Barton | Starking Hardy Giant | Parent |
| Cherokee | Schley | Evers | Parent |
| Halbert | Texas native | Open-pollinated | Parent |
| Mahan | Schley seedling | Open-pollinated | Parent |
| BW1 | Barton seedling | Open-pollinated | Parent |
| Success | Mississippi seedling | Open-pollinated | Parent |
| Major | Kentucky native | Open-pollinated | Parent |
| Evers | Texas seedling | Open-pollinated | Parent |
| Clark | Texas native | Open-pollinated | Parent |
| Odom | Mississippi seedling | Open-pollinated | Parent |
| Western | Texas native | Open-pollinated | Parent |
| Oklahoma | Oklahoma native | Open-pollinated | Parent |
| Moore | Florida seedling | Open-pollinated | Parent |
| Nugget | Texas native | Open-pollinated | Parent |

## Results and Discussion

The number of aphids at the site in Burleson County increased the second week of July in 2008 and the third week of July in 2009. Water-sensitive cards were deployed shortly thereafter to collect and measure honeydew production. Overall honeydew production was less in 2008 than in 2009; among sampling dates within a year, honeydew production was greatest on 22 July 2008 and 7 August 2009

(Table 2). ANOVA analyses resulted in significant separation of some of the means on every date except 29 July 2008 after the peak of honeydew production was observed on 21 July. Pawnee ranked least, the three cultivars lacking either as a parent ranked next, and cultivars with Cheyenne parentage ranked greatest in total honeydew production. Statistically significant cultivar groupings were relatively consistent across sampling dates, indicating assignment of a relative ranking based on honeydew could be used to designate susceptibility to blackmargined aphid.

Table 2. Gallons per Acre of Blackmargined Aphid Honeydew Estimated from Water-sensitive Cards Exposed for 2-Hours under Pecan Cultivars on Six Dates

| Pecan | 2008 | | | 2009 | | | |
|---|---|---|---|---|---|---|---|
| cultivar | 15 July | 22 July | 29 July | 21 July | 3 Aug. | 7 Aug. | Mean |
| Pawnee | 0.0171a | 0.0380a | 0.0313a | 0.0606a | 0.0776a | 0.1238a | 0.0565a |
| 75-8-5 | 0.0238ab | 0.0652ab | 0.0450a | 0.0717a | 0.1179abc | 0.1695abc | 0.0820ab |
| 79-9-6 | 0.0238ab | 0.0624ab | 0.0488a | 0.0885a | 0.1830cd | 0.2457c | 0.1090bc |
| 81-1-7 | 0.0241 | 0.0489a | 0.0316a | 0.0899a | 0.1426 | 0.1528 | 0.0825 |
| 82-15-21 | 0.0249ab | 0.0347a | 0.0420a | 0.0721a | 0.1026ab | 0.1460ab | 0.0710a |
| 79-7-44 | 0.0254ab | 0.0496a | 0.0462a | 0.0405a | 0.0795a | 0.1395a | 0.0635a |
| 77-2-8 | 0.0361ab | 0.0768ab | 0.0596a | 0.0187a | 0.1548abc | 0.2320c | 0.1146c |
| 78-1-23 | 0.0400bc | 0.1094c | 0.0495a | 0.0974a | 0.2097d | 0.2204bc | 0.1212c |
| 79-9-38 | 0.0447c | 0.0980bc | 0.0480a | 0.1028a | 0.1798bcd | 0.1959abc | 0.1115bc |
| *P* value | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| *F* value | 4.069 | 6.978 | 1.715 | 1.912 | 7.263 | 6.269 | 11.562 |
| df | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

*Means followed by the same letter are not significantly different using ANOVA and means separated using Tukey's HSD test at $P < 0.05$ (PASW Statistics 2009).

The relative differences among all cultivars tested at the site in Burleson County showed progeny with a Cheyenne parent had significantly more honeydew than did Pawnee and two of the three other cultivars that lacked a Cheyenne parent (Fig. 1). This indicated that Cheyenne when used as a parent may confer to progeny greater susceptibility to aphids.

The number of aphids at Brownwood increased the first week in August in 2009, and water-sensitive cards were deployed shortly thereafter to collect and measure honeydew production. Overall, honeydew production was greater than that observed in Burleson County in either year, and was greatest at Brownwood on 20 August (Table 3). ANOVA analyses resulted in significant separation of some of the means on every sampling date. The four progenies of a Cheyenne-Pawnee cross ranked first, third, sixth, and eleventh in total honeydew production, which encompasses the entire range of aphid susceptibility observed in the test; similarly, the four progenies of a Wichita-Pawnee cross resulted in two ranking less than the mean and two greater than the mean in total honeydew production. Pawnee ranked less than the mean, and Mandan and Wichita ranked greater than the mean in honeydew production. Statistically significant cultivar groupings were relatively consistent across sampling dates (although more variable than observed in the data from Burleson County), again indicating the assignment of a relative ranking based on honeydew could be used to designate susceptibility to blackmargined aphid.

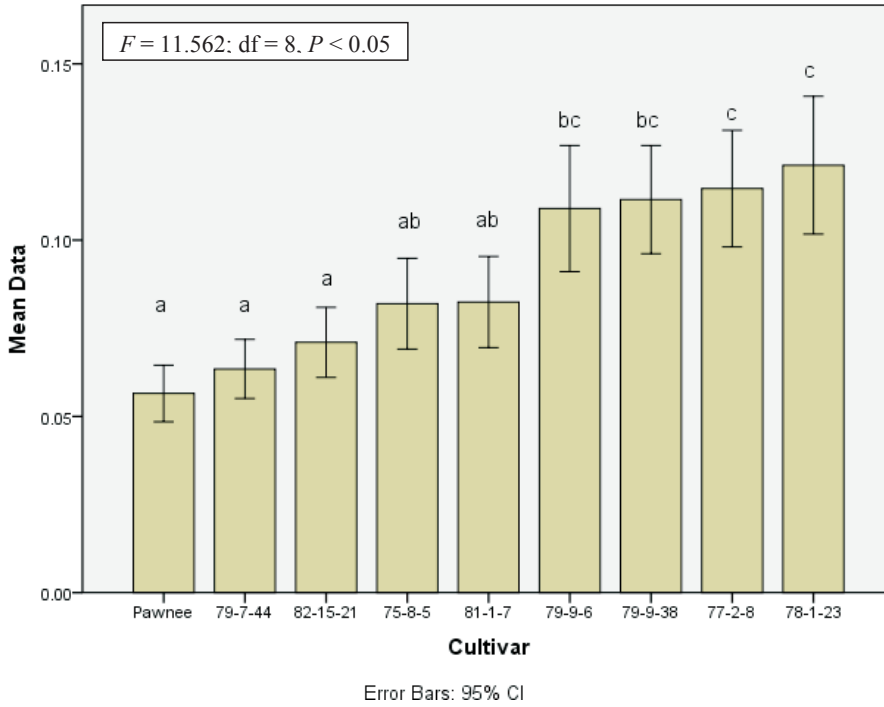


Fig. 1. Honeydew production on nine pecan cultivars near Snook, Burleson County, Texas, in 2008-2009.

Table 3. Mean Number of Gallons per Acre of Honeydew by Blackmargined Aphid for 2-Hour Exposure of Pecan Cultivars on Six Dates in 2009

| Pecan | 12 Aug. | 13 Aug. | 19 Aug. | 20 Aug. | 27 Aug. | 28 Aug. | Total |
|---|---|---|---|---|---|---|---|
| 86-2-2899 | 0.0698a | 0.0723a | 0.3015ab | 0.3548bc | 0.3306cd | 0.239bcd | 0.2337bc |
| 86-2-1803 | 0.0733a | 0.0725ab | 0.2389a | 0.2592a | 0.2508ab | 0.1524a | 0.1736a |
| 86-3-356 | 0.1047ab | 0.1184ab | 0.2420a | 0.2083a | 0.2023a | 0.162ab | 0.1727a |
| 86-2-2645 | 0.1143ab | 0.1221cd | 0.3918bc | 0.3880c | 0.2691ab | 0.1958abc | 0.2423c |
| Wichita | 0.1246bc | 0.1312cd | 0.3844bc | 0.3833c | 0.3947d | 0.2723d | 0.2792c |
| 86-3-6 | 0.1366bc | 0.1043ab | 0.2131a | 0.2164a | 0.2155ab | 0.1659ab | 0.1767a |
| Pawnee | 0.1372bc | 0.1218bc | 0.2540a | 0.2859ab | 0.2388ab | 0.1332a | 0.1928ab |
| 86-3-624 | 0.1667c | 0.1728ef | 0.3670bc | 0.3567bc | 0.1910a | 0.1636ab | 0.2366bc |
| Mandan | 0.1693c | 0.1644de | 0.3837bc | 0.4346c | 0.2393ab | 0.1744abc | 0.2574c |
| 86-2-63 | 0.1703c | 0.1897fg | 0.3588b | 0.3717c | 0.2422ab | 0.1701abc | 0.2803c |
| 86-3-8 | 0.2358d | 0.2207f | 0.4604c | 0.5212d | 0.2890bc | 0.2503cd | 0.3351d |
| *P* value | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| *F* value | 19.283 | 18.773 | 14.786 | 26.780 | 12.191 | 6.263 | 23.320 |
| df | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

*Means followed by the same letter are not significantly different using ANOVA and means separated using Tukey's HSD test at $P < 0.05$ (PASW Statistics 2009).

The relative differences observed among all cultivars tested at Brownwood showed a consistency in individual susceptibility to blackmargined aphid, but much variability in the role a particular parent may play in conferring that susceptibility. The Cheyenne-Pawnee progeny in particular illustrated this variability, which was also manifested to a lesser degree in the Wichita-Pawnee progeny (Fig. 2). This indicated that resistance of each individual progeny may be more important than the lineage used to produce it.

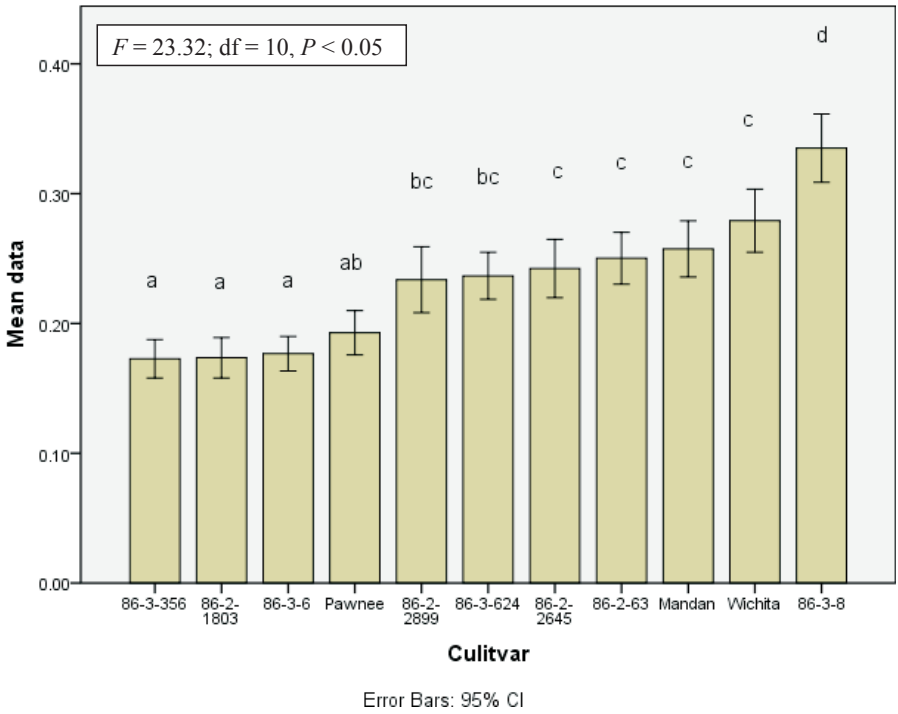


Fig. 2. Honeydew production on 11 pecan cultivars at Brownwood, Brown County, Texas, in 2009.

To breed a resistant plant, the trait must be identifiable, heritable, compatible, and durable (Harris 1980). We developed a method to identify trees resistant to blackmargined aphid. The water-sensitive card method provided an estimate of the number of aphids in each tree and facilitated the relative ranking of resistance of the cultivars. This method was faster and apparently as efficient as counting the numbers of aphids on the surfaces of multiple leaflets on multiple trees throughout the season (Thompson and Grauke 1998). Pawnee, previously reported to be the most resistant to yellow aphid (Thompson and Grauke 1998, Thompson et al. 2000), produced consistently less honeydew. Multiple trees of the same genotype

in each of the tests allowed increased confidence in the rankings based on the water-sensitive cards. As an evaluation tool, questions remain to be answered; could the evaluation be done on individual seedlings in a nursery, saving the time, effort, and expense of propagating a pecan susceptible to aphids? A trait also needs to be heritable. The variation in the tests is consistent with heritability of resistance. The cultivars that had Cheyenne as a parent in the orchard in Burleson County had the greatest amounts of honeydew. At Brownwood, cultivars with both Cheyenne and Pawnee as parents segregated across the entire spectrum of resistance and susceptibility in patterns consistent with genetic mechanisms of inheritance and could be explained by either qualitative or quantitative traits. If a biparental mapping population was evaluated, the mechanism of inheritance could be better interpreted, contributing to the identification of parents useful in breeding resistance or in development of molecular markers related to the trait. An aphid-resistant cultivar must have a combination of traits compatible with adoption by the pecan industry. Pawnee is known to have resistance to aphids and also has high-quality and early maturing nuts. A corollary question is how important is resistance to aphids in the selection of valuable pecan cultivars? Evaluation in a nursery might eliminate seedlings based on susceptibility to aphids, preventing release of such cultivars as Cheyenne. Finally, the trait must be durable. Pawnee has maintained a useful level of resistance to aphids since released in 1984, implying durability. Ultimately, the relative value of a trait, the timing of its evaluation in the evaluation process, and its weight in the decision concerning release is in the hands of the breeder. This research provides a valuable tool to the breeder -- a fast and efficient method for evaluating resistance to blackmargined aphid.

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