FIELD STUDIES OF THREE SPECIES OF APHIDS ON PECAN: AN IMPROVED CAGE FOR COLLECTING HONEYDEW AND GLUCOSE-EQUIVALENTS CONTAINED IN HONEYDEW[1]

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ABSTRACT

Cages were designed for isolating single aphids and for collecting honeydew excreted by aphids. The cages were easy to construct and use and protected the aphids and honeydew from rainfall and predators. The cages did not cause condensation of leaf transpiration within. Glucose-equivalents in honeydew excreted by each instar of *Monellia caryella* (Fitch), *Monelliopsis pecanis* Bissell, and *Melanocallis caryaefoliae* (Davis) were measured. Honeydew of *M. caryella* contained 9 and 13 times more glucose-equivalents than *M. pecanis* and *M. caryaefoliae,* respectively. All instars of each aphid species produced similar levels of sugar; however, successive instars tended to produce slightly more honeydew. *Monellia caryella* was found to be the most detrimental to the energy reserve of pecan trees, assuming equal numbers of each species.

Key Words: *Monellia caryella* (Fitch), *Monelliopsis pecanis* Bissell, *Melanocallis caryaefoliae* (Davis), honeydew, glucose-equivalents, aphid cage.

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INTRODUCTION

Feeding by *Monellia caryella* (Fitch), *Monelliopsis pecanis* Bissell, and *Melanocallis caryaefoliae* (Davis) (Homoptera: Aphididae) on the foliage of pecan trees damages the leaf vascular system (Tedders and Thompson 1981) and reduces leaf area and leaf chlorophyl content (Tedders et al. 1982). These aphids also compete with the tree for photosynthates (Wood et al. 1985) while sooty mold growth on aphid excrement (honeydew) shades the leaves from sunlight needed for photosynthesis (Tedders and Smith 1976). Cumulative evidence indicates that large numbers of feeding aphids seriously interfere with the quality and quantity of pecan nut production (Wood and Tedders 1982; Dutcher et al. 1984; Dutcher 1985; Tedders and Wood 1985).

Honeydew excreted by aphids is rich in carbohydrates (Tedders et al. 1982) and likely represents a major source causing losses in nut production. The quantity of honeydew excreted by each species has not been measured except for *M. caryella* which produced an average of 0.092 g for the first 25 days after birth (Tedders 1978). This study was designed to better understand the effects of aphid competition with the tree for photosynthates and to determine the amount of sugar contained in honeydew excreted by aphids from their birth until adulthood.

For this study we needed small, light-weight cages equipped with a device that collected honeydew excreted by individual aphids located on the lower surfaces of

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single pecan leaflets. Dixon (1970) first used lightweight cages constructed from spring-loaded hair clips and aluminum foil cups to collect honeydew from aphids on plant roots. Llewellyn (1972) modified this cage to collect honeydew produced by the lime aphid, *Eucallipterus tiliae* L. on lime, *Tilia vulgaris* Hayne, foliage.

Isolation of aphids on pecan trees in the field is difficult. Cages attached to single leaflets are often unsatisfactory because weight from the cage and winds cause the leaflet to detach from the leaf rachis. Rainfalls contaminate or wash away honeydew collections. Cages that are tight enough to prevent aphid escapes sometime condense leaf transpiration on the inside of the cage contaminating the honeydew or interfering with the aphid. Pressure exerted by cages that are tight enough to prevent aphid escape sometimes damage the veins of leaflets. Ants and other insects often penetrate aphid cages to collect honeydew or to attack the aphid. We report here a redesigned spring-loaded cage that was suitable for collecting honeydew from individual aphids on pecan trees.

MATERIALS AND METHODS

Materials used for cages were spring loaded hair clips (47 mm long) (Tip-Top Division, Faberge Inc., Omaha, NE 68102), 25.4 mm wide by 1.5 mm thick, double-faced foam mounting tape (Household and Hardware Products 3M Company, St. Paul, MN 55101), 16 ga. copper wire, 79 mesh/cm nylon screen, and Parafilm® (American Can Co., Greenwich, CT 06830). Foam tape was cut into 25.4 mm^2 pieces (Fig. 1A1) and the exposed sticky surface covered with parafilm. The paper backing on the foam square was left in place until the cages were used. A hole (14 mm diam.) was cut with a cork hole borer through the center of each foam square. Insert rings (14.5 mm OD) were constructed by wrapping 16 ga. copper wire around an 11 mm diam. dowel. The wire coil was then cut along one side and the resulting rings were flattened. Retainer rings (17 mm OD) were similarly constructed by wrapping 16 ga. copper wire around a 15 mm dowel. Both insert and retainer rings were covered on one side with 79 mesh/cm nylon screen affixed with cyanoacrylate glue (Fig. 1A2, 1A3). Retainer rings were fitted with a centered cross bar of 16 ga. copper wire, also glued in place. Retainer rings were glued on the nylon covered side to the inside of the hair clip (Fig. 1A4).

Fig. 1B illustrates the arrangement of the cage components on a pecan leaflet blade. For attachment of a cage to a leaflet, the paper backing was removed from one side of the foam tape square and the adhesive side of this component appressed to the lower surface of a leaflet. An insert ring was pressed into the hole in the foam square (nylon covered side away from the leaflet) thus forming the aphid containing chamber. The hair clip with retainer ring was then clipped over the smaller ring and leaf blade with the large ring resting on the parafilm. The parafilm prevented contact between the retainer ring and adhesive surface of the foam but allowed a good seal and traction with the foam square. The cross bar in the retainer ring prevented the insert ring from becoming dislodged. This arrangement provided a cage with a replacable insert for collecting honeydew. Pressure from the hair clip served to keep the foam square closely appressed to the leaflet blade to prevent aphid escapes and detachment of the cage from the leaflet.

To prevent rain from wetting the cages, the narrow side of polyethylene bags, 20.5 × 38.5 cm (flattened dimensions), were cut open to form oblong sleeves from the same dimensions. A sleeve was placed over the compound leaf and attached

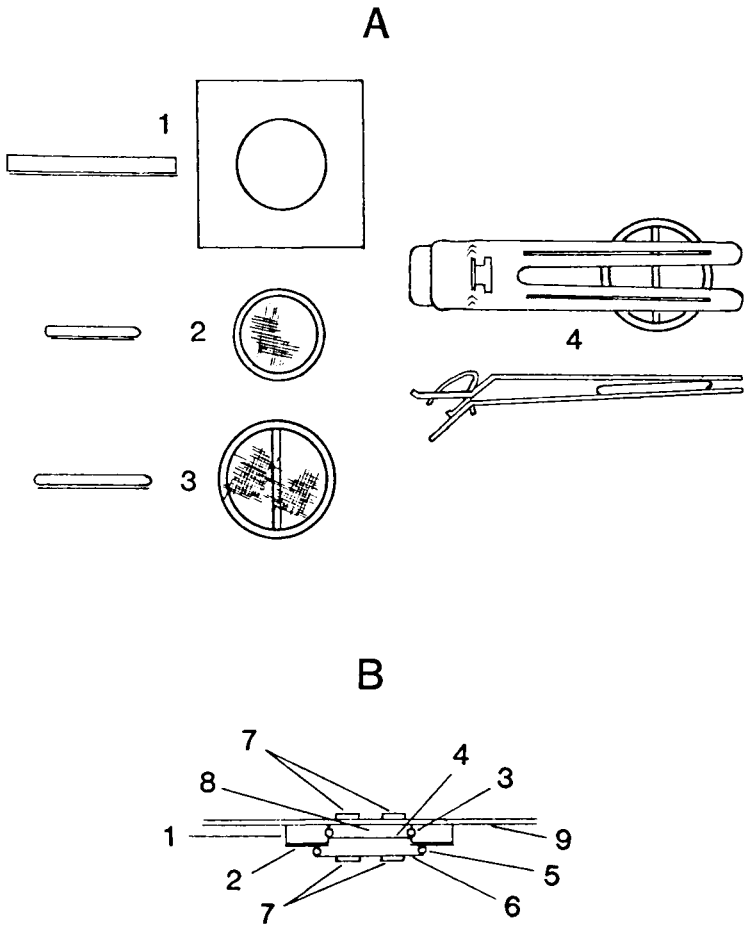

Fig. 1. Ant excluding aphid cage for collecting honeydew. (A) component parts: 1 – left, foam mounting tape (1.5 mm thick) with lower covering of parafilm; 1 – right, same (25.4 mm^2 with 14 mm diam. hole; 2 – left, honeydew collecting insert ring (16 ga. cu. wire) with covering of 79 mesh/cm nylon screen on lower surface; 2 – right, same (14.5 mm diam. OD); 3 – left, ant excluding retainer ring (16 ga. cu. wire) with 79 mesh/cm nylon screen cover on lower surface; 3 – right, same (17 mm diam. OD) showing retainer cross bar; 4 – above, spring loaded hair clip with attached ant excluding retainer ring; 4 – below, same showing attachment of retainer ring to lower tines of clip. (B) Cross-section of assembled aphid cage on pecan leaflet; 1, foam mounting tape; 2, parafilm cover; 3, insert ring; 4, nylon screen; 5, retainer ring; 6, nylon screen; 7, tines of hair clip; 8, aphid chamber; 9, leaflet blade.

cage and closed around the proximal end of the leaf rachis with cotton cord. On sunny days, the sleeve was gathered up around the basal end of the leaf rachis and fastened. With the approach of rain, the sleeve was pulled down over the entire leaf. Natural droop of the leaves combined with weight from the sleeve and cage caused the open end of the sleeve to face downward.

An aphid imago placed in each cage was examined every few hours until a birth occurred. Then the imago was removed, leaving a single first instar nymph. A clean ring was then inserted in the cage. Ring inserts with honeydew were removed each 24 hours thereafter, sealed in glass vials, labeled and frozen at 0°C until all collections were made. Twenty such cages for each aphid species were placed on the foliage of fruiting 'Stuart' pecan trees during September. Insert rings were pre-washed in hot 70% ethanol before use. Ethanol extracts from unused insert rings were examined before the test began to determine if the copper ring, nylon screen, and cyanocrylate glue would contaminate the honeydew.

Sugar analysis was done colormetrically using Nelson's modification of Somogyi's method (Nelson 1944). Ring inserts with honeydew were soaked in hot 70% ethanol to dissolve sugars. The ethanol extracts were placed in test tubes and dried at 70°C in a water bath. One ml of 50 mM sodium acetate buffer (pH 4.7) was added with 100 units of invertase (Sigma, St. Louis, MO) to convert nonreducing sugars to the reducing form. Tubes were then incubated at 37°C for 1 hour; at which time 1 ml of copper reagent (Hodge and Hofreiter 1952) was added and heated to 80°C for 15 min and cooled for 30 min. Samples were mixed with 2 ml of arsenomolybdate reagent and diluted with water. Sugar levels were then determined colormetrically by measuring absorbance at 500 nM. Optical density was compared with a standard curve of glucose for quantitation and results expressed as glucose-equivalents. The sugar levels in honeydew excretions were analyzed for species and instar differences based on a completely randomized design with 20 replications. Treatment means were separated by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Llewellyn's cage was unsatisfactory for our use because water from leaf transpiration collected inside the cage. However, the weight of the spring-loaded hair clip was satisfactory and pressure from the spring did not damage the leaflet veins.

The redesigned cages were easily attached to leaflets and ring inserts with honeydew were easily replaced without injuring the confined aphid or permitting excessive escapes. The 79 mesh nylon screen on the insert rings captured all droplets of honeydew but did not cause condensation from leaf transpiration. Honeydew usually accumulated on one spot on the nylon screen, indicating limited movement by aphids. The polyethylene sleeves excluded even heavy rains and did not cause condensation problems within the cages. The foam squares sealed nicely around even the largest leaflet veins without causing vein damage and prevented even first instar aphid escapes. Cages weighed ca. 3.5 g and did not cause leaflet detachment even on days with wind gusts of 24 kmph. Double screening provided by the retainer rings prevented ants from collecting the honeydew and aphids were not lost to parasites or predators. Only one to three aphids of each species died or was damaged during the testing period. These were replaced. The cage system

developed in this study makes possible the long-term study of individual aphids and a system useful for the determination of aphid energetics. Alcohol extracts from unused insert rings did not produce contaminants.

Honeydew excretion differed with aphid species in that *M. caryella* excreted 9 and 13 times more glucose-equivalents than did *M. pecanis* and *M. caryaefoliae,* respectively (Fig. 2). While excretion by *M. pecanis* tended to be greater than *M. caryaefoliae,* it was not statistically significant except at the $P = 0.15$ level. There was also a tendency for increased excretion of glucose-equivalents of honeydew with progressive aphid instar, but was not statistically significant except at the $P = 0.15$ level. Thus aphids of any stadium, within each of the three species, might be expected to excrete a similar level of sugar. This does not mean that individuals of different instars extract equal amounts of energy from the tree because efficiencies of extraction of sugars and organic nitrogen from phloem sap, aphid respiration and growth rates, and amount of sap removed must also be considered.

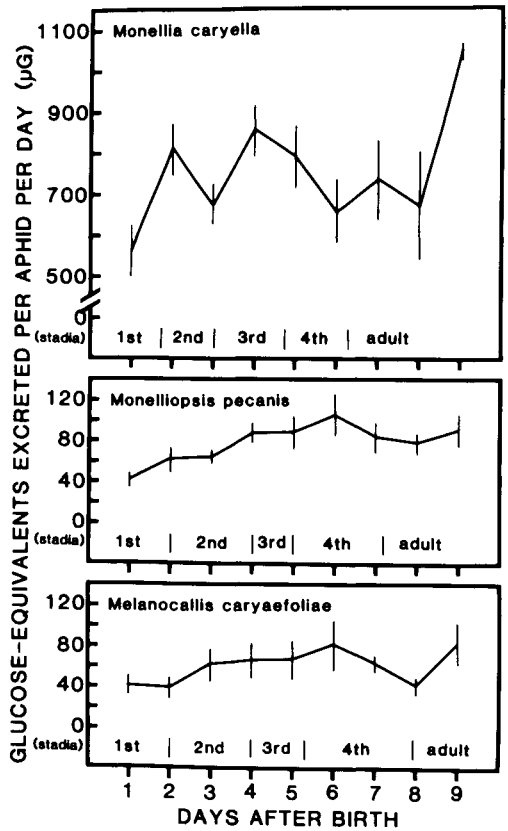

Fig. 2. Glucose-equivalents excreted per day by each instar of *M. caryella, M. pecanis,* and *M. caryaefoliae* from time of birth until adulthood. Instar means were not statistically different at $P = 0.05$). *Monellia caryella* was significantly different from *M. pecanis* and *M. caryaefoliae* ($P = 0.05$).

These data indicate that since *M. caryella* excrete about 9 times more sugar than *M. pecanis,* they might be expected to be more detrimental to the tree's energy reserve levels, assuming equal numbers. However, *M. pecanis,* numbers during a growing season usually exceed number of *M. caryella* by about 4 or 5 times (Tedders 1978). Based upon this information alone, seasonal infestations are roughly twice as damaging. *Monellia caryella* excrete about 13 times more sugar than *M. caryaefoliae.* However, the chlorosis caused by *M. caryaefoliae* is the most damaging of the three. Fortunately, *M. caryaefoliae* rarely occur in numbers larger than 0.3 × that of *M. caryella.*

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