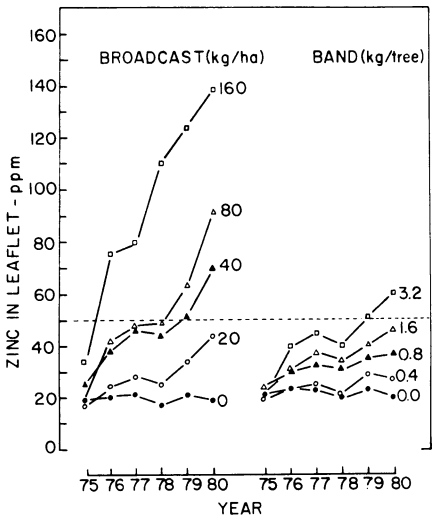


Fig. 1. Zinc concentrations in pecan leaflets from soil applications applied as broadcast and band. The horizontal, broken line represents the lower limit (50 ppm) of the Zn sufficiency range (3).

each tree and the treated area in a square to restrict cross-feeding between trees. The subsoil strip was 1.8 m wide and 0.75 m deep.

Leaflet samples for Zn analysis were taken annually. The sampling procedure (3) and the time of sampling (7) were as recommended for pecan. The air-dried leaflets were oven-dried for 72 hr at 70°C, ground, redried, and analyzed for Zn by a Jarrell-Ash plasma emission spectrometer (5).

Zinc uptake during the first growing season of the study (1975) was not appreciably different from the control, except for the broadcast rate of 160 kg (Fig. 1). This rate is considerably greater than that normally applied by growers. Thus, the lack of substantial uptake from other rates confirms research (1, 4, 10) and grower experience that substantial Zn uptake often does not occur in the first season of application. This lag in uptake reemphasizes (8) the importance of applying foliar Zn to Zn-deficient trees during the lag period following soil application.

Zinc concentration in the leaflet increased with the rate of Zn applied to the soil during the second season and thereafter in the case of broadcast application, and during the third season and thereafter in the case of the band application (Fig. 1). At equivalent rates of Zn per tree, Zn concentration in leaflets from broadcast application was superior to band application after the first year of application.

Effectiveness of the treatments can be further evaluated by comparing their associated Zn concentrations in leaflets with the recommended sufficiency concentration of 50 to 100 ppm (3). The 160-kg broadcast rate in the second year following application, and the 40- and 80-kg broadcast rates, in the fifth year of the study produced a Zn concentration above the lower limit of the sufficiency range, (Fig. 1). The 3.2-kg rate was the only band rate that produced a Zn concentration above the lower limit of the Zn sufficiency value, and this occurred in the fifth year. Thus, high Zn rates or a long period of time with lower rates may be required to produce Zn sufficiency in the pecan leaflets when Zn deficiency is present. The lag and relatively slow uptake following soil application point out the importance of applying Zn to the soil before the Zn concentrations in leaflets drop below the sufficiency range.

Increasing Zn uptake with time from a single application (Fig. 1) has been reported (4). The seasonal effect likewise has been observed (4, 6, 10). The seasonal effect was especially apparent in 1977 and 1978 (Fig. 1). Rainfall in the spring of these 2 seasons was less than in the other 4 seasons. Hunter's data (4) also suggest that soil moisture in early spring may influence Zn uptake. Both the seasonal effect and the increasing uptake with time are factors that should be carefully considered when making diagnostic recommendations; otherwise, Zn applications may be made unnecessarily.

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# Effects of an Infestation of Blackmargined Aphid on Carbohydrates in Mature 'Stuart' Pecan[1]

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*Additional index words.* sugars, starch, irregular bearing, fruit development, insect damage, *Carya illinoensis, Monellia caryella*

***Abstract.*** **Twenty-one days of foliar feeding in late spring by the blackmargined aphid [*Monellia caryella* (Fitch)] on a mature 'Stuart' pecan tree [*Carya illinoensis* (Wang.) K. Koch] reduced soluble sugars and starch in leaves to 82% and 79%, respectively, of the aphid-free control. Chlorophyll levels were unaffected. Sugars were reduced to 75% of the control in both 1- and 2-year-old branches. Starch in 1-year-old branches was reduced to 71%, but was unchanged in 2-year-old branches.**

Irregular bearing of pecan has been shown to be largely associated with reserve carbohydrate levels (8, 10, 17, 18). Foliar diseases (15, 19), defoliation (9, 10), and overcropping (2, 9) may contribute to alternate bearing by reducing carbohydrate reserves. The effect of insects on alternate bearing has been little-studied, and studies of their effects on pecan trees have dealt with those that either damage the fruit or consume or damage the foliage or roots (6, 11). Little is known of the effects of parasitic foliar-feeding insects, such as aphids, on carbohydrate reserves and fruit development. The blackmargined pecan aphid, [*Monelliopsis nigropunctata* (Granovsky)] and the black pecan aphid [*Melano-*

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*callis caryaefoliae* (Davis)] injure the vascular systems of leaves (12) and excrete honeydew that supports sooty mold growth, which reduce photosynthesis (12, 13). These 3 aphids have been shown to greatly reduce growth (14), carbohydrates, and chlorophyll (15) levels in greenhouse-grown pecan seedlings. *M. caryaefoliae* has been shown to cause economic loss (6), but *M. caryella* and *M. nigropunctata* have not. The purpose of this study was to investigate the effect of a late-spring infestation of *M. caryella* on leaf chlorophyll and carbohydrate levels in pecan leaves, fruit, and shoots.

Aphids were allowed to infest a mature 'Stuart' tree growing under near-optimum fertilizer and pest management. Immediately prior to application of aphids, the tree was treated with Pyrenone [piperonyl butoxide (60%) plus pyrethrins (6%)] at 0.5 ml/liter water to kill only insects on the tree. Pyrenone was used because of its very short residual life. Branches possessing 3 fruits per cluster were selected in the 4 cardinal directions and covered by a white nylon organdy type cage (40 × 60 cm) to retain the aphids. These cages reduced light energy reaching the foliage by 17%. On May 27, 1981, 6 blackmargined aphids (fourth instar nymphs and adults) were inserted into each treatment cage, where they fed on the foliage and reproduced. The experimental design consisted of the aphid treatment and an aphid-free control (cage only), both replicated 10 times. Individual replicates were positioned on the same major branch. During the study period, rainfall was 12 cm and average temperature was 26°C. On June 16, leaves, fruit, 1-year-old shoots, and 2-year-old shoots were cut from the tree, removed from the cage, washed with cold water to remove any aphid honeydew, and frozen at −20°. After lyophilization, samples were ground in a Wiley Mill (40-mesh) and stored at room temperature until analysis.

Leaf chlorophyll content was determined by homogenizing 100 mg of tissue for 3 min in cold 80% (v/v) acetone. After filtration and dilution with 80% acetone, the absorbance of the extract was measured at 645 and 663 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. Concentrations of chlorophylls *a* and *b* were estimated using the formula of Arnon (1).

Sugar and starch concentrations were determined in each plant component by assaying colorimetrically through the use of anthrone. Samples were extracted 12 hr with 80% (v/v) ethanol at 60°C. The ethanol extract was analyzed for sugar by reacting an aliquot with anthrone and sulfuric acid and assaying spectrophotometrically (625 nm) for glucose equivalents based on the method of McCready et al. (5) as modified for woody tissue (3). After ethanol extraction, the tissue residue was digested with amyloglucosidase (7) to break down the starch to glucose. An aliquot was then evaluated for glucose equivalents using the anthrone procedure as described above.

After 21 days of foliar feeding by blackmargined aphids, carbohydrate losses were evident in leaves and branches, but not fruit (Table 1). Aphid feeding reduced soluble sugars and starch in leaves to 82% and 79%, respectively, of levels in leaves without aphids. Feeding also reduced carbohydrate levels in both 1- and 2-year-old branches (Table 1). Soluble sugars in branches of both ages was about 75% of noninfested branches. Starch levels in 1-year-old branches was reduced to 71% of the control, but was unchanged in 2-year-old branches.

Aphid feeding had no measurable effect on carbohydrates in developing fruit (Table 1) or on fruit size or dry weight. This is probably because pecan fruit normally grows very slowly during May and June. Rapid fruit or kernel growth did not occur until several seeks after the termination of the study; consequently, there would have been relatively little carbohydrate movement to the fruit. Feeding for a prolonged time or during the rapid kernel growth of fruit development (16) could be expected to reduce pecan fruit size and kernel development.

Carbohydrate losses are attributed primarily to their removal by feeding aphids, because chlorophyll content did not differ between treatments. In a previous study with pecan seedlings, both the level of carbohydrates and chlorophyll in developing leaves was greatly reduced by blackmargined aphids (15). The foliage in this study was fully expanded and relatively mature. This suggests that chlorophyll levels are more easily affected when aphids feed on young leaves rather than on mature ones.

This study demonstrates that a brief infestation of blackmargined aphids can significantly decrease carbohydrates in pecan leaves and shoots and raises the possibility that foliar-feeding aphids can have an important effect on carbohydrate reserves. This in turn could influence fruit development as well as irregular bearing. This is especially evident when one considers that aphids can infest a tree at very high levels for a large part of the growing season, and that blackmargined aphids are only 1 of 3 species commonly found on pecan (11). This study only reflects their influence over a few days in a relatively nonstressing portion of the growing season. It is not known what levels of aphids can be tolerated without significant damage to pecan production or kernel quality. This study suggests that aphids may be a much more serious insect pest than previously thought.

Table 1. The effects of 2 weeks of feeding by blackmargined aphid *(Monellia caryella)* on sugars and starch in leaves and shoots of 'Stuart' pecan.

| Aphid treatment | Carbohydrate concn. (mg/g dry wt) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaves | | 1-yr-old branches | | 2-yr-old branches | | Fruit | |
| | Sugar | Starch | Sugar | Starch | Sugar | Starch | Sugar | Starch |
| Control | 17 b[z] | 19 b | 25 b | 45 b[y] | 31 b | 48 a | 27 a | 188 a |
| Blackmargined aphid | 14 a | 15 a | 19 a | 32 a | 23 a | 45 a | 23 a | 192 a |

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Mean separation of starch in 1-year-old branches was at P = 7%.

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# Objective Measurement of Chilling Injury in the Mesocarp of Stored Avocados[1]

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*Additional index words. Persea americana*

***Abstract.*** **A method to measure the severity of chilling injury (CI) in stored avocados (*Persea americana* Mill.) is described that gives better results than visual appraisal. Colored metabolites are extracted from chilled mesocarp and measured in a colorimeter.**

The postharvest life of avocado fruit can be extended by refrigeration. However, in common with many tropical and subtropical species, low temperature (~ 0 to 10°C) storage causes physiological dysfunction (8) known as chilling injury (CI). The presence of CI results in decreased market value and, ultimately, in complete wastage. The chief symptom of CI observed in avocado fruit is a gray or dark-brown discoloration of the mesocarp (edible pulp) (1,5). This discoloration is due to an accumulation of oxidized phenolic compounds (7).

Certain physiological responses to CI in plants, such as abnormal respiration rates (2) and electrolyte leakage from cells (9), can be measured by laboratory techniques. However, these methods are generally impractical for routine CI assessment in stored fruit. In the absence of suitable quantitative methods, subjective assessments of CI severity based on visual rating or arbitrarily defined classifications such as "acceptable" and "not acceptable" have been used (10, 11). Such methods only provide a 2-dimensional assessment of a disorder having a 3-dimensional distribution.

In a study of enzymatic browning in freshly cut avocados, Kahn (6) reported that there was differential susceptibility to browning among avocado cultivars, based on visual appraisal of browning rates. These studies have been extended by Golan and Sadovski (4) who determined browning potential by measuring the rate of color change in prescribed target areas of exposed mesocarp using a color difference meter. This measurement technique was considered unsuitable for adaptation to measurement of CI because of the uneven distribution of symptoms throughout the mesocarp of individual fruit (1).

This paper describes a method for assaying CI based on extraction and measurement of the soluble colored metabolites present in the mesocarp of chilled avocado fruit. Some of the characteristics of extracts obtained by the method are described, as well as data showing the severity and distribution of CI symptoms.

Mature 'Fuerte' avocado fruit were harvested and grouped randomly into treatments consisting of 5 fruit which were stored for various times at 5°C, then ripened at 20°. Ripe fruit were bisected longitudinally, inspected visually for mesocarp discoloration, and rated subjectively for CI by several assessors using a scale of 0 to 5, with 0 = no visible injury and 5 = severe injury.

Fruit halves were cut transversely into 4 sections of equal thickness (~25mm), and each mesocarp section, with the skin removed, was mixed by hand to a uniform paste. Mesocarp samples (1 g) were then blended with a solution (5 ml) of 10 chloroform:10 methanol:9 water according to a modified Bligh and Dyer technique (3). Homogenates were then centrifuged (at about 700 × *g*) for 15 min. The water-methanol layer containing the colored metabolites was decanted and its absorbance was measured in a simple photometer without a filter (Eel Portable Colorimeter). The values obtained were used as an index of CI severity. The absorbance o extracts in loosely stoppered vials remained stable for at least 2 hours at room temperature.

Extract pH was found to vary depending on the section within individual fruit from which it was derived. Moreover, after their pH was adjusted with NaOH and HCl, absorbance of individual extracts showed pH dependence (Table 1). However, pH of extracts was always within the range 6.25 to 7.80 where absorbance was not markedly affected. Hence, the use of a pH buffer solution in the extraction procedure was considered unnecessary.

Serial dilution of a strongly colored extract showed that absorbance was linearly related to extract concentration ($r^2$ = 0.995). Likewise, absorbance was proportional to variations in the ratio of sample weight to extractant volume. This colorimetric technique, therefore, determines relative differences in the concentration of soluble colored metabolites extracted from chilled avocado fruit.

From visual appraisal, the severity of CI symptoms increased from slight (mean visual rating 1.2) after 2 weeks storage to severe (rating 5) after 8 weeks at 5°C. However, as shown previously (1), the symptoms were initially more severe at the stylar end compared to the pedicel end, especially after 2 weeks storage when there was no injury apparent in the pedicel end of the fruit. Because of this variable symptom distribution, a single visual rating value does not provide a realistic measure of CI. The data from the objective measurement of CI (Table 2) clearly quantify the increase of CI index (severity) with storage time and also the gradient of CI

Table 1. Effect of pH on absorbance of extract from chilled avocado mesocarp (pH adjusted with HCl and NaOH).

| Extract pH | Absorbance |
|---|---|
| 2.02 | 0.50 |
| 6.25 | 0.72 |
| 6.60[z] | 0.76 |
| 6.90 | 0.75 |
| 7.35 | 0.74 |
| 7.65 | 0.73 |
| 7.80 | 0.74 |
| 11.80 | 1.30 |

[z]Unadjusted extract.

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