

symptoms of epinasty when the total sulfide level reached 9 to 11 ppm in the root rhizosphere. The concn of sulfides in the rhizosphere was always 5 to 14 times higher than sulfide levels measured 2 cm from the roots in all experiments set up to make such a dual measurement. Plants that collapsed, even in tests of only 5 days duration, invariably died when transplanted to aerated sandy soil for 10 days. The tap roots of all collapsed seedlings that were checked had an excess of sulfides extending to the crown, as indicated by dark blue stained deposits of methylene blue in phloem and xylem. Sulfide levels appeared to be excessive in distal areas of the root before there was active transport toward the crown.

Discussion

Toxicity of H₂S to citrus roots under waterlogged conditions appeared to be associated with the molecular concn of dissolved H₂S in the root rhizosphere. The generation of H₂S, presumably by sulfate reducing bacteria in the root rhizosphere, was logarithmic as indicated by a doubling of the H₂S concn every 24 hr. The importance of the root rhizosphere as a source of H₂S under anaerobic conditions was demonstrated by the generation of sulfides in the circulating solution in controlled experiments. The root rhizosphere is known as a zone of high bacterial activity. Sulfate reducing bacteria function best during anaerobiosis on an energy source of lactic and citric acids (3), compounds reported to be exudates from plant roots (7).

Hydrogen sulfide toxicity has a concn-time relationship. There was more root damage at 2.3 ppm molecular H₂S in 7-day tests (2) than 2.8 ppm in 5-day tests. The killing of citrus root tissue by H₂S as indicated by stained dark methylene blue areas suggests that sulfides must be in excess, presumably after overcoming all oxidative systems, before the tissues are beyond the point of regenerating a functional root system.

Differential permeability to the H₂S molecule rather than

the more active sulfide ion can probably be explained by the fact that the molecule is small, fat soluble, and readily penetrates plant roots (1). It is suggested that the bisulfide and sulfide ion may not diffuse into roots because of the possibility of a high hydration number. Differential permeability occurs with certain 0.5 mil PVC membranes (5) which are permeable only to the H₂S molecule.

Citrus apparently is reasonably tolerant to O₂ deficiency *per se* in the rhizosphere. Seven-day exposures to O₂ deficiency in the multicelled apparatus did not seriously injure roots (2). It has been suggested that citrus may have an internal downward O₂ transport system as indicated by analyses for air spaces in citrus roots (6). The presence of O₂ in air spaces could contribute to a delay in the transport of toxic concn of H₂S in citrus roots.

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Pecan Nutlet Set and Carbohydrate Level of Various Tissues in the Spring as Affected by Fungicide Sprays¹

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Abstract. Fentin hydroxide and benomyl increased nutlet set in 1969 but not in 1970. Fungicide sprays had little effect on starch content of wood tissue in late March of the year following application. Starch and sugar content of 6 wood tissues in March and nutlet set was not associated with leaf scorch index the previous fall. Starch content of wood tissues in late March ranked as follows from greatest to least: Roots > 2.5 cm diam, trunk, scaffold limbs, new wood, 1-year-old wood, and roots < 1.3 cm diam. Soluble sugar content ranked: Roots < 1.3 cm diam, 1-year-old wood, new wood, roots > 2.5 cm diam, trunk, and scaffold limbs.

Annual production is a goal of most pecan growers, however, trees must be sprayed if this goal is to be met (18). Spraying to control insects and diseases aids in leaf retention (3) and consequently increases photosynthesis (10, 11). Previous reports (18) have shown that spraying with fungicides that control the scab fungus also increases nutlet set the following year. The report herein gives additional results of the effects of the same and additional fungicides on nutlet set in a year when nutlet set

was very heavy (1969) and medium (1970).

Spraying to control the scab fungus also reduces incidence of leaf scorch, a malady responsible for early defoliation of pecans (6, 20). Early defoliation also reduces nutlet set the next year (8, 17). It appears logical then that these sprays should increase the reserve carbohydrate (CHO) content of the tree which perhaps controls nutlet set. Smith and Waugh (15) studied CHO content of small roots of pecan and found that starch appeared to be the most important reserve material, and high levels of starch in the fall were associated with large crops the following year. Further evidence that starch is an important reserve CHO

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is the disappearance of starch in current shoots during the nut-filling period (5).

The location of CHO in storage organs of pecan has not been studied extensively, but studies of other tree crops have shown higher CHO concn in roots than in trunk tissues of sugar prunes (1) and higher than any above ground wood tissue of apple (13). LeClerc Du Sablon (9) studied CHO reserves in several deciduous trees and found roots to be higher in CHO than stems and varied more during the season. Reserves were greatest at leaf abscission and least in spring during rapid shoot growth. Smith and Waugh's work (15) suggests that seasonal trends for pecans are similar with an additional exhaustion of reserve CHO during the nut-filling period.

We noticed that trees sprayed with fungicides retained green, healthy foliage until frost on November 15 while trees receiving only insecticides had severely scorched leaflets, and many of them had abscised before frost. We undertook to learn 1) the effect that these fungicides had on CHO content of the tree, 2) if the early defoliation and severe scorch reduced the CHO content, and 3) if CHO content and nutlet set were related. The effect that fungicides had on pecan scab, leaf scorch, and kernel quality is reported in a contiguous paper (19).

Materials and Methods

We used 'Schley' trees in a grove of mature 'Stuart' and 'Schley' pecans (*Carya illinoensis* Koch.) in a mixed planting located near Tifton, Georgia for the study. Spray application procedures are described elsewhere (19).

Fungicides used were fentin hydroxide (triphenyltin hydroxide) (47.5% a.i.) (Du-Ter); dodine (*n*-dodecylguanidine acetate) (65% a.i.) (Cyprex); captafol (cis-N-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide) (80% a.i. WP or .48 kg/liter EC) (Difolatan); and benomyl ([methyl 1-(butyl-carbamoyl)-2-benzimidazole-carbamate] (50% a.i.) (Benlate).

Nutlet counts were made on July 14 to 16, 1969 and June 2 to 5, 1970 to determine the effect of fungicide sprays applied in 1968 and 1969, respectively. The nutlets on 25 terminals taken at random at a ht of about 6 m (20 ft) on the north, south, east, and west side of each tree were counted. Six or more trees were used for each treatment. Counts for each tree were averaged and treatments were tested by analysis of variance with Duncan's multiple range test to compare means.

Leaf scorch was measured by counting the number of scorched and missing leaflets on 25 adjacent leaves on each of 4

sides of the tree on Oct. 29 to Nov. 5.

Wood samples consisting of new wood (new terminal shoots grown the previous season), 1-year-old wood, scaffold limbs, trunk, large roots (> 2.5 cm diam), and small roots (< 1.3 cm diam) were collected from 6 trees of each treatment on March 25, 1970. The scaffold limb, trunk, and large root tissue samples consisted of borings from a 2.5-cm-diam hole which included both outer cortex (bark) and wood tissue. The other samples were whole tissue samples. Samples were placed in aluminum cans with friction tops and brought to the laboratory where tissues were killed by drying at 100°C for 90 min then further dried at 70°C overnight. This procedure reduces CHO losses to near that obtained by freeze drying (16). Samples were ground to pass a 60-mesh screen and stored in air tight glass bottles for analyses. The anthrone assay procedure of McCready et al. (12) as modified for wood tissue by Dowler (4) was used. Soluble sugars were extracted with hot 80%, then 60%, ethanol. The residue that was solubilized by incubation for 24 hr with 4.8 N perchloric acid at 37°C was called starch.

Differences in sugar or starch were tested for each tissue by a computerized least squares analysis of variance with Duncan's multiple range test (UGA 3090CT). Correlations between sugar and starch in each tissue with leaf scorch rating and nutlet set were computed by using the BMD03D program (2).

Results and Discussion

Nutlet set. Data for 1967 and 1968 (18) is included in Table 1 for comparison. Fentin hydroxide sprays to control the scab fungus also increased nutlet set over unsprayed trees for 3 consecutive years, but did not increase nutlet set the fourth year (1970). Dodine increased set in only 1 of 3 years. (It was not included in 1967 spray tests.) All fungicides applied in 1966 increased nutlet set over the check in 1967 (18), but 1967 was a year of low nutlet set with practically none being set on trees that received no fungicide the previous year. Nutlet set was good to excellent in the next 3 years even on trees that received no fungicide. Benomyl (without surfactant) caused significant increases in nutlet set in 1969 but not in 1970. The data indicate that increases in nutlet set due to fungicide are not always obtained if nutlet set is high. Nutlet set in 1970 was expected to be low in check plots compared with fungicide sprayed plots because leaf scorch on them, was severe in 1969 and they lost their leaves much earlier than trees sprayed with fungicides (19). Damage from other leaf diseases was not noticed and was considered negligible. Symptomatology of the

Table 1. Effect of fungicides on pecan nutlet set the year after application.

Fungicide treatment ^z	Rate of active ingredient g/tree/spray	Nutlet set No./terminal shoot			
		1967	1968	1969	1970
Dodine-65% (Cyprex)	89 (.196 lb)	0.69b ^w	----	1.90a	1.28a
Dodine-65% (Cyprex) massive dormant then regular schedule beginning June 24 ^y	531 (1.170 lb) then 89 (.196 lb)	----	----	2.06ab	----
Fentin hydroxide-47.5% (Du-ter)	26 (.057 lb)	1.34c	2.61b	2.75d	1.44a
Captafol-80W (Di folatan) massive dormant then regular schedule beginning June 24 ^y	719 (1.583 lb) then 109 (.240 lb)	----	----	2.47cd	----
Captafol-80W (Di folatan)	109 (.240 lb)	----	----	2.63 cd	----
Captafol (Difolatan-4-F) ^x	136 (.300 lb)	----	----	----	1.48a
Captafol (Difolatan-4-F) ^x	163 (.359 lb)	----	----	----	1.03a
Benomyl-50% (Benlate) no surfactant	25 (.055 lb)	----	----	2.71d	1.30a
Benomyl-50% (Benlate) + Du Pont surfactant F (34 g/tree)	25 (.055 lb)	----	----	2.48cd	1.07a
No fungicide	----	0.01a	1.99a	2.26abc	1.63a

^zRegularly scheduled sprays were applied April 22, May 13, June 3, June 24, July 15, and August 5 in 1968 and April 29, May 20, June 9, June 30, July 23, and August 12, 1970.

^yMassive dormant applications were made at bud break on March 26 with Volk Supreme spray oil (2 liters/100 liters) used as a surfactant for the captafol dormant spray. No further treatments were made until June 24.

^xChevron Spray Sticker at 71 ml/tree was used as a surfactant.

^wDuncan's multiple range test, within years, at 5% level.

scorched condition that we observed is described in another paper (19). Foliage on trees receiving any of the fungicides remained healthy and appeared active until frost on November 15. The nuts on trees not receiving fungicides, however, were severely damaged by scab and many abscised prematurely. Those that matured were poorly filled, and the shucks dehiscid long before those of fungicide sprayed trees. Although yield records were not taken, yields from trees not sprayed with fungicides were much lower than that for the fungicide sprayed trees. If CHO reserves are major factors responsible for the

or the control. No other treatment differences were significant. Total CHO (starch + sugar) averaged over all tissues showed no significant treatment effect.

There was no evidence of a reduction of CHO caused by the early leaf loss of trees not sprayed with fungicides. There was also no correlation between nutlet set and starch or sugar content of the tissues. Neither was there a significant correlation for leaf scorch index in 1969 with starch or sugar content of any of the tissues or with nutlet set in 1970 (Table 4). Perhaps the above facts account for the absence of a significant

Table 2. Effect of fungicide sprays in 1969 on anthrone detectable sugar in various wood tissue of pecan, March 23, 1970.

Fungicide treatment	Rate of active ingredient g/tree/spray	Percent sugar						Mean
		New wood	1-year-old wood	Scaffold limbs	Trunk	Roots > 2.5 cm	Roots < 1.3 cm	
Dodine-65% (Cyprex)	89 (.196 lb)	7.5c ^Y	7.3ab ^Y	4.8a	6.2a	5.4a	10.5c	7.0c
Fentin hydroxide-47.5% (Du-ter)	26 (.057 lb)	6.5bc	8.3b	4.9a	8.2b	6.2a	9.8bc	7.3c
Captafol (Difolatan-4-F) ^Z	136 (.300 lb)	6.0b	6.8ab	4.4a	4.9a	5.8a	9.4bc	6.2a
Captafol (Difolatan-4-F) ^Z	163 (.359 lb)	5.7ab	6.8ab	4.1a	5.0a	5.3a	8.8ab	6.0a
Benomyl-50% (Benlate)	25 (.055 lb)	5.3ab	7.0ab	4.5a	5.2a	6.2a	9.4bc	6.3ab
Benomyl-50% (Benlate) + Du Pont surfactant F (34 g/tree)	25 (.055 lb)	4.7a	6.3a	4.6a	5.8a	5.9a	7.9a	5.9a
No fungicide	- - -	6.3b	6.8ab	4.9a	6.3a	6.8a	9.5bc	6.8bc
Tissue means ^X		6.0f	7.0g	4.6e	6.0f	5.9f	9.3h	

^ZChevron Spray Sticker at 71 ml/tree was used as a surfactant.

^YDuncan's multiple range test for spray treatment means, within columns, at 5% level.

^XDuncan's multiple range test for tissue means, bottom row, at 5% level.

formation of pistillate flowers in spring, then the CHO level of trees receiving the various treatments must have equalized. It is possible that the CHO used to produce the higher yield, higher quality, and later maturity of nuts from the fungicide sprayed trees compared with those receiving no fungicides might have reduced the advantage that they had in healthier foliage and later leaf retention in the fall.

Carbohydrate reserves. Soluble sugars were higher for the mean of all tissues when trees were sprayed with dodine or fentin hydroxide the previous season than for all other treatments except the non-sprayed check (Table 2). This relationship can be observed for most of the tissues, but differences were not always significant. Benomyl (with surfactant) caused lower sugars in new wood and small root tissue than that found in trees not sprayed with fungicides.

Fungicide sprays had little effect on starch content of the various tissues (Table 3). Trees sprayed with benomyl (without surfactant) contained significantly more starch in the new wood tissue than those sprayed with dodine, the high rate of captafol,

difference in starch level in the wood tissue of fungicide sprayed trees compared with those not sprayed with fungicides, and for the lack of significant correlation of leaf scorch with sugars and starches. There is also the possibility that CHO level is not the controlling factor for female flower formation. Studies with apple showed that defoliation and defruiting did not affect CHO level of shoots or spurs the next spring and that there was no correlation between CHO content and fruit set (7). The differences in sugar content of the fungicide sprayed trees is difficult to explain because visual differences in foliage were not detected in the fall.

The amount of starch and sugar in the various tissues differed greatly. Starch was highest in the large roots and least in the small roots. Murneek (13) reported that starch in apple was highest among small roots followed by large roots; however, his small roots ranged from 1 to 6 years old while ours were < 1.3 cm in diam. Obviously most of his small roots would have been classified as large roots in our study. Smith and Waugh (15) in studies of seasonal variation of CHO in pecan roots used only

Table 3. Effect of fungicide sprays in 1969 on anthrone detectable starch in various wood tissues of pecan, March 23, 1970.

Fungicide treatment	Rate of active ingredient g/tree/spray	Percent starch						Mean
		New wood	1-year-old wood	Scaffold limbs	Trunk	Roots > 2.5 cm	Roots < 1.3 cm	
Dodine-65% (Cyprex)	89 (.196 lb)	7.5a ^Y	6.6a	9.0a	8.4a	11.9a	3.1a	7.8a
Fentin hydroxide-47.5% (Du-ter)	26 (.057 lb)	8.0ab	7.2a	9.8a	8.9a	14.8a	2.8a	8.6a
Captafol (Difolatan-4-F) ^Z	136 (.300 lb)	8.6ab	6.5a	9.3a	9.2a	15.7a	4.3a	8.9a
Captafol (Difolatan-4-F) ^Z	163 (.359 lb)	7.7a	7.0a	9.0a	9.1a	11.7a	4.3a	8.1a
Benomyl-50% (Benlate)	25 (.055 lb)	9.6b	7.5a	9.3a	10.0a	14.4a	2.4a	8.4a
Benomyl-50% (Benlate) + Du Pont surfactant F (34 g/tree)	25 (.055 lb)	8.1ab	6.8a	8.2a	9.9a	15.1a	2.2a	8.4a
No fungicide	- - -	7.3a	6.6a	8.1a	9.4a	12.0a	3.5a	7.8a
Tissue means ^X		8.1g	6.9f	9.0gh	9.3h	13.6i	3.2e	

^ZChevron Spray Sticker at 34 ml/tree was used as a surfactant.

^YDuncan's multiple range test for spray treatment means, within columns, at 5% level.

^XDuncan's multiple range test for tissue means, bottom row, at 5% level.

Table 4. Correlation coefficient matrix for sugar and starch in various pecan tissues, nutlet set, and leaf scorch index.

Variable 1	Percent sugar						Percent starch						Nutlet set 1970
Variable 2	New wood	1-yr-old wood	Scaffold limbs	Trunk	Large roots	Small roots	New wood	1-yr-old wood	Scaffold limbs	Trunk	Large roots	Small roots	
Percent sugar:													
1-yr-old wood	.52a ^z												
Scaffold limbs	.21	.39a											
Trunk	.33a	.54a	.20										
Large roots	.12	.20	.23	.04									
Small roots	.64a	.47a	.29	.30	.05								
Percent starch:													
New wood	-.38a	-.05	-.22	.04	-.15	-.16							
1-yr-old wood	-.13	.30	.23	.33a	-.10	.04	.39a						
Scaffold limbs	.12	.23	-.20	.12	-.11	-.05	-.01	.08					
Trunk	-.06	.16	.11	-.01	.06	-.16	.02	.05	.12				
Large roots	-.02	.15	.20	.17	-.35a	.09	.27	.35a	.11	.43a			
Small roots	-.14	-.12	-.09	-.14	-.34a	-.26	-.04	-.18	.33a	-.02	.11		
Nutlet set, 1970	.08	.10	.09	.09	.17	-.04	.12	.08	-.04	-.03	.04	.10	
Leaf scorch index, 1970	.20	-.01	.18	.21	.27	-.10	-.26	-.06	-.15	-.02	-.13	.12	.27

^zCoefficients with the letter a are significant by Duncan's multiple range test at 5% level; least significant r, .31.

roots 1 to 1.3 cm in diam, which according to our study, was the tissue containing the least amount of starch. Others have found higher starch in root tissue than in trunk tissue of other trees (1, 9, 13). The high starch content of trunk and scaffold limb tissue of pecan indicates that these organs might also be storage organs (Table 3).

Soluble sugars were highest in the small root tissue followed by 1-year-old wood and new wood. Murneek (13) also observed that younger above-ground wood tissues of apple contained more sugars than the older above-ground tissues. Since the pecan samples were taken just prior to bud break, concn of sugars in these areas of high growth activity might account for the high levels found. Also, during cold weather starch in the peripheral regions of trees is hydrolyzed into sugars (14).

The data of Dowler and King (4) for peaches agreed with ours for pecans in that twigs contained more sugars than scaffold limbs or the trunk. Their scaffold limbs and trunk tissue did not include bark as did ours. Bark tissue from the scaffold limbs and trunk of peaches contained twice as much sugar as twig tissue. Starch in pecan trunk and scaffold limb tissue was higher than that of the younger tissues on March 23 whereas Dowler's data showed the opposite for peaches on March 16. Earlier data for peaches, however, showed the same trend as for pecans. Since peach buds break much earlier than those of pecan in the spring, it appears that starch in pecan tissues in late March is comparable to that in peaches in early March or even winter.

The sugar level in the various tree parts was positively related, but only correlation coefficients (r's) for sugar in new wood and 1-year-old wood tissue with other plant parts were significant (Table 4). The highest r in the correlation matrix was that for sugar in new wood and sugar in small roots thus indicating that these 2 tissues are reacting similarly with respect to sugar utilization. Sugar and starch were negatively correlated with each other in both tissues, but the r for sugar with starch in small roots was not significant. Sugar in large roots was negatively correlated with starch in both large and small roots.

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