

Nonstructural Carbohydrate Contents of Trees Affected with Texas Live Oak Decline

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ABSTRACT

Tainter, F. H., and Lewis, R., Jr. 1982. Nonstructural carbohydrate contents of trees affected with Texas live oak decline. *Plant Disease* 66:120-122.

Nonstructural carbohydrates were compared in previously wilted (declining) and healthy *Quercus virginiana* in central Texas in 1978. Total carbohydrate and reducing sugar contents were significantly greater in leaves and twigs of declining trees than in those of healthy trees. Starch content was also significantly greater in leaves but not in twigs of declining trees. However, total ethanolic extractives from both leaves and twigs were significantly greater in the healthy trees. Radial growth was significantly greater in healthy trees than in declining trees. Nonstructural carbohydrate content and radial growth were affected by the relative health of trees.

Understanding Texas live oak (*Quercus virginiana* Mill.) decline represents a substantial challenge. Documentation of the presence of *Ceratocystis fagacearum* (Bretz) Hunt in the Dallas area (1,5) and the recent confirmation of its involvement in the decline syndrome in central Texas (6,7) are contrary to conceptions of where the fungus can survive. The ability of this fungus to infect trees and survive far beyond the previously known range of

the disease represents one of the more intriguing problems since *C. fagacearum* was identified as the causal agent of oak wilt in 1944 (4).

Red oak (*Erythrobalanus*) trees infected with *C. fagacearum* usually die during the first year of infection. Ecologic studies of *C. fagacearum* nearest to the Texas live oak decline sites were done in northern Arkansas (10,11), on the southwesterly edge of what was formerly thought to be the range of the disease. There, three *Erythrobalanus* species were colonized by *Hypoxylon* spp. shortly after wilting caused by oak wilt. Recovery of *C. fagacearum* from infected trees was always low but dropped to zero within several months. This reduction in recovery correlated closely with invasions by *Hypoxylon* spp. and subsequent

removal of nonstructural carbohydrates from trunk sapwood and branches (11). The term "nonstructural" distinguishes between carbohydrates such as starch and free sugars that are located in the cytoplasm of host cells and carbohydrates such as cellulose that are components of host cell walls.

Because *Hypoxylon* spp., especially *H. atropunctatum*, are prevalent in Arkansas and apparently adapted to the relatively high summer temperatures there, the spread of oak wilt in a southerly direction appeared limited by this natural biologic control. Although little is known about the biology of *H. atropunctatum*, it is known to be an aggressive invader of stressed trees, even those stressed by oak wilt. The high prevailing temperatures that enhance growth of *Hypoxylon* inhibit growth of *C. fagacearum*. The result is that the sapwood is depleted of nonstructural carbohydrates within 1-5 mo and *C. fagacearum* dies, usually before it can produce pressure pads, sporulate, and be spread overland.

Hypoxylon spp. are present in Texas (13) but do not appear to influence *C. fagacearum* spread as they do in Arkansas. Their impact on nonstructural carbohydrates in *Q. virginiana* is unknown. The present research attempts to elucidate further the live oak decline

Accepted for publication 11 May 1981.

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0191-2917/82/02012003/\$03.00/0

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syndrome and compares the relative contents of nonstructural carbohydrates in declining *C. fagacearum*-infected and healthy live oak trees growing in Texas.

MATERIALS AND METHODS

Eleven declining live oak trees and 11 nearby healthy ones were sampled in September 1978 in Kerrville State Park, TX. Both healthy and declined trees averaged about 30 cm diameter at breast height. Four samples each of leaves and small twigs were collected from each tree, one set from each cardinal direction in the crown. The tissues were immediately placed in bottles containing 150 ml of 80% ethanol. Upon arrival at the laboratory, the samples were removed, oven-dried at 80 C for 24 hr, and ground in a Wiley mill to pass through a 40-mesh screen. Total nonstructural carbohydrates, except starch, were extracted with 80% ethanol for 6 hr in Soxhlet extractors. Aliquots were assayed for total soluble carbohydrate by the phenol-sulfuric acid method (2) and for reducing sugars (8). Starch was extracted from the ethanol-extracted residue of ground tissue (3). All determinations were performed in triplicate.

During preliminary analysis, other extractives were obviously being removed by the Soxhlet extractions. These extractives, which were typically dense green for leaves and dense brown for twigs, interfered with subsequent quantifications. Deproteinization (9) removed all such colorations and a small but predictable amount of carbohydrate from the samples. The linear relationships $\hat{y} = 0.078x + 1.405$ ($R^2 = 0.78$ for $n = 80$) and $\hat{y} = 0.044x + 1.602$ ($R^2 = 0.64$ for $n = 86$) were determined for total carbohydrate and reducing sugar contents, respectively; they can be used to adjust the values given in the results, which are the deproteinized values (x).

RESULTS

C. fagacearum was isolated from the 11 declining trees in April and June 1978. These trees were in four active oak wilt infection centers, but they survived over summer. The symptoms observed in September were thin crowns, small chlorotic leaves, defoliation, suckering, and dieback. *Hypoxyylon* was not isolated nor observed in the trees. It was also not observed on dead live oaks within the four infection centers. The 11 healthy trees had full crowns, normal leaves, and no dieback. They were all located within 50–100 m of infected trees. Neither *C. fagacearum* nor *Hypoxyylon* was isolated from the healthy trees in 1978, and they remained healthy through 1979.

Total carbohydrate, reducing sugar, and starch contents of leaves were all significantly greater ($\alpha = 0.01$, $n = 44$) in *C. fagacearum*-infected than in healthy trees (Fig. 1), representing a 46, 32, and 76% increase, respectively, over those in

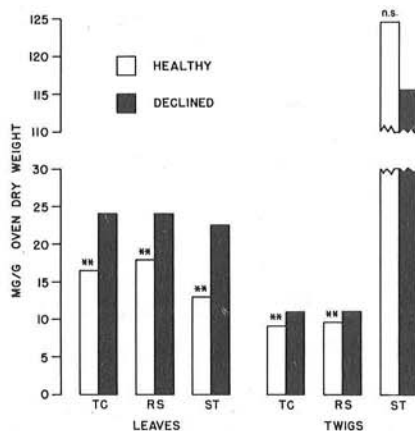


Fig. 1. Nonstructural carbohydrate contents of live oak trees affected with Texas live oak decline. TC = total carbohydrates, RS = reducing sugars, ST = starch, ** = α at 0.01 for $n = 44$, n.s. = not significant.

healthy trees. In twigs, total carbohydrates and reducing sugars were significantly greater ($\alpha = 0.01$, $n = 44$) in *C. fagacearum*-infected than in healthy trees (Fig. 1), representing increases of 19 and 16%, respectively. Starch contents of declining trees and healthy trees were not significantly different.

The total amount of material extracted from each sample was determined gravimetrically following extraction. In healthy trees, this was 124.4 and 103.0 mg/g oven-dry weight for leaves and twigs, respectively; for declining trees, it was 94.5 and 77.1 mg/g for leaves and twigs, respectively. These amounts in both tissue types were significantly different ($\alpha = 0.01$, $n = 44$) between healthy and declining trees.

There was an effect of *C. fagacearum* infection on diameter growth. The average radial growth from 1978 of 2.72 mm for healthy trees and 1.95 mm for declining trees was significantly different ($\alpha = 0.05$, $n = 11$). There was no effect of side of bole from which core was taken on growth.

DISCUSSION

Although a time-sequence measurement of changes in carbohydrate content was not possible with these naturally infected trees, nonstructural carbohydrate content of declining live oak trees was obviously quite different from that of healthy live oak trees.

The greater concentrations of the three carbohydrate forms extracted from leaves than from twigs of declining live oak trees could have resulted from impaired but not complete blockage of translocation of soluble carbohydrates out of the leaves. Approximately 32–76% of the nonstructural carbohydrate was apparently pooled in the leaves and presumably would be lost when the leaves were cast. With this loss in available food reserves and the fewer, smaller leaves, it is certainly reasonable to expect diameter

growth to decrease and each new flush of leaves to be subsequently more sparse.

The apparent relatively greater concentrations of nonstructural carbohydrates in declining trees may relate at least partially to their composition. Infection of tissues with *C. fagacearum* would cause metabolic disruption and degradation of host cell cytoplasm, with the increased metabolic turnover producing more chemicals with reducing groups. These would include amino acids, fats, etc. Deproteinization does not remove all of these compounds. Although the unremoved portions may not be sugars, they would react as such because they contain reducing groups.

Leaf and twig tissues of declining trees were also likely suffering moisture stress because of impaired translocation of water through partially blocked xylem (12), explaining why gravimetric determination of extractives showed cell cytoplasm of declining trees to be less extractable. First, there was less cytoplasm; second, there were fewer cytoplasmic solubles; and third, the solubles were likely more tightly bound to endoplasmic reticulum.

It is possible that *C. fagacearum* infecting live oaks in south central Texas is weakened by the high summer temperatures prevalent there and its virulence reduced. It is seldom isolated during the summer (7), and it appears to reinvade the aboveground portions of trees from the roots during the following winter. As a result, insufficient damage is caused during the infection syndrome to facilitate attack by *Hypoxyylon*. Because *Hypoxyylon* does not invade these trees, they remain alive much longer than would be expected, although in a declining condition. Elevated nonstructural carbohydrate contents are symptomatic of these declining trees.

ACKNOWLEDGMENTS

This research was supported in part by the Southern Forest Experiment Station, U.S. Forest Service. Most of the work was done while the senior author was with the Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

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