JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION
VOL. 36, NO. 1 AMERICAN WATER RESOURCES ASSOCIATION FEBRUARY 2000

SOLUBLE SUGAR COMPOSITION OF POND-CYPRESS: A POTENTIAL HYDROECOLOGICAL INDICATOR OF GROUND WATER PERTURBATIONS[1]

Sydney T. Bacchus, Toshihide Hamazaki, Kerry O. Britton, and Bruce L. Haines[2]

ABSTRACT: Pond-cypress, a deciduous conifer, is a dominant canopy species in depressional wetlands of the southeastern Coastal Plain (SCP). Extensive premature decline and death of pond-cypress trees in central Florida have been attributed to hydroperiod alterations due to excessive withdrawals of ground water from the Floridan aquifer. One factor identified in the decline process is basal decay, which may be related to the presence of *Botryosphaeria rhodina* and *Fusarium* species (nonaggressive, facultative fungal pathogens). These fungi have been cultured from sapwood tissue of declining pond-cypress associated with ground water mining, but not from pond-cypress away from ground water mining areas. In this experiment, differences in soluble (nonstructural) carbohydrate composition of branch tips were evaluated for one- and two-year old, nursery-grown (unsheltered) pond-cypress, following a year of growth under treatment conditions (control, fungal inoculation, water stress, and fungal inoculation plus water stress) in a growth chamber. Results from two methods of wet chemical analysis were compared (trimethylsilyl methylglycoside – Method A, and alditol acetate – Method B). Three pentoses (arabinose, rhamnose, and xylose) and three hexoses (galactose, glucose, and mannose) were identified in branch tips from both age classes. A fourth hexose (fucose) also was identified in samples from the younger trees. The acidic sugar, galacturonic acid, was identified in both age classes using Method A. Results suggest that prolonged water stress is correlated with greater relative concentrations of the neutral soluble sugars rhamnose ($P = 0.02$), xylose ($P = 0.02$), and galactose ($P = 0.02$), in addition to the acidic sugar galacturonic acid ($P = 0.01$), for Method A, and arabinose ($P = 0.02$) for Method B. These results also suggest that in the absence of water stress, the fungal pathogen *B. rhodina* does not penetrate to the sapwood of the trees, and that inoculation with this fungal pathogen is not correlated with differences in relative concentrations of nonstructural, soluble carbohydrates, based on Method A analysis. Empirical evidence suggests that pond-cypress trees in depressional wetlands respond similarly to anthropogenic perturbations of ground water, but not to natural periods of drought in the absence of such perturbations. Therefore, pond-cypress appear to be integrators of groundwater perturbations. Greater concentrations of the soluble sugars identified in this study in pond-cypress branch tips may be hydroecological indicators of such anthropogenic perturbations as unsustainable yield from the regional aquifer and adverse impacts from aquifer storage and recovery (ASR) activities in the SCP.

(KEY TERMS: ASR and aquifer yield impacts; depressional wetlands; hydroecological indicators; predisposition; soluble sugars; *Taxodium ascendens* Brong.; water resources planning; water stress.)

INTRODUCTION

Pond-cypress (*Taxodium ascendens* Brong.), a deciduous conifer, is the dominant tree species in most forested depressional wetlands in the southeastern Coastal Plain (SCP). These depressional wetlands have become established in relict sinkholes that probably formed during the fluctuations of sea level in the Pleistocene epoch. They generally have a more direct contact with the underlying karst aquifer than surrounding areas. These depressional wetlands are associated with fracture traces, solution/collapse features, in addition to the various degrees of breached semiconfining units (Bacchus and Brook, 1996; Brook and Allison, 1986; Watson *et al.*, 1990) of the Floridan aquifer system.

The Floridan is a regional carbonate ground water system that extends throughout Florida and the SCP of Georgia, South Carolina, and Alabama, USA (Johnston and Miller, 1988). Premature death and decline of pond-cypress have been observed in Florida, Georgia, and South Carolina, where unsustainable ground water withdrawals are occurring, or have occurred (Bacchus, 1994; 1999; unpublished data). In

[1]Paper No. 97123 of the *Journal of the American Water Resources Association*. **Discussions are open until October 1, 2000.**
[2]Respectively, Institute of Ecology, University of Georgia, Athens, Georgia 30602; Department of Fishery and Wildlife Science, New Mexico State University, P.O. Box 30003, MSC 4901, Las Cruces, NM 88003; USDA Forest Service, Forestry Sciences Laboratory, Athens, Georgia 30602; and Department of Botany, University of Georgia, Athens, Georgia 30602 (E-Mail/Bacchus: sbacchus@arches.uga.edu).

one county of west-central Florida, excessive ground water withdrawals have destroyed approximately 6,880 hectares (17,000 acres) of wetlands, many of which were dominated by pond-cypress. These withdrawals also resulted in damage to private wells, initially estimated at four million dollars, and increased sinkhole formation (House Committee on Natural Resources, 1994). The increase in sinkhole formation represents irreversible damage to the matrix (structure) of this regional aquifer. This damage, including the premature death and decline in pond-cypress from water stress, was determined to be the result of anthropogenic perturbations of ground water (ground water mining), rather than natural droughts (Bacchus, 1994; 1997; 1998; Quattlebaum, 1997; Southwest Florida Water Management District, 1996).

Many naturally-occurring plant species, such as pond-cypress, have evolved effective mechanisms to cope with natural cycles of drought. Volaire (1995) provides an example of the role of carbohydrate reserves in drought adaptation. Drought is a meteorological phenomenon of insufficient precipitation that may result in water stress to plants growing under conditions to which they are not adapted, such as introduced landscape plants and most agricultural plants (Kozlowski et al., 1991; May and Milthorpe, 1962; McWilliam, 1986; Salisbury and Ross, 1992; Wang and Stutte, 1992).

Responses of plants to water stress can be grouped in two broad categories. Type I includes short-term hydroecological responses, such as leaf drop (typical for pond-cypress), which generally are reversible. Type II includes long-term hydroecological responses and those that generally are irreversible, such as infection by fungal pathogens (Bacchus, 1996).

Infection by *Botryosphaeria rhodina* and *Fusarium* species has caused significant damage and death in water-stressed commercial and landscape trees, with the latter causing the roots to decay (Brown and Britton, 1986; Ocamb and Juzwik, 1995). Under conditions of no water stress, these fungi are considered nonaggressive, facultative fungi. These fungi were found in the sapwood at the bases of many pond-cypress associated with areas of anthropogenic ground water perturbations and experiencing premature death and decline. Since these trees lacked wounds, such as mechanical and pruning damage, the fungi were presumed to have penetrated the bark and roots, which are barriers to fungi when the host is not stressed. These fungi were not found in the sapwood of pond-cypress not associated with ground water mining areas (Bacchus, unpublished data).

Decay of the roots (root rot) and base (butt rot) of pond-cypress eventually result in windthrow (leaning and falling of the tree prior to death of the canopy). Premature decline of the trees is a process that may continue for 15 to 20 years after the onset of hydroperiod perturbations, before culminating in windthrow or the death of the trees. However, damage to the trees and the aquifer may become irreversible much earlier. For example, in north-east Florida, wells extracting water from the Floridan considerable distances from the east coast became contaminated with salt water from zones underlying the Floridan after only a brief period of pumping. Vertical fractures through the semiconfining unit permitted contamination of the aquifer in this case (Spechler, 1994; Spechler and Phelps, 1997).

Current approaches for evaluating impacts from ground water mining primarily use routine hydrologic approaches to predict responses of the regional and surficial aquifers. However, estimations of hydraulic conductivity can vary approximately six orders of magnitude from lab scale (pore/microfissures = 10^{-7}) through borehole scale (macrofissures = 10^{-5}) to regional scale (karstic networks = 10^{-2}), with ± two orders of magnitude error factor at each scale (Ford and Williams, 1989). Consequently, determining regional responses of karst aquifers is difficult using existing hydrological approaches, particularly when multiple sources of anthropogenic perturbations occur. Additionally, this approach provides no information regarding what impacts the perturbations may have on associated living systems.

A more precise and accurate method is needed for early detection of damage to the regional aquifer and surficial aquifer perturbations by anthropogenic activities such as ground water mining and aquifer storage and recovery (ASR). The latter is a relatively new concept intended to reduce impacts of excessive withdrawals from ground water resources (Pyne, 1989). Garcia-Bengochea and Muniz (1989) define ASR as storage of excess waters through wells into confined aquifers for recovery during water shortages.

More than half of the operational ASR systems are in the Floridan aquifer in Florida (Pyne, 1989). In karst aquifers such as the Floridan, rapid introduction of artificial recharge, and subsequent withdrawals, common in ASR activities, can initiate or exacerbate both structural (e.g., suffosion) and geochemical problems (e.g., contamination of potable ground water from injected effluent or other highly eutrophic waters via fractures). Hydroperiod perturbations from ASR activities (localized drawdowns and rebounds of the surficial aquifer) are predicted to result in adverse synergistic responses and water stress in associated wetland vegetation due to alternating periods of too little and too much water. Currently, no monitoring is being conducted in wetlands associated with ASR activities in the SCP, to evaluate adverse potential hydroecological impacts.

Early detection of stress from these ground water perturbations in depressional wetland plants could be a useful tool. However, methods commonly used to monitor plant communities primarily determine the presence, absence (death), or dimensions of plants, rather than detect Type I and early stages of Type II hydroecological stress responses of plants to hydroperiod perturbations. Consequently, these methods are ill-suited for determining the onset/early stages of environmental damage and damage to the aquifer associated with ground water mining from karst systems (Bacchus, 1996). For example, Wargo (1988) describes inadequacies of current approaches to determine tree vigor, whereas Bacchus (1996) discusses and ranks the effectiveness of some of the most common assessment methods for detecting Type I and II hydroecological stress responses to hydroperiod perturbations. The interactions between the karst aquifer matrix and ground water flow, including responses to ground water withdrawals, have been addressed by numerous authors (Brook, 1985; Brook and Allison, 1986; Bush and Johnston, 1987; Ford and Williams, 1989; McConnell and Hacke, 1993; Spechler and Phelps, 1997; Watson *et al.,* 1990).

In this study, we examined the potential usefulness of soluble, nonstructural carbohydrates in branch tips as an indicator of water stress and fungal infection in pond-cypress. If successful, this approach could provide a sensitive, objective means for early detection of groundwater perturbations such as unsustainable yield from the regional karst aquifer and adverse impacts from ASR activities. Early detection would provide an opportunity to prevent irreversible damage to the regional and surficial aquifers, and to the environment. This approach also could be applicable for monitoring ASR sites.

Branch tips were selected primarily for two reasons. First, pond-cypress is a deciduous tree that may drop its leaves during the growing season in response to reduced water availability. Second, the carbohydrates (e.g., soluble, nonstructural sugars) are perceived to be more stable in branch tips than the same components in leaves. These carbon-based compounds can vary diurnally within leaves and by leaf position on the branch (Dickson, 1986; and Larson and Dickson, 1986). Soluble sugars commonly are analyzed by two wet chemical methods (A and B), as described under "Laboratory Methods" below. However, only results for Method A generally are reported, with Method B used within the laboratory for confirmation of results (Parastoo Azadi, CCRC, pers. comm., 1995). We elected to analyze the data from both methods, for a comparison of results.

METHODS

Pre-Treatment Procedures

Twelve two-year old and 24 one-year old pond-cypress trees of uniform size were selected from a commercial nursery in central Florida where the trees had been grown (unsheltered) from seed collected at a nearby research site (Tosohatchee State Reserve, Orange County, Florida). Trees were repotted in steam-sterilized potting mixture (1: 1:1 Coastal Plain sand:sphagnum peat moss:pine bark mini-nuggets) to a depth of 20 cm in sterilized plastic containers (35.6 x 30.5 x 30.5 cm). Nitrogen, phosphorus, and potassium were provided by distributing 31 g of Osmocote 14-14-14 over the soil surface in each container. The containerized trees were placed randomly in an environmental growth chamber (1.33 m wide, 2.48 m long, and 2.00 m tall) for a 14-day acclimation period, under 92 percent relative humidity and 12-hour photoperiods of moderate light. Temperature, humidity, and day length conditions simulated those of central Florida (with a month time lag), where the trees originated. These conditions were maintained for the acclimation period and throughout the year (1994) of the experiment. The containers in the chamber were rotated every two days throughout the acclimation period and the experiment. An "S" pattern rotation was used to minimize differences in temperature, light, and humidity gradients within the chamber. All trees were watered with 2 L per container of tap water acidified to a pH of 4.7 (± 0.2) to simulate *in situ* pH conditions.

Experimental Design

The experimental design included four treatments: (1) control, (2) fungal inoculation and adequate water, (3) no fungal inoculation and water stress, and (4) fungal inoculation and water stress. The sample size per treatment was six for one-year old trees (three per 19 L container) and three for two-year old trees (one per 19 L container). A random number table was used for both treatment assignment and repositioning of containerized trees in the growth chamber.

For the fungal treatments, the aqueous suspension of inoculum was applied with a natural hair paint brush to a zone extending from the soil surface (but preventing contact between the brush and the soil) to a height of 7.5 cm for one-year old trees and 15 cm for two-year old trees in Treatments 2 and 4. For the trees not inoculated, sterilized deionized water was brushed onto the stems. The fungal inoculum was an isolate of *B. rhodina* (in the anamorph state) collected from sapwood tissue of mature, prematurely declining pond-cypress trees at the Starkey Well Field in Pasco County, Florida. After approximately two weeks of culture on acidified potato-dextrose agar (APDA), the conidia and hyphae were scraped from the agar using a rubber policeman, and transferred into sterilized tap water. The aqueous suspension was strained through cheese cloth. Few conidia were present.

For the two water stress treatments, water was withheld repeatedly until symptoms of wilt or abscission were apparent in the leaves (approximately two weeks, soil water potential < -2.0 MPa), then 2 L of acidified water was added to each container, without permitting direct contact of the water with the stems of the trees. Moist soil conditions (soil water potential > -0.01 MPa.) were maintained for trees in the remaining two treatments by adding 2 L of acidified water to each container on alternate days, in the same manner as described above. This watering regime was repeated for the duration of the experiment.

Initially, the pre-dawn water potential of each tree was estimated with a pressure bomb (Scholander *et* al., 1965) following the periods of water stress. One of the youngest, fully expanded short shoots was used from each one-year old tree and three of the youngest, fully expanded short shoots were used from each two-year old tree. However, water potential of short shoots from water stress treatments did not differ from other treatments at pre-dawn, mid-day, or end of the daily photoperiod. Consequently, measurements of water potential in short shoots were discontinued (data not shown). This lack of decline may be due to the ability of this species to maintain water potentials in the short shoots while water potentials become more negative in the numerous, highly reduced leaves attached along the short shoots.

Pre-dawn soil water potentials were determined using a tensimeter and tensiometers (Marthaler *et* al., 1983; Storm and Younos, 1984). The time domain reflectometry (TDR) technique (Topp *et al.,* 1980), with pairs of stainless steel rods 20 cm in length installed 5 cm apart, in an equivalent position as the tensiometers, was used to determine soil-moisture potentials electromagnetically in containers with the water-stressed trees (Treatments 3 and 4), because this technique provides accurate measurements of soil moisture at greater suction than tensiometers. The TDR rods remained in place throughout the experiment.

Laboratory Methods

At the conclusion of the experiment, the distal 2 cm of each branch was removed and dried at 45°C for 48 hours to determine nonstructural soluble sugar composition. Branch tips were pooled by container, then ground with a Wiley mill using a 20 mesh screen. The Complex Carbohydrate Research Center (CCRC) in Athens, Georgia, selected a representative sample of approximately 100 mg from each pooled sample for analysis by two methods to determine soluble sugar composition of the branch tips. Method A was the preparation and gas chromatography-mass spectrometry (GC-MS) analysis of trimethylsilyl (TMS) methylglycosides. In this method, samples were subjected to methanolysis for 16 hours at 80°C, followed by N-acetylation using methanol, pyridine, and acetic anhydride. The samples then were silylated with Trisil-Z and analyzed by a Hewlett-Packard 5985 GC-MS system using a DB1 column (0.25 mm x 0.25 mm i.d., J & W Scientific). The initial oven temperature of 160°C was maintained for 3 minutes then increased at 30°C min^{-1} to 260°C, then held for 15 minutes. Ionization for electron impact-mass spectrometry (EI-MS) was performed at 70 eV and at a source temperature of 200°C.

Method B was the preparation and analysis of alditol acetates. An additional aliquot of the initial sample was used for preparing an alditol acetate derivative. The sample was hydrolyzed with 2M trifluoroacetic acid (TFA) at 121°C for 2 hours, dried and reduced with sodium borodeuteride at room temperature for 1 hour. This mixture was acetylated using acetic anhydride in pyridine at 121°C for 20 minutes and analyzed by a Hewlett-Packard 5985 GC-MS system using an SP-2330 column (30 m x 0.25 mm i.d., Supelco, Inc.). The initial oven temperature of 80°C was maintained for 2 minutes, then increased at 30°C min^{-1} to 220°C, and then at 6°C min^{-1} to 240°C. Ionization for EI-MS was performed as described in Method A. Peaks were quantified by injecting either 20 or 50 µg of inositol as an internal standard. Results were provided by CCRC as mol percent total carbohydrates, and the proportion of each soluble, neutral sugar and acid present, relative to the total carbohydrates. Values for soluble sugars and acids were normalized to represent their relative concentration for the dry mass of each sample. Galacturonic acid cannot be determined by Method B.

Statistical Methods

The chemical data were analyzed using the randomized block, two-way factorial analysis of variance (ANOVA), general linear model procedure of SAS (SAS Institute Inc., Cary, North Carolina), for each chemical method (A and B). The two age classes were the blocks, while the factors were water stress and fungal inoculation. Least squares means (LSMEANS) was used to obtain t-tests for comparison of means. Values for pentoses were calculated by summing the values for arabinose, rhamnose, and xylose as percent dry mass of each sample. Values for hexoses were calculated by summing the values for fucose, galactose, glucose, and mannose as percent dry mass of each sample. The ratio of pentoses to hexoses was determined by dividing the sum of the values for all pentoses by the sum of the values for all hexoses. All significant differences were based on a < 0.05.

RESULTS

Pond-cypress trees subjected to prolonged water stress throughout this experiment exhibited wilting, abscission of short shoots, and production of fewer and smaller new leaves. Control trees and trees subjected to nonwounding inoculation with the fungal pathogen did not exhibit these conditions. Pretreatment isolations revealed that *B. rhodina* was associated with all of the bark samples and none of the sapwood samples from randomly-selected trees. For post-treatment isolations, *B. rhodina* was associated with all of the bark samples and sapwood samples from both age classes of trees in water stress treatments, but not from the remaining treatments (data not shown), providing additional support that *B. rhodina* penetrates and infects interior woody tissue under conditions of prolonged water stress.

Table 1 provides the relative concentrations and standard errors of nonstructural, soluble sugars, acids, and total carbohydrates present in the pond-cypress branch tips at the conclusion of the experiment. Six neutral, soluble sugars were identified in both age classes of pond-cypress branch tips. Half of the soluble sugars were pentoses (arabinose, rhamnose, and xylose). The remaining soluble sugars were hexoses (galactose, glucose, and mannose). A fourth hexose, fucose, was identified only in branch tip samples from the younger age class of trees. Fucose may have been present in the older trees in concentrations below the level of detection (Parastoo Azadi, CCRC, pers. comm., September 2, 1998). The acidic sugar, galacturonic acid, also was identified in all samples.

Results of the statistical analysis of chemical data are summarized in Table 2. Significant differences between the age classes occurred for rhamnose ($P = 0.01$), xylose ($P = 0.02$), and fucose ($P = 0.001$) using Method A, and for arabinose ($P = 0.011$, rhamnose ($P = 0.01$), and fucose ($P = 0.0001$) using Method B. No significant differences occurred between the age classes for total pentoses, total hexoses, the pentose:hexose ratio, or total carbohydrates for either method.

Branch tips from trees subjected to prolonged water stress had significantly greater concentrations of rhamnose ($P = 0.02$), xylose ($P = 0.02$), galactose ($P = 0.02$), and galacturonic acid ($P = 0.01$) using Method A, and significantly greater concentrations of arabinose ($P = 0.02$) using Method B. For trees subjected to non-wounding fungal inoculation, no significant differences were found in nonstructural carbohydrates using Method A, but concentrations of arabinose were significantly greater ($P = 0.02$) using Method B. No significant interactions were identified using either method of chemical analysis (Table 2).

Results were significantly different for the two methods for all constituents except rhamnose, mannose, and total carbohydrates (Table 2). Concentrations of arabinose ($P = 0.001$), xylose ($P = 0.001$), total pentoses ($P = 0.001$), fucose ($P = 0.001$), and the pentose: hexose ratio ($P = 0.0001$) were significantly greater using Method B. However, concentrations of galactose ($P = 0.01$), glucose ($P = 0.0001$), and total hexoses ($P = 0.003$) were significantly less using Method B.

DISCUSSION

Water stress can occur from abnormal deficits or excesses of water, both of which can be associated with ground water mining and ASR activities. In the former, drawdown of the surficial aquifer results from induced recharge during periods of greatest pumping, but may rebound when pumping is reduced. With ASR, surficial aquifer levels may be increased artificially during injections, then drained during withdrawals. In both cases, the seasonality and magnitude of drawdown and rebound will not be consistent with natural conditions. Stress from excess water also may occur as the relict sinkholes are reactivated by repeated abnormal pulsing, resulting in subsidence in the interior of the wetlands, and subsequent “drowning” of the existing vegetation.

Plant responses to these types of water stress include premature and extended closure of stomata leading to reduced transpiration, premature senescence and shedding of leaves, and all forms of reduced

TABLE 1. Relative Concentrations of Soluble Sugars, Acids, and Carbohydrates in Branch Tips from Two Age Classes of Pond-cypress Trees After a Simulated Annual Cycle of Treatments.1

Solutes	Treatments: Control	Fungus	Water Stress	Fungus+Water Stress
One-Year Old Trees[2] - Method A				
Pentoses (P):				
arabinose	4.6 (3.2)	3.2 (0.4)	3.6 (0.2)	2.0 (0.2)
rhamnose	0.4 (0.0)	0.6 (0.1)	0.7 (0.1)	0.4 (0.1)
xylose	2.9 (0.1)	5.1 (1.3)	5.2 (0.2)	3.3 (0.2)
Total P	7.9 (3.2)	8.9 (1.8)	9.5 (0.0)	5.7 (0.5)
Hexoses (H):				
fucose	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.0)
galactose	2.3 (0.2)	5.0 (3.4)	5.9 (1.4)	3.6 (0.5)
glucose	7.3 (2.1)	10.0 (1.4)	6.3 (1.0)	4.5 (0.2)
mannose	1.8 (0.2)	3.2 (1.1)	3.3 (0.1)	1.9 (0.1)
Total H	11.5 (2.2)	16.1 (3.6)	15.7 (0.6)	10.2 (0.9)
galact. acid	1.6 (0.4)	2.1 (0.5)	2.4 (0.7)	2.7 (0.3)
Totalcarbo.	21.0 (5.6)	30.4 (0.0)	27.6 (1.4)	18.7 (1.7)
One-Year Old Trees[2] - Method B				
Pentoses:				
arabinose	4.1 (0.8)	5.8 (0.2)	6.5 (0.2)	4.0 (0.0)
rhamnose	1.5 (0.2)	2.3 (0.5)	2.4 (0.1)	2.7 (0.8)
xylose	5.9 (0.9)	9.8 (0.4)	8.5 (1.7)	5.6 (0.2)
Total P	11.5 (1.9)	17.9 (1.1)	17.3 (1.6)	10.3 (2.6)
Hexoses:				
fucose	0.5 (0.1)	0.7 (0.1)	0.8 (0.0)	0.5 (0.0)
galactose	2.1 (0.3)	2.2 (0.3)	2.7 (0.1)	2.1 (0.5)
glucose	4.2 (2.3)	5.1 (0.8)	3.1 (0.7)	2.9 (0.7)
mannose	2.8 (1.0)	4.5 (0.1)	3.5 (0.5)	2.6 (0.7)
Total H	9.5 (3.7)	12.5 (1.1)	10.2 (0.3)	8.0 (1.7)
Totalcarbo.	30.3 (3.9)	25.5 (3.7)	17.1 (0.9)	27.4 (8.8)
Two-Year Old Trees[3] - Method A				
Pentoses (P):				
arabinose	2.2 (0.2)	2.8 (0.7)	1.2 (0.4)	7.1 (0.9)
rhamnose	0.5 (0.0)	0.6 (0.2)	2.7 (0.5)	1.3 (0.2)
xylose	2.2 (0.8)	3.9 (0.1)	5.8 (1.6)	7.3 (0.5)
Total P	4.9 (0.7)	7.3 (0.9)	9.7 (2.6)	15.6 (1.4)
Hexoses (H):				
galactose	3.6 (0.6)	3.9 (0.1)	7.7 (1.7)	8.7 (1.1)
glucose	6.2 (0.8)	5.6 (0.7)	4.5 (0.8)	6.4 (1.5)
mannose	2.4 (0.4)	2.1 (0.8)	2.8 (0.0)	3.7 (0.5)
Total H	12.3 (1.7)	11.5 (0.2)	15.0 (1.0)	18.8 (2.6)
galact. acid	1.3 (0.9)	2.6 (0.5)	3.3 (0.5)	4.7 (1.0)
Totalcarbo.	18.5 (1.5)	21.0 (2.0)	28.0 (4.0)	39.3 (1.5)
Two-Year Old Trees[3] - Method B				
Pentoses:				
arabinose	5.9 (1.1)	8.3 (0.4)	8.0 (0.8)	15.7 (1.7)
rhamnose	0.3 (0.1)	0.5 (0.0)	1.1 (0.1)	1.0 (0.2)
xylose	4.6 (0.4)	5.1 (0.0)	5.8 (0.8)	11.8 (0.5)
Total P	10.6 (1.5)	13.7 (0.7)	14.9 (1.7)	28.5 (1.2)
Hexoses:				
galactose	2.6 (0.6)	2.6 (0.8)	4.7 (0.8)	4.2 (0.6)
glucose	3.3 (0.0)	2.6 (0.0)	3.1 (0.5)	3.6 (0.3)
mannose	2.0 (04)	2.1 (0.6)	5.3 (0.9)	4.0 (0.4)
Total H	7.8 (0.1)	7.3 (1.4)	13.1 (2.3)	11.7 (1.3)
Totalcarbo.	17.4 (1.0)	20.4 (0.8)	32.6 (5.8)	38.9 (2.9)

[1]Mean dry mass subsampled from pooled branch tips, with one standard error in parentheses.
[2]Age at beginning of experiment, mean = two pooled samples of three trees each.
[3]Age at beginning of experiment, mean = three pooled samples of one tree each.

TABLE 2. Summary of ANOVA Results from Two Methods of Chemical Analysis for Relative Concentrations of Soluble Sugars, Acids, and Carbohydrates in Pond-Cypress Branch Tips from Two Ages of Trees Subjected to Prolonged Water Stress and Fungal Inoculation.

Solutes	Year	Water Stress	Fungal Inoculation	Water + Fungal Inoculation
Method A[1]				
Pentoses:				
arabinose	ns	ns	ns	ns
rhamnose	0.01	0.02	ns	ns
xylose	0.02	0.02	ns	ns
Total P	ns	ns	ns	ns
Hexoses:				
fucose	0.001	ns	ns	ns
galactose	ns	0.02	ns	ns
glucose	ns	ns	ns	ns
mannose	ns	ns	ns	ns
Total H	ns	ns	ns	ns
P/H ratio	ns	ns	ns	ns
galacturonic acid	ns	0.01	ns	ns
Total carbohydrates	ns	ns	ns	ns
Method B[2]				
Pentoses:				
arabinose	0.01	0.02	0.02	ns
rhamnose	0.04	ns	ns	ns
xylose	ns	ns	ns	ns
Total P	ns	ns	ns	ns
Hexoses:				
fucose	0.0001	ns	ns	ns
galactose	ns	ns	ns	ns
glucose	ns	ns	ns	ns
mannose	ns	ns	ns	ns
Total H	ns	ns	ns	ns
P/H ratio	ns	ns	ns	ns
galacturonic acid	NA	NA	NA	NA
Total carbohydrates	ns	ns	ns	ns

Method A vs. Method B

Solutes	Method	p values
Pentoses:		
arabinose	B>A	0.001
rhamnose	A=B	ns
xylose	B>A	0.002
Total P	B>A	0.001
Hexoses:		
fucose	B>A	0.001
galactose	A > B	0.01
glucose	A > B	0.0001
mannose	A=B	ns
Total H	A > B	0.003
P/H ratio	B > A	0.0001
galacturonic acid	NA	NA
Total carbohydrates	A = B	ns

[1]Trimethylsilyl methylglycoside method.
[2]Alditol acetate method.

growth (e.g., reduced leaf expansion and leaf area). Reduced viability of seeds subjected to water stress have been reported, and seeds of cypress are killed by desiccation (Kozlowski et al., 1991). Pond-cypress in areas of the SCP where excessive ground water withdrawals are occurring or have occurred exhibit reduced leaf area, reduced viability of pollen, and premature senescence and shedding of leaves, in addition to other symptoms attributed to water stress (Bacchus, unpublished data). Similar vegetative responses were observed in trees assigned to water stress treatments in this study.

Water stress generally increases susceptibility of plants to attack by insects and pathogenic fungi. Bidwell (1974) summarizes resistance of plants to microbial infection and insect attack. Outbreaks of leaf-eating insects can be promoted by water stress due to chemical changes that improve the nutritional quality of the leaves (Mattson and Haack, 1987). Evidence suggests that under extreme stress, conditions probably are unfavorable for both insects and hosts, and extremely weak hosts are avoided (Amman *et al.,* 1988; Scriber, 1977). For example, leaf attacking beetles appear to favor pond-cypress trees subjected to moderate water stress. This conclusion is based on the extent and severity of beetle damage in areas of Florida where anthropogenic ground water perturbations have occurred (Bacchus, unpublished data). These activities can alter wetland hydroperiods significantly (Bacchus, 1998).

In general, controlled experiments evaluating biochemical responses of plants to prolonged water stress and fungal infection are lacking. Most water-stress research evaluating carbohydrate response has involved herbaceous species, with leaf tissue as the primary focus (Barta, 1988; de Ruiter *et al.,* 1992; Miller *et al.,* 1989; Pressman *et al.,* 1989; Richardson *et al.,* 1992). Responses in leaves are of limited use for deciduous trees such as pond-cypress, which respond to water stress by dropping their leaves. No previous research has evaluated biochemical responses of pond-cypress to prolonged water stress and non-wounding fungal inoculation, and studies that have evaluated the impacts of water stress on conifers have involved evergreen species (Koppenaal *et al.,* 1991; Zwiazek, 1991). The knowledge that chemical changes associated with water stress in other plant species can facilitate microbial infection and insect attack was a key factor in the selection of nonstructural soluble sugars in this study to evaluate as an indicator of decline in pond-cypress from prolonged water stress.

Invasion by certain fungi appear to be favored by water stress (Appel and Stipes, 1986; Bier, 1959; Brown and Britton, 1986; Chapela and Boddy, 1988; Crist and Schoeneweiss, 1975; Parker, 1961; Schoeneweiss, 1978a,b). Reduced water transport to shoots caused by vascular disease and polysaccharide slimes produced by certain bacteria are more evident when plants are subjected to water stress (Bidwell, 1974). *Fusarium* species, vascular wilt fungi, were isolated from sapwood tissue of mature pond-cypress trees associated with areas of hydroperiod perturbations in central Florida but did not appear in sapwood cultures from trees in areas without apparent ground water perturbations (Bacchus, 1998).

Complexes of *Fusarium* species, including the same species found in central Florida pond-cypress with decayed roots and bases of trunks, were isolated from north-central U.S. nursery-grown eastern white pine seedlings with root rot (Ocamb and Juzwik, 1995). Penetration and infection of the pine roots by this fungi are attributed, in part, to stress associated with nonoptimal nursery irrigation (Cynthia M. Ocamb, USDA Forest Service, oral comm. May 6, 1996).

Susceptibility of trees to attack by nonaggressive fungal species has been attributed to ground water withdrawals in west-central Florida (Kenneth Webber, Florida Division of Forestry, written and oral comm., January 5, 1995, and September 11, 1995, respectively). Similar responses in peach trees grown commercially in Georgia were attributed to water stress (Brown and Britton, 1986). Cessation of irrigation following peach harvests may be an important factor in allowing *B. rhodina* and similar opportunistic fungi to penetrate to interior wood of the peach trees. This fungal species, in addition to *Fusarium* species, later were isolated from sapwood tissue of mature pond-cypress trees associated with areas of hydroperiod perturbations in central Florida (Bacchus, unpublished data).

Ouf *et al.* (1991) reported concentrations of galactose and xylose in viscous substances produced by *Erwinia caratouora.* This bacterium was found to cause reversible wilting of tomato cuttings, possibly by plugging conducting vessels of the host. Symptoms of wilt were evident in pond-cypress and other trees in areas of prolonged ground water withdrawals and in water stress treatments in this experiment. This bacterium could contribute to greater concentrations of galactose and xylose in host tissue, as was observed in water stress treatments at the conclusion of this experiment. Bacteria have been cultured from sapwood of pond-cypress in areas of excessive ground water withdrawals (Bacchus, unpublished data), but were not identified in this experiment.

Our results using two wet chemical analysis methods showed that seven neutral, soluble sugars (pentoses: arabinose, rhamnose, xylose; and hexoses: fucose, galactose, glucose, mannose) occurred in branch tips of pond-cypress grown in a chamber that simulated an annual cycle of temperature, humidity, and day length for central Florida. Although Method

B is used as a complementary method of analysis, to confirm values for soluble sugars determined by Method A, Method A reportedly provides a better representation of all soluble carbohydrates present in a sample because it measures both neutral (e.g., pentoses and hexoses) and acidic sugars (galacturonic acid and glucouronic acid). Method B detects only neutral sugars, not uronic acid residues. However, Method B may solubilize neutral sugars more thoroughly than Method A (Parastoo Azadi, CCRC, written comm., October 12, 1998).

The assumption that Method B provides greater solubilization of sugars was consistent with our results, with the exception of galactose and glucose, which were greater using Method A, and rhamnose and mannose, which were equivalent for both methods. The greater concentrations of galactose and glucose using Method A contributed to the significantly greater concentrations of total hexoses when Method A was used.

Significant differences in concentrations for individual sugars in the two, similar age classes of pond-cypress could be an artifact of sample storage time. Samples from the older trees were analyzed at the termination of the experiment, whereas samples from the younger trees were stored more than a year, until additional funds for chemical analysis were available. However, despite the time lag in chemical analysis of the two sample sets, both were analyzed by the same chemist using the same equipment. Other factors that might account for the differences are the small concentrations of some sugars and small sample sizes (due to limited space in the growth chamber). Finally, the differences could be due to physiological changes that occur in pond-cypress trees after their initial period of growth and development. This is thought to be the case for fucose, since concentrations were greater in the younger age class.

The lack of significant differences between age classes when values for individual sugars were summed by type (pentoses and hexoses), and for the ratio of these two types, suggests that analysis of soluble sugars by these groups may be a more versatile and reliable means of evaluating changes in pond-cypress branch tips than using individual sugars.

Results of this experiment suggest that prolonged water stress is correlated with greater relative concentrations of nonstructural, soluble sugars rhamnose, xylose (pentoses), and galactose (a hexose), in addition to the acidic sugar galacturonic acid, if Method A is used for analysis, and arabinose (a pentose), if Method B is used for analysis. Inoculation with the fungal pathogen B. *rhodina* did not result in significant differences in relative concentrations of nonstructural, soluble carbohydrates, based on Method A analysis. However, changes in concentrations of carbohydrates as a result of water stress may have permitted colonization of the sapwood by the fungal pathogen.

Pond-cypress have survived countless natural droughts since the Pleistocene epoch. Additionally, field observations of pond-cypress that are associated with ground water mining areas and those that are not have confirmed that pond-cypress trees in depressional wetlands respond differently to anthropogenic perturbations of ground water than to natural periods of drought in the absence of such perturbations (Bacchus, unpublished data; Southwest Florida Water Management District, 1996). Pond-cypress in depressional wetlands appear to be integrators of anthropogenic ground water perturbations, and the soluble nonstructural sugars identified in this study may provide hydroecological indicators of perturbations such as unsustainable yield from the regional aquifer and ASR activities in the SCP. Additional research is needed to (1) evaluate responses of mature pond-cypress in *situ* and (2) compare how responses vary seasonally and with stress periods of different durations and magnitudes.

CONCLUSIONS

Currently, no precise or accurate means is available for early detection of adverse impacts to the matrix of the regional karst aquifer, the surficial aquifer, and associated organisms due to anthropogenic ground water perturbations in the SCP such as ground water mining and ASR. Results of this experiment suggest that greater concentrations of nonstructural, soluble sugars in pond-cypress branch tips are correlated with prolonged water stress. Pond-cypress are dominant species in most forested depressional wetlands of the SCP. These depressional features are relict sinkholes, with more direct connections with the underlying regional aquifer. Pond-cypress appear to be one of the most sensitive species to anthropogenic ground water perturbations, functioning as integrators of these perturbations. Therefore, monitoring nonstructural, soluble sugars in pond-cypress branch tips may provide a precise and accurate means for early detection of adverse impacts to the matrix of the regional karst aquifer, the surficial aquifer, and associated organisms due to anthropogenic ground water perturbations in the SCP. Future research should include analysis of branch tips from mature pond-cypress trees *in situ* at areas of ground water mining and ASR activities, in addition to areas without significant ground water perturbations. Seasonal variations, in addition to variations in magnitude and duration of water stress also should be evaluated.

ACKNOWLEDGMENTS

Rod Will and Paula Spaine provided comments on the initial experimental design. David Drylie of Green Images in Christmas, Florida donated the pond-cypress trees. Kathy Cantwell, Katherine Nelson, and B. P. Nelson transported the trees to Georgia. Ronald Roncadori provided guidance regarding fungal pathogens and access to laboratory, supplies, and his assistant, Nancy Harrison. Robert Teskey, Rod Will, and Jay Brown, Warnell School of Forest Resources, UGA, provided the growth chamber; technical assistance with chamber operations; and containers, soil components, and technical assistance with soil preparation, respectively. S. Liu and Rod Will maintained watering and rotation regimes during brief periods when the senior author was conducting research in Florida. Critical reviews of the manuscript were provided by Jeffrey Dean, David Himmelsbach, Robert Windham, and three anonymous reviewers. Stephen Rathbun contributed comments on the statistical aspects of analysis. Russell Carlson and Parastoo Azadi, Complex Carbohydrate Research Center, provided technical assistance and analyzed the samples. The study was supported in part by grants from the U.S. Department of Agriculture, Forest Service (Contract No. 12-11-008-876); the Florida Department of Environmental Regulation; and the Eugene and William Odum Foundation; in addition to contributions by the U.S. Department of Energy-Funded (DE-FG09-93ER-20097) Center for Plant and Microbial Complex Carbohydrates. We are grateful to the people and organizations listed above for contributions to this project. The reference of specific products and companies does not constitute a promotion of these products and companies.

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