

Near-infrared spectroscopy of a hydroecological indicator: new tool for determining sustainable yield for Floridan aquifer system

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Abstract:

Pond-cypress (*Taxodium ascendens* Brong.) is a dominant canopy species in depressional wetlands of the south-eastern Coastal Plain. Unsustainable withdrawals from the karst Floridan aquifer system have caused premature decline and death of pond-cypress trees, presumably owing to altered hydroperiods (which alter the flow of water and nutrients in trees). There has been no scientifically based means to determine sustainable yield from this regional aquifer system or to detect early stages of physical/ecological damage associated with groundwater mining and aquifer storage and recovery (ASR, which also can alter natural hydroperiods). In this study, the relationship between visual symptoms (indicators) of stress or premature decline, and spectral reflectance was evaluated using dried, milled branch tips collected from natural stands of mature pond-cypress. Depressional systems evaluated represented four of the six aquifer system subregions where subsurface perturbations from groundwater mining: (i) were presumed not to be occurring (reference wetlands); (ii) may be occurring but are not documented; and (iii) have been confirmed. Sampled trees were assigned to one of three stress classes (1, no/minimal; 2, moderate; 3, severe) based on the visual indicators. Partial least squares–linear discriminant analysis of second derivative spectral transformations in the visible/shortwave near-infrared (NIR) region (400–1100 nm) and the NIR region (1100–2500 nm) was used to evaluate the samples in assigned classes.

Class 1 samples were discriminated from combined class 2 and 3 samples in the NIR region with 100% and 97% accuracy for consecutive winter sample periods (before bud-break). The percentage of correctly classified samples in this spectral region was lower (85%) for summer samples (full leaf-out). Second-derivative models for the NIR region developed from the winter data sets predicted assigned classes for alternate winter's samples with an accuracy of 97% and 100%. High correlation between spectral reflectance of dried, milled branch tips collected from mature pond-cypress in winter and visual indicators of premature decline suggests *in situ* pond-cypress are hydroecological indicators of anthropogenic subsurface hydroperiod perturbations. This approach provides objective means for early detection of unsustainable aquifer yield and adverse impacts from ASR activities in the south-eastern Coastal Plain. Used in conjunction with hydrological monitoring and modelling, the hydroecological indicators should provide the means with which sustainable yield in the south-eastern Coastal Plain can be achieved and maintained. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS aquifer storage and recovery; depressional wetlands; groundwater mining impacts; hydroecological indicator; near-infrared reflectance spectroscopy; premature tree decline; water resources protection

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INTRODUCTION

Background

In 1994, the Florida House Committee on Natural Resources issued a report stating that destruction of approximately 6880 ha (17 000 acres) of wetlands had occurred in a single county in west-central Florida owing to unsustainable groundwater withdrawals. At the time the report was released, approximately \$4 million had been spent to repair damage to private wells from groundwater mining in that area (House Committee on Natural Resources, 1994). Groundwater mining, like structural mining (e.g. phosphate rock, lime rock, sand, coal), results in the removal and export of a natural resource. The export of mined groundwater may occur via: (i) piping or trucking (e.g. bottled water) to other watersheds, subregions, regions, states or countries for potable, industrial, or agricultural use; (ii) discharge on to impervious surfaces (e.g. roads, parking lots) then flowing as stormwater runoff into surface waters (e.g. exported to the sea or as evaporation); and (iii) injected into other aquifers or areas (e.g. treated sewage effluent). The costs of other damage related to these unsustainable groundwater withdrawals remain unassessed, but are considerable.

Sustainable yield of an aquifer is not confined to the mere volume of water that can be extracted, but involves all of the physical, chemical and ecological aspects of that aquifer yield. Physical components include not only maintaining aquifer levels, but also maintaining the structural integrity of the aquifer, its natural recharge capabilities and the plants and animals that rely on aquifer discharges (Brown *et al.*, 1999; Klijn and Witte, 1999; Meinzer, 1927; Rosenberry *et al.*, 2000; Sharp, 1988; Winter, 2000). Those factors can be critical in sustaining the chemical qualities of the aquifer water. Finally, in Florida and the remaining south-eastern Coastal Plain, various ecosystems also are linked inextricably to the regional aquifer system.

Categories of damage associated with unsustainable aquifer yield (groundwater mining) in Florida and the remaining south-eastern Coastal Plain, for which damages have not been assessed, can include any combination, or all of the following: (i) irreversible damage to the aquifer matrix and concomitant increased potential for groundwater contamination; (ii) large-scale wildfires with subsequent degradation of air quality and debilitation of transportation corridors, and destruction of forest resources, organic soils (releasing bound mercury), wildlife habitat and property; and (iii) destruction of 'protected' natural areas. A subsequent legal case, in which scientific evidence was submitted (Quattlebaum, 1997), and a report issued by the Southwest Florida Water Management District (1996) both concluded that the damage (including premature decline and death of pond-cypress (*Taxodium ascendens* Brong.) trees in west-central Florida) was the result of unsustainable groundwater withdrawals from the Floridan aquifer system in that subregion, rather than from natural causes such as drought. Bacchus (1999, Figure 1) provides photographic examples of damage that has occurred on public and private property as a result of groundwater mining in west-central Florida.

Wildfires historically were common and remain crucial for the maintenance of some types of south-eastern Coastal Plain forests. Although the destruction of natural stands of wetland and upland trees and pine plantations by catastrophic wildfires in west-central Florida has been attributed to extensive groundwater mining (Southwest Florida Water Management District, 1996; Quattlebaum, 1997), the connection between groundwater mining from the regional Floridan aquifer system and catastrophic destructive wildfires in the remainder of the state has not been made. For example, catastrophic wildfires have destroyed extensive areas of pond-cypress, other species of wetland trees, and pines in State Forests and on private property in east-central Florida in the vicinity of four municipal well fields, with no post-burn recovery of the trees. Rapid recovery of pond-cypress trees has been observed following similar wildfires in wetlands in other areas of Florida not associated with extensive groundwater mining (see Bacchus, 1999, figure 2 for examples of both scenarios). Those findings and observations suggest that groundwater mining is a critical factor in the continued maintenance of south-eastern Coastal Plain forest stands where the present-day approach of prescribed burns is applied. A more detailed discussion regarding groundwater mining impacts in the south-eastern Coastal Plain is provided by Bacchus (2000).

Many of the Florida wetlands referenced above are, or were, dominated by pond-cypress, a deciduous conifer native to the south-eastern Coastal Plain. Historically, natural stands of cypress characteristically

did not exhibit symptoms of stress, and generally were disease-free (Donald Marx, Forest Pathologist, USDA Forest Service, personal communication, 1992). Premature death and decline of pond-cypress have increased in magnitude and extent in west-central Florida since the 1994 report referenced above. Approximately 20 years may elapse, however, between the initiation of wellfield withdrawals and subsequent premature death of the trees (Rochow and Rhinesmith, 1991; Bacchus, unpublished data). Bacchus (1996) classified stress responses in trees exposed to anthropogenic perturbations of hydroperiod (e.g. water levels, duration, seasonality) into two general categories. Short-term responses (type I; e.g. leaf drop) generally are reversible, whereas long-term responses (type II; e.g. fungal decay) generally are irreversible. Efforts for early detection and documentation of such damage to the trees in west-central Florida by agencies regulating the water resources have been unsuccessful (Rochow, 1994). The lack of success is because initial stress responses are too subtle to detect with the monitoring approaches used by water regulatory agencies for field evaluation. Furthermore, methods commonly used to monitor plant communities are not designed to detect stress responses. Water stress and subsequent premature decline can occur from hydroperiod alterations that result in abnormal deficits or excesses of water, or altered timing of water level fluctuations.

Bacchus *et al.* (submitted) provide a summary of plant responses to water stress. A more extensive discussion of various types of premature decline and dieback in trees is presented by Manion and Lachance (1992). Houston (1992) emphasizes the role of environmental stresses, such as unusually severe or protracted periods of water shortage or temperature increase; periods of extreme winter cold (sometimes followed by warmth); early or late frosts; and outbreaks of defoliating or sucking insects (singly, or in concert) that precede the onset of symptoms for diseases in trees. Buds, twigs, stems, or roots of the stressed trees then are killed by one or more secondary pathogenic organisms. He further summarizes the following important relationships: (i) dieback often results from the effects of the stress factor(s) alone; (ii) stress alone, if sufficiently severe, prolonged, or repeated, can cause continued or repeated dieback and death; (iii) usually the decline phase is the consequence of organism invasion of stress-altered tissues; and (iv) where and when the dieback phase occurs is closely related to the location and timing of the triggering stress(es). Stresses such as mechanical wounding and soil compaction (associated with premature decline of urban and silvicultural stands) are not addressed in this paper because they are not factors influencing these natural pond-cypress wetlands. Likewise, stresses such as air pollution and acid rain are not addressed because the pattern of damage is not consistent with air-dispersed contaminants.

Hydrogeology

The Floridan aquifer system is a regional karst (carbonate) groundwater resource that extends throughout Florida, into the lower portion of the south-eastern Coastal Plain in Georgia, South Carolina and Alabama (Johnston and Miller, 1988). Pond-cypress wetlands are underlain by this regional karst aquifer. Throughout its extent, the Floridan is characterized by horizontal and vertical solution cavities and fractures which breach the so-called 'confining' units (Brook and Allison, 1986; Watson *et al.*, 1990; McConnell and Hacke, 1993; Spechler and Phelps, 1997). These features are common in karst aquifers throughout the world (Ford and Williams, 1989). Pumpage from the Floridan is supplied primarily by the diversion of natural outflow from the aquifer system and by induced recharge rather than from storage, and may hasten the development of sinkholes (Bush and Johnston, 1987).

Aquifer storage and recovery (ASR) is being promoted as a relatively new water supply concept in the USA (Pyne, 1989), and has been proposed as one means for reducing impacts of excessive withdrawals from groundwater resources but the ecological impacts have been ignored. Garcia-Bengochea and Muniz (1989) define ASR as storage of excess waters through wells into confined aquifers for recovery during water shortages. The authors' focus, however, was in south Florida, in a regional karst aquifer system that is semiconfined, or 'leaky'. In 1989, seven ASR systems were reported to be operational in the USA, four of which were using the limestone matrix of the Floridan aquifer system in Florida (Pyne, 1989). The rate of fracture enlargement/extension and dissolution/sinkhole development should be expected to increase in

conjunction with ASR activities. This would be true particularly for the former, if the foreign ion effect was a factor (Ford and Williams, 1989), as might be expected when vertical fractures are linked to strata of saline groundwater, or when fresh water is injected into aquifers bearing brackish or saline water, as is the case at some ASR sites. The increased recovery of injected water, reported in subsequent years of operation from some ASR wells, in addition to associated adverse environmental impacts (Bacchus, unpublished data) provides support for the presumption that ASR activities increase interconnection between aquifer layers, to the detriment of the aquifer system and associated ecosystems.

Depressional wetlands in the south-eastern Coastal Plain are associated with fracture traces, solution/collapse features and various degrees of breached semiconfining units (Brook and Allison, 1986; Watson *et al.*, 1990; Bacchus and Brook, 1996). Preferential flow is known to occur within the fractures in response to pumping (Brook, 1985; Spechler and Phelps, 1997). Similar responses would be predicted where solution/collapse features and breached semiconfining units co-occur with depressional wetlands.

Pyne (1989) lists several potential advantages of ASR, but none of the potential disadvantages. In karst aquifers such as the Floridan aquifer system, rapid introduction of recharge, and subsequent withdrawals could initiate or exacerbate both structural (e.g. aquifer matrix collapse, suffosion) and geochemical problems (e.g. contamination of potable ground water via continual fluctuations in the heads of interrelated aquifers). Concomitant problems in depressional wetlands associated with ASR could induce more pronounced anthropogenic perturbations of natural hydroperiods than those expected from groundwater withdrawals alone, even in the absence of increased subsurface subsidence and detrimental changes in water chemistry. Hydroperiod perturbations from ASR may result in adverse synergistic responses in associated organisms or wetland vegetation from water stress owing to alternating periods of too little and too much water, in conjunction with localized drawdowns and rebounds of the surficial aquifer. For example, chronic water stress increased the susceptibility of young pond-cypress to invasion by the facultative (opportunistic) fungal parasite *Botryosphaeria rhodina* (Cooke) Aux. in a controlled study (Bacchus *et al.*, 2000).

Current approaches

The primary approach to determining sustainable yield from the Floridan aquifer system has been monitoring groundwater levels and incorporating the data into hydrological models. Numerous problems are associated with this approach. First, estimations of hydraulic conductivity can vary approximately six orders of magnitude from the laboratory scale (pore/microfissures = 10^{-7}), through the borehole scale (macrofissures = 10^{-5}), to the regional scale (karstic networks = 10^{-2}), with a plus or minus two orders of magnitude error factor at each scale (Ford and Williams, 1989). Second, long-term, pre-pumping (baseline) hydrological data generally are unavailable, particularly for ecosystems that appear to respond most rapidly and severely to groundwater perturbations (e.g. pond-cypress wetlands). Consequently, the natural hydroperiod of ecosystems such as depressional wetlands, lakes and streams within the cone of influence of groundwater mining areas are not documented and cannot be determined after pumping is initiated. Third, observation wells generally are located in uplands, with the presumption that aquifer responses in those areas represent aquifer responses in other areas, such as depressional wetlands. This presumption is not supported in karst aquifers such as the Floridan aquifer system (see Ford and Williams (1989) for a discussion regarding order-of-magnitude increases in vertical hydraulic conductivity associated with dissolution features). Furthermore, the actual lateral extent of groundwater perturbations is difficult to determine because of the many subsurface pathways available for groundwater flow in karst systems and the dynamic nature of karst aquifers. An additional problem arises from the lack of well-documented pre-pumping ground elevations, and the failure to resurvey elevations routinely after groundwater mining is initiated. Observation wells or staff gauges can provide misleading information if the elevation of those devices is lowered owing to subsidence, without adjustment of the water-level data to reflect the sinking of the well or staff gauges, or the lowering of the land surface. Finally, data on changes in groundwater levels do not provide information regarding how those changes affect plants and animals whose ecosystems inextricably are linked to groundwater resources.

Current assessments of vegetation changes also are ill-suited for evaluating early stages of stress. Increases in diameter of tree boles often is used to indicate growth, and has been interpreted by water regulatory agencies as a positive change for pond-cypress in well fields. The thick, shaggy bark of pond-cypress trees, however, is not conducive to accurate diameter measurement. Under natural (acidic, oligotrophic) conditions, pond-cypress grow very slowly. Therefore, errors in diameter at breast height (DBH) measurements obtained using standard metal DBH tapes (which do not stretch during measurement, but provide DBH values from the circumference of trees) exceeded standard annual growth increments (Bacchus, unpublished data). Installed dendrometer bands provide more accurate long-term measurements of diameter growth, but also would be susceptible to false inferences of growth as the cambium of the tree dies and the thick bark separates prior to sloughing off. This was the case at the cypress creek wellfield in west-central Florida where pond-cypress trees were monitored using standard DBH tapes. Trees in one of the 'augmented' wetlands were reported to be in good condition based on large increases in diameter measurements. By the next year the bark was falling off as those trees died.

Changes in canopy trees that result from groundwater mining can be subtle and difficult to quantify. In an attempt to avoid the difficulty of detecting change in canopy species, changes in herbaceous species have been monitored. The problem with this approach, as applied at the west-central Florida sites, is that adverse impacts to the wetlands are presumed only if the percentage of 'upland' species increases. A high percentage of 'aquatic' plant species in the wetlands is interpreted as an 'unaffected' condition. Those depressional wetlands, however, commonly are regarded as relict (palaeokarst) sinkholes, which formed during the significant fluctuations of Pleistocene sea-level, then filled with sediment and natural debris (Jackson, 1997). In addition to creating new sinkholes, groundwater mining may enlarge or 'reactivate' relict sinkholes by increasing flow through infilled sediments and debris, and resulting in preferential subsidence from oxidized/compacted organic sediment and suffosion. Consequently, depressional wetlands with high or increasing percentages of 'aquatic' species would represent those experiencing the most severe adverse impacts (from subsidence), owing to deeper and more prolonged standing water during wet seasons and periods of reduced pumping. In those cases, the trees are subjected to water stress from excessive levels or duration of standing water.

Williams (1985) illustrates the progressive increase in vertical hydraulic conductivity from the outer perimeter of these depressions, inward. The significance of increased vertical hydraulic conductivity in depressional wetlands is addressed by Bacchus (2000), and can result in three general 'zones' of response within a single depressional wetland. The interior zone is most susceptible to subsidence, and water stress from anthropogenic increases in hydroperiod during some parts of year. The exterior, perimeter zone is most likely to be affected by water stress from decreases in hydroperiod owing to groundwater mining. The middle zone is the most buffered area of these wetlands. No current monitoring regime accounts for those potential differences in response within individual depressional wetlands.

Physical and chemical changes in forest canopies in areas other than the south-eastern Coastal Plain have been quantified by computer analysis of hemispheric photographs, as summarized by Kull (1998). Similar indirect measurement of canopy architecture, such as leaf area index (LAI) and foliage inclination has been made using LI-CORTM instruments such as the LAI-2000 (Welles and Norman, 1991). For both of those instruments, samples with less light are interpreted as more vigorous or dense canopies, and vice versa. Those upward-looking approaches, however, are not suitable to monitor pond-cypress wetlands in areas of groundwater mining because although the actual canopy of the pond-cypress declines in response to chronic water stress, photographs and light measurements can record pseudo-increases in canopy cover. This is caused by the thick growth of Spanish moss and lichens on canopy branches, which proliferate on branches during the advanced stages of decline (see Bacchus, 1999, figure 3). The canopy also may become obscured by shrub and subcanopy vegetation of opportunistic plant species (including upland species) that become established or increase in density in response to the thinning tree canopy. In severe cases, it becomes virtually impossible to position the camera or LI-CORTM instrument above the encroaching layer to photograph or assess the canopy from below (Bacchus, unpublished data).

Windthrow (leaning and falling of the trees caused by basal decay and root rot) further complicates attempts to monitor canopy conditions via aerial photography and satellite-sensor imagery. Another constraint for the use of satellite-sensor imagery to detect changes in the canopy of depressional wetlands has been the relatively small size of those wetlands. This is demonstrated in the Landsat Thematic Mapper images in figure 2 of Curran *et al.* (1990a). Finally, the differing responses in the zones referenced above, and the increasing influence of conditions below the canopy, as canopy closure decreases with prolonged water stress, results in a problem with interference by groundcover reflection (refer to Curran *et al.*, 1990b).

Recent improvements in spatial-scale resolution of satellite sensors occurred with the successful launching of Ikonos in September 1999, and Quickbird 2 in October 2001 (approximately the time this study was completed). Imagery from those sensors became available commercially in late 1999 and May 2002, respectively. Although those satellite sensors are considered to have a spatial resolution of approximately 1 m (1.1 m and 0.6 m, respectively), in reality, that spatial resolution is for the panchromatic mode only. The panchromatic mode is similar to black and white photography, and does not detect multispectral data, such as reflectance in the infrared (IR) and near-infrared (NIR) regions. Multispectral data are used to evaluate canopy conditions in tree stands, as described above. The spatial resolutions for multispectral data from those satellite sensors are 4 m and 2.4 m, respectively. Considering the small size of many of the depressional wetlands in the south-eastern Coastal Plain, the tendency for the stress responses to vary within concentric zones of those wetlands, and weather-related problems associated with satellite-sensor images, multispectral images even at the spatial scale available from Ikonos and Quickbird may not be suitable for detecting stress-responses in depressional wetlands that are described above.

Low-level aerial photographs provide another approach for downward-looking measurements of tree canopy. For example, low-level aerial photographs (true colour and colour IR) were taken in the spring and autumn for several years to establish baseline conditions in the canopy of a south-eastern USA wetland forest in preparation for monitoring water stress effects of an impoundment on the trees, in a study conducted by the US Army Corps of Engineers. The objective was to develop a readily available and inexpensive method for early detection of decline, on a forest-wide basis. Spectra for NIR, red and green were quantified for plots and dates. The lack of success of this approach was attributed to inconsistent quality of the colour IR photography, seasonal differences in tree phenology, and possibly differences in water levels and other ambient conditions when the photographs were taken (Mary Davis, personal communication, 2000). Rochow (1994) also found low-level aerial photography insufficient for early detection of damage to forested wetlands caused by groundwater mining in Florida.

Visual crown-rating methods for determining vigour and decline, such as those used in Europe and adopted for use in southern USA by Anderson and Belanger (1986), can overcome such instrument-related problems because the human eye can distinguish residual canopy from other light-blocking components in or under the canopy. Wargo (1988) provides a more detailed discussion of the inadequacies of current approaches to estimate general tree vigour. More sensitive, objective approaches are needed to detect early symptoms of stress before damage to the aquifer system, public and private property and the environment becomes severe and irreversible.

Developing a new approach

As a first step to developing a new approach for early detection of stress from prolonged water deficits, Bacchus *et al.* (2000) evaluated differences in composition of non-structural soluble sugars in branch tips from young pond-cypress subjected to controlled water stress conditions in a growth chamber experiment. An annual cycle of temperature, humidity and day length was simulated for the central Florida area where the trees originated. Prolonged water stress was correlated with greater relative concentrations of five sugars (arabinose, galactose, galacturonic acid, rhamnose, xylose) determined by wet-chemical analysis. Additionally, all of the 18 water-stressed trees became infected by the facultative fungal pathogen, *Botryosphaeria rhodina*. Colonization of plants by opportunistic fungi, such as *Botryosphaeria* species, increases with host stress (Crist

and Schoeneweiss, 1975; Schoeneweiss, 1978a,b). The role of decreased bark moisture in promoting infection by facultative fungi has been referenced since the early 1900s (Parker, 1961). Pusey (1989) describes the influence of water stress on susceptibility of non-wounded peach bark to infection by *Botryosphaeria dothidea*.

Subsequently, a NIRSystems™ 6500 monochromator with a spinning cup module was used for collection and analysis of spectral data from the same pond-cypress samples analysed by wet-chemical methods in the growth chamber study (Bacchus *et al.*, in review). Best-fit models developed using modified partial least squares (Mod PLS) models suggested a high correlation between NIR reflectance (1100–2500 nanometers (nm)) and wet-chemical results for the same five sugars identified previously, in addition to glucose, mannose, total pentoses (five-carbon sugars), total hexoses (six-carbon sugars), the pentose:hexose ratio, and total carbohydrates.

The PLS regression models use all of the NIR wavelengths, but with variable weighting. The regression vector plots provide a good indication of the relative importance of various spectral ranges. The ranges of greatest significance are those in the plots that have large positive or negative values. The NIR reflectance spectra, however, have many overlapping peaks and do not have baseline resolution of bands. Therefore, other analyses would be required to identify the most important wavelengths. Overfitting in PLS regression models is minimized by using factor-based regression and cross-validation to determine the number of factors. The correlation relationships would be expected to hold true for specimens that are similar to the ones in the study.

Xylose, a significant sugar in both studies referenced above, is one of the main carbohydrate constituents in wood. Previous research has identified strong correlations between the per cent of xylose, lignin and glucose in birch wood predicted by NIR spectroscopic analysis and the concentrations of those monosaccharides measured using wet-chemical techniques (Wallbäcks *et al.*, 1991). Galactose, arabinose and mannose also were present in those birch wood samples. Wet-chemical analysis of plant samples is time consuming, generates chemical waste products and is expensive (approximately \$250 per sample for commercial analysis). Although spectral analysis requires the same initial milling procedure used in wet-chemical analysis, it eliminates the time required for chemical extractions. Therefore, spectral analysis generally requires less time than wet-chemical analysis, in addition to producing no waste products, and not consuming the sample, so that multiple analyses may be conducted. The cost of data acquisition using spectral analysis is approximately 25% to 50% of the cost of data acquisition using wet-chemical analysis. An additional constraint with wet-chemical analysis is that the amounts are given as percentages of the dry substance, so that if one constituent decreases, that induces a relative increase in the amount of carbohydrates, making interpretation of the chemical significance for the calculated PLS components difficult (Wallbäcks *et al.*, 1991).

Various chemical and physical characteristics (e.g. nitrogen, lignin, moisture, chlorophyll content, leaf area index and biomass) have been correlated with visible and NIR reflectance of expanses of grasses (Tucker, 1979; Gross *et al.*, 1987), numerous non-vascular and vascular plants (summarized by Tucker *et al.*, 1980) and forest canopies (Card *et al.*, 1988; Marten *et al.*, 1989; Wessman *et al.*, 1989; Curran *et al.*, 1990a,b; 1995; McLellan *et al.*, 1991; Moss and Rock, 1991; Egnell and Orlander, 1993; Thorn, 1993; Johnson *et al.*, 1994; Bolster *et al.*, 1996; Martin and Aber, 1990, 1997; Hallett *et al.*, 1998; Aber *et al.*, 2000). The majority of those studies, including recent spectroscopic evaluations of forest stands to provide estimates of forest productivity, focus on analysis of foliage. The Accelerated Canopy Chemistry Program (ACCP) was initiated to determine the theoretical and empirical basis for remote sensing of nitrogen and lignin concentrations in vegetation canopies of different ecosystems. Data sets from that investigation primarily consist of AVIRIS images, laboratory chemical analysis of field samples, laboratory spectra, and chemical analyses from minicanopy experiments and modelling data. Most recent investigations of that nature (refer to Aber *et al.*, 2000) have focused on forest productivity and foliar chemistry at the whole-stand level. Chemical characteristics (including nitrogen content) of pond-cypress foliage varied significantly, both spatially (according to canopy position) and temporally within individual trees (Bacchus, unpublished data). For this and other reasons described previously, pond-cypress foliage was determined to be unsuitable for analysis at both the direct level, and the remotely sensed (whole-stand, canopy) level.

Branch tips provide several potential advantages for monitoring. First, the chemical composition of foliage in natural stands can vary greatly owing to diurnal and seasonal fluxes, foliage age, and canopy position. Second,

branch 'dieback' has been documented at the sites where premature decline and death of pond-cypress are occurring (see examples in Bacchus, 1999, figure 4). This type of premature decline involves the gradual death of the branch tips as the essential nutrients are translocated to the core of the tree during periods of prolonged, chronic stress. As the distal branch increments die and are shed, the process is repeated in the next increment, if the stress continues. Chronic stress has been shown to prevent accumulation of storage carbohydrates in twigs (Waring, 1991), providing additional support for use of branch tips to assess chronic stress. Therefore, branch tips from deciduous trees collected prior to bud-break in the winter could maximize detection of chemical differences resulting from differences in carbohydrate reserves in the branch tips of stressed and unstressed trees. These carbohydrate reserves are channelled into the new growth produced in the spring.

Barton (1989) describes the unique advantages of using the NIR region of the electromagnetic spectrum for sample analysis. The majority of chemical components of a sample have NIR absorption properties that can be used to differentiate one component from another. Spectroscopy has been used to detect changes in wood chemistry, initially in the mid-infrared (MIR) region (Liang *et al.*, 1960; Marchessault, 1962; Harrington *et al.*, 1964; Michell *et al.*, 1965; Michell, 1994) and later in the NIR region (Easty *et al.*, 1990; Wright *et al.*, 1990; Wallbäcks *et al.*, 1991; Michell, 1995). Application generally has been to tree plantations and commercial products, rather than to evaluate stress in natural stands of trees. Therefore, although spectral analysis is not a new method for evaluating the condition of vegetation or wood characteristics, this approach does not appear to have been used to evaluate responses of natural systems to anthropogenic stresses.

The preliminary research on young pond-cypress trees by Bacchus *et al.* (2000; see also Bacchus *et al.*, in review) suggests that spectral analysis of pond-cypress branch tips using the NIRSystems™ 6500 can provide a more scientifically-based and sensitive means of detecting responses to groundwater perturbations than the current approaches. Spectral analysis of pond-cypress from natural stands has not been reported previously. The research described below implements a multidisciplinary approach, incorporates hydrogeological knowledge of aquifer response to groundwater mining in karst systems (e.g. Floridan aquifer system) with knowledge of pathological and physiological responses of trees to stress, and the ability of NIR spectroscopy and chemometrics to detect subtle differences such as changes in chemical composition associated with prolonged stress. The International Chemometrics Society defines chemometrics as 'the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods.'

The first objective of this research was to use pond-cypress trees to develop a simple, rapid, inexpensive, yet scientifically-based, approach for detecting areas where unsustainable aquifer yield from the regional Floridan aquifer system may be occurring, via a classification system based on visual indicators of stress commonly used by forest pathologists. The second purpose was to develop an objective means of validating determinations made by using the simple, inexpensive classification approach. In this manner, pond-cypress could be used as a hydroecological indicator for monitoring and early detection of anthropogenic hydroperiod perturbations in the south-eastern Coastal Plain. Results from this research, used in conjunction with hydrological monitoring and modelling, could provide a scientifically-based mechanism for determining sustainable yield of the Floridan aquifer system, and a means of adjusting groundwater withdrawals before irreversible structural damage to the aquifer and damage to public and private property occurs at new sites of withdrawals, or intensifies at sites of existing withdrawals. Likewise, the results of this study, used in conjunction with hydrological monitoring and modelling, also could provide a mechanism for monitoring ASR sites to prevent similar damage from associated groundwater perturbations.

STUDY AREAS

The United States Geological Survey (USGS) has divided the Floridan aquifer system into six subregions (Krause and Randolph, 1989). The primary criterion for selection of study areas for this research was to represent as many of those subregions as possible. Within each subregion, the primary criteria for selection of wetlands included: (i) no evidence of surface perturbations of hydroperiod, such as draining by ditching, or

flooding by discharge from stormwater ponds; (ii) perceived protection from common modes of destruction, such as timbering and construction; (iii) permission for long-term access; and (iv) the presence of a minimum of three mature, dominant canopy trees in a given stress class that were of similar dimension to selected trees in other wetland sites.

Historic aerial photographs, 7.5 min USGS topographic quadrangle maps, and preliminary ground reconnaissance were used to select 41 depressional pond-cypress systems throughout Florida and the south-eastern Coastal Plain as potential sites for inclusion in this study. Following more detailed ground reconnaissance, 15 pond-cypress wetlands were selected in nine study areas extending from the Okefenokee Swamp in south-central Georgia, west to the St Marks National Wildlife Refuge (SMNWR) in the panhandle of Florida, and south to Big Cypress National Preserve (BCNP) in south Florida. The general location of the study areas is shown in Figure 1. The distribution of those study areas represents four of the six subregions of the Floridan aquifer system, as designated by USGS.

Study area 1 is within USGS Subregion D, in the Okefenokee Swamp in Georgia, and includes the three most northern wetland sites (two within the Okefenokee National Wildlife Refuge (ONWR), in Charlton County, and one adjacent to ONWR, in Ware County). The remaining wetlands are located in Florida. Study area 2 (SMNWR, in Wakulla County (R)) is within the unassigned USGS Subregion in the Florida panhandle, where groundwater data are insufficient for modelling purposes. This site represents the northern reference wetland. Study area 3 is within USGS Subregion F near the eastern boundary (adjacent to Subregion E), in the Withlacoochee State Forest (WSF), and adjacent to the eastern boundary of the Northern Tampa Bay Water Use Conservation Area (WUCA). This site represents the central reference wetland. Study areas 4 through 7 (denoted as 4 in Figure 1) are distributed throughout the central portion of USGS Subregion F. This subregion includes the most extensive data regarding interactions between the Floridan and surficial aquifers, in the Northern Tampa Bay WUCA, where numerous wellfields are extracting water from the Floridan aquifer system. Study area 4 includes two sites in the privately owned Barthle Ranch (B-1, B-2), in

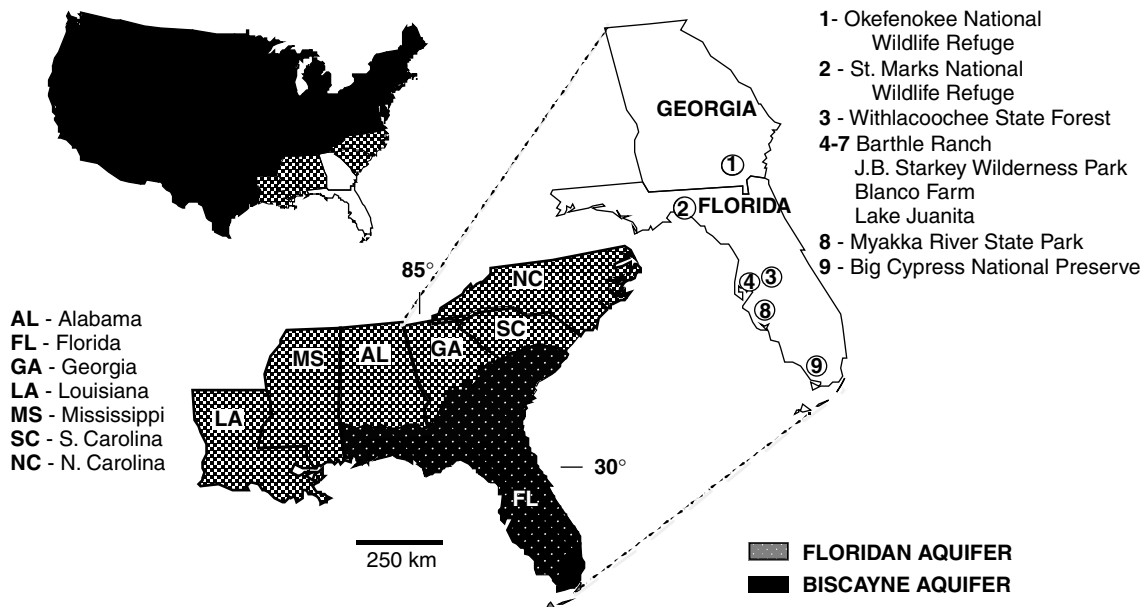


Figure 1. The seven south-eastern Coastal Plain States containing depressional pond-cypress wetlands (LA, MS, AL, GA, SC, NC and FL); the approximate extent of the Floridan aquifer system in Florida, Georgia, South Carolina and Alabama (modified from Miller, 1986); and the general location of the nine study areas: (1) Okefenokee National Wildlife Refuge; (2) St Marks National Wildlife Refuge; (3) Withlacoochee State Forest; (4–7) Barthle Ranch; J. B. Starkey Wilderness Park, Blanco Farm, Lake Juanita; (8) Myakka River State Park; (9) Big Cypress National Preserve

Pasco County. Study Area 5 includes two sites in the publicly owned J. B. Starkey Wellfield and Wilderness Park (SWP), in Pasco County (S-1, S-2). Study area 6 includes one site in the privately owned Blanco Farm (I), in Pasco County. Study area 7 includes one site in privately owned Lake Juanita (J), in Hillsborough County. Study area 8 (Myakka River State Park (MRSP), in Sarasota County (M)) is in the southern portion of USGS Subregion F (near the boundary with Subregion G), and within the Southern Water Use Caution Area (SWUCA) where groundwater mining occurs for agricultural and municipal use. Study area 9 is within the south-western portion of USGS Subregion G, in BCNP, Collier County (P-1, P-3, P-4). This site represents the southern reference wetland.

These depressional systems range in size from approximately 2 to >150 ha (5 to >375 acres), with the majority of the wetlands less than 10 ha (25 acres) in size. More detailed information, including approximate Universal Transverse Mercator (UTM) coordinates for the 15 wetlands selected, and the approximate size of each wetland, is provided in Bacchus (1999, appendix A). Maps with more precise locations are provided by Bacchus *et al.* (1997). Federal permits were obtained to conduct research within the ONWR, the SMNWR and the BCNP. State and regional permits were obtained to collect data and samples from the WSF, SWP and MRSP sites. Permission was granted to conduct the research on the privately owned tracts.

METHODS

Collection and preparation of branch-tip samples

The primary sample period was late winter, prior to bud-break. Winter 1996 samples were collected from all sites within a two-week period between the end of December 1995 and 1 January 1996. Winter 1997 samples were collected from all sites during the last two weeks of December 1996. For comparison purposes, samples also were collected within a two-week period in late summer (August) 1996, after full leaf-out. Sample trees from the selected wetlands were dominant mature canopy pond-cypress of similar dimension. Also for comparison purposes, branch-tip samples were collected from the single mature bald-cypress (*T. distichum* (L.) Richard) tree that was planted at the pre-drawdown edge of Lake Juanita.

The selected trees were assigned to one of the three stress classes, based on visual indicators (symptoms) of premature decline. Trees assigned to class 1 did not exhibit any significant degree of key indicators used in this study. Those indicators included: a proliferation of male cones, Spanish moss or lichens (foliose and fruticose); branch dieback; canopy decline; windthrow; and basal decay. Proliferation of cones should not be interpreted as viable reproductive material. Seeds from proliferating female cones of conifers examined in Europe were found to be inviable (Butin, 1995), and examinations of male cones in this study suggested inviability (Bacchus, unpublished data). A proliferation of cones in conifers commonly is associated with root and butt rot (basal decay) diseases, and is referenced as 'distress cones' by forest pathologists (Foster and Wallis, 1974). Trees assigned to class 2 exhibited a moderate degree of one or more of the symptoms of premature decline, except windthrow or basal decay (which infers both butt rot and root rot for this study). Trees assigned to class 3 exhibited windthrow, basal decay, or a severe degree of one or more of the other key indicator symptoms. Photographic examples of those indicators of premature decline (including trees other than the pond-cypress used in this study) are provided by Bacchus (1999, figures 1a, 1b, 2b, 2c, 3c, 4 and 6).

The tip was removed from three branches in the lower third of the canopy of each tree, and trimmed to the distal 5 cm sample. Winter 1996 samples were refrigerated until processed. Summer 1996 and winter 1997 samples were frozen after collection, until processed. The samples were oven-dried at 45 °C for 48 h. The three branch tips from each tree were pooled, using a Wiley mill with a 20 mesh screen. Some samples from class 2 and class 3 trees, however, could not be milled fine enough to pass through the 20 mesh screen. Because of the small amount of branch tissue available for analysis, all milled tissue from the pooled samples was incorporated into the sample, including fragments that did not pass through the 20 mesh screen. Additional details of the selection and identification of the trees, and collection, storage and preparation of the samples are provided by Bacchus (1999).

Collection and analysis of spectral data

As this research involves analytical spectroscopy, the terminology used follows that discipline rather than the discipline of remote sensing with satellite-sensor imagery. The visible region ranges from 400 to 700 nm, the shortwave NIR region is 700–1100 nm, and the NIR region is 1100–2500 nm. Collection of diffuse reflection spectral data in those regions was summarized by Bacchus *et al.* (in review). The spectral data were transferred to Unscrambler 6.1 (CAMO, Trondheim, Norway) for analysis. Partial least squares–linear discriminant analysis (PLS–LDA) was used to discriminate samples assigned to the three classes. Both first and second derivative Savitzky–Golay cubic equation transformations were evaluated (Savitzky and Golay, 1964; Madden, 1978). After derivative transformation, the region of the spectrum that contained noise (primarily the visible region) was deleted; the remaining spectral data primarily were the shortwave NIR region (approximately 600–1100 nm) and the NIR region (1106–2492 nm), based on the two regions assessed by the silicon and lead sulphide detectors, respectively. Those two regions were normalized independently using multiplicative signal correction (MSC), as described by Isaksson and Naes (1988), with the spectral replicates of each sample averaged following normalization. Two-class PLS–LDA models were developed for each of the two regions for first and second derivative transformations. A more detailed description of the procedural steps for collection and analysis of spectral data is provided by Bacchus (1999, appendix B and C). The two models developed using the winter 1996 and winter 1997 data sets were cross-validated as a test of robustness.

Representative spectra

Representative spectra generated by each step of the evaluation process are provided by Bacchus (1999, figures 7 and 8). The representative spectra included the untransformed $\log(1/R)$ spectra generated by every tenth, unaveraged samples from the winter 1996 data set; the first and second derivative transformations of the same data; and, for comparison purposes, the same treatments of Summer 1996 data. The discontinuity in the spectra that occurs at 1100 nm is the result of analysis of the 400–1100 nm region by a silicon detector and analysis of the 1100–2500 nm region by a lead sulphide detector. Therefore, that segment of the NIR region at the break between the two detectors was deleted before MSC normalization of the data. Slight spectral disturbance occurred in the 1100–1112 nm region after derivative transformation of the spectra. The shortwave NIR (including the 600–700 nm segment of the visible region), and NIR regions remaining after modifications described above, were normalized independently. The representative spectra for those two normalized regions described above for winter and summer 1996 are shown by Bacchus (1999, figure 8). The NIR region includes most of the major analytical overtone and combination vibrations of chemical bonds in organic solids. The shortwave NIR region includes the tails of electronic absorptions from the visible side, as well as some important second and third overtones.

RESULTS AND DISCUSSION

Data analysis and cross validation of the classes

Variable scattering resulting from samples with differing texture can be resolved by use of second derivative transformation of spectral data (Williams and Norris, 1987). Michell (1988, 1989) reported that second derivative transformation of spectral data has facilitated interpretation of wood samples using both MIR (2500–25 000 nm) and NIR spectra, particularly in the latter case, where more overlapping bands are present. Characteristically, the second derivative mode has provided sharper, more resolved bands, with inverted peaks for NIR spectra of actual samples, cellulose and xylan, milled wood lignin, and hot water extractives from wood in Australia (Michell and Schimleck, 1996).

Wallbäcks *et al.* (1991) characterized pulp from birch trees (species unspecified), using three different spectroscopic methods. They concluded that NIR and the multivariate calibration technique, partial least squares (PLS) regression, had the advantage of being able to minimize the problem of overlapping signals

from the three main polymer constituents (cellulose, hemicellulose and lignin), in addition to handling cases in which the number of samples is small compared with the number of variables used for characterization. They found that a three-component model based on NIR data described 99% of the variance in the chemical composition matrix. Bolster *et al.* (1996) also found that the PLS calibration technique produced consistently lower standard error of calibration with both first and second difference equations during their evaluation of nitrogen, lignin and cellulose in foliage from numerous species of non-coniferous deciduous trees and coniferous evergreen trees in the north-eastern USA.

Kemsley (1996) concurred that PLS methods are a valuable alternative to principal components analysis (PCA) for compressing high dimensional data such as NIR, but treated PLS as a data reduction method prior to performing linear discriminant analysis (LDA). He evaluated PCA and PLS scores as variables in LDA, concluding that PLS reductions yield scores that maximize the between-groups variance. Fewer compressed PLS dimensions were required and higher prediction success rates were obtained using PLS scores than PCA scores. The percentage correctly classified provides the means of testing the predictive ability of the model. The importance of PLS is its ability to compress the most relevant, but not necessarily the largest, variations into the first few dimensions. Therefore, the first score is the single best discriminator in PLS, whereas in PCA, this is not generally the case (Kemsley, 1996). Consequently, PLS–LDA was selected for evaluation of the samples in this study.

The composite sample for each tree provides the same precision of estimation as would be obtained by taking the mean of separately analysed branch tips. Therefore, pooling samples from an individual tree reduces processing time, and provides comparable information regarding among-tree variation locally (within wetlands/sites) and regionally (among sites). Variance resulting from sample packing during scanning was accounted for by averaging the two scans per composite sample. Initial PLS–LDA analyses for each sample period included the bald-cypress sample.

The bald-cypress sample was the most extreme class 1 sample in the NIR region for all three sample periods (Figure 2a–c), suggesting that bald-cypress branch tips are chemically different from pond-cypress branch tips. Investigations over a 3 year period prior to the sample periods in this study provided evidence that a reproductive isolation mechanism exists between bald-cypress and pond-cypress, supporting distinction of the two taxa (Bacchus, unpublished data). Godfrey (1988) described similar observations and recognized pond-cypress as a distinct species; however, some taxonomists do not support this distinction. Nomenclature in this paper follows Godfrey (1988). More extensive investigation of the NIR spectra generated from bald-cypress and pond-cypress branch tips may provide additional support for the distinction of these taxa. Other species of trees have been distinguished successfully using IR analysis of wood. For example, Furumoto *et al.* (1999) were able to classify two species of pine (an evergreen conifer) using IR measurements, regardless of the condition of the wood chips. After the initial cross-validation analyses of the data with the bald-cypress sample, the transformed, normalized data were re-evaluated without the bald-cypress samples. Classification results for the NIR region improved after elimination of the bald-cypress samples, improving classification results (Figure 2d–f).

The second derivative spectral transformations in the NIR region using PLS–LDA accurately discriminated all class 1 from the combined class 2 and 3 branch tips for 1996 and 1997 winter samples (Table I, Figure 2d and f). No class 2 or 3 samples were identified as class 1 samples (false +) for the winter 1996 data set. Two false + identifications, however, occurred for the winter 1997 data set (Table I). Consequently, the percentage of samples correctly classified in the NIR region for the winter samples was 100% and 97%, respectively. For summer 1996 samples, second derivative data in the same spectral region produced seven false + and one false – identifications, resulting in only 85% of the samples correctly classified (Table I).

Four and six PLS components, respectively, were used in the models for winter 1996 and winter 1997 (Table I). The number of components generated in the cross-validation analyses of these data sets is within the range specified as acceptable by the American Society for Testing and Materials, under E 1655-94, for validation of a multivariate infrared model. The regression coefficients of the second derivative NIR reflectance spectra for class 1 versus the combined class 2 plus class 3 samples collected in winter 1996,

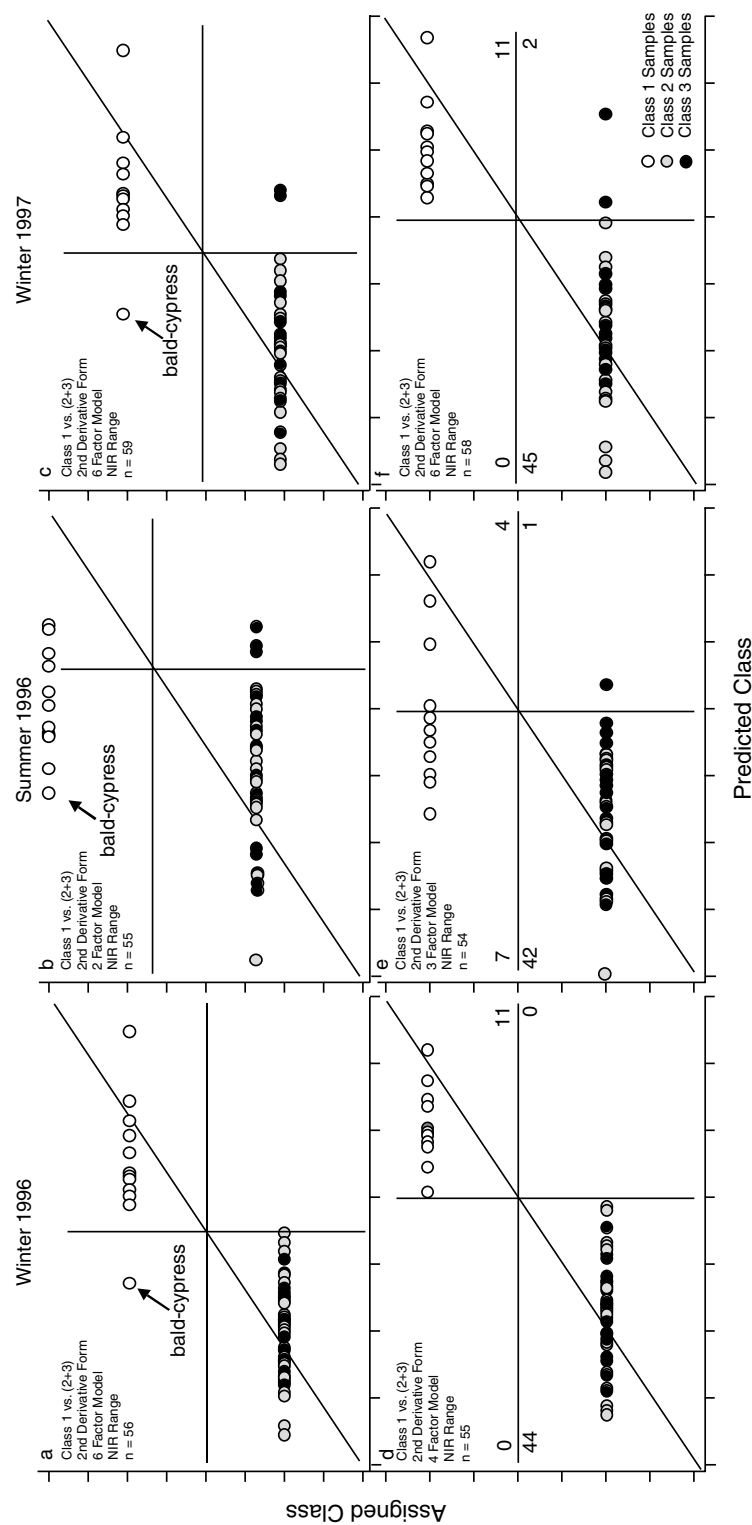


Figure 2. Predicted class versus assigned class discriminating class 1 (unstressed) versus combined class 2 (moderately stressed) and class 3 (severely stressed) pond-cypress trees for second derivative NIR reflectance spectra (1106–2492 nm) of dried, milled branch tips collected in: (a) winter 1996, (b) summer 1996, and (c) winter 1997 with bald-cypress samples included, and for the same periods without bald-cypress samples (d–f, respectively)

Table I. Summarized cross-validation results for classification of visual indicators of stress with NIR reflectance spectra (1112–2429 nm) of dried, milled pond-cypress branch tips using PLS–LDA. Class 1 samples (no/minimal stress) were compared with a combination of class 2 (moderate stress) and class 3 (severe stress) samples

Season	<i>n</i>	Class 1 versus Classes 2 and 3						Percentage Correctly Classified
		Derivative	Components	True+	True–	False+	False–	
Winter 1996	55	1st	4	11	43	0	1	98
		2nd	4	11	44	0	0	100
Summer 1996	54	1st	5	6	40	5	3	85
		2nd	3	4	42	7	1	85
Winter 1997	58	1st	6	10	44	1	3	93
		2nd	6	11	45	0	2	97

Sample sizes for individual classes were winter 1996: $n_1 = 22$, $n_2 = 50$, $n_3 = 38$; summer 1996: $n_1 = 22$, $n_2 = 44$, $n_3 = 42$; winter 1997: $n_1 = 22$, $n_2 = 48$, $n_3 = 44$ ($n = 3$ branch tips pooled per tree). Classification performance was evaluated by calculating the percentage of samples correctly classified: $[(\text{true} +) + (\text{true} -)]/n \times 100$. True+ = class 1 identified as class 1; true– = class 2 or 3 identified as class 1; false– = class 1 identified as class 2 plus 3; and false+ = class 2 or 3 identified as class 1

Table II. Wavelengths (nm) of NIR spectral peaks from regression correlations (second derivative transformations) of dried, milled, pond-cypress branch tips. Greatest peaks are underlined. Assignments to similar regions of the spectrum, based on analyses of other plant species by previous researchers, are provided for comparison

Winter 1996	Summer 1996	Winter 1997	Assignments	References
1378		1378		
<u>1410</u>		<u>1410</u>		
1440		1428	Lignin, O–H bond stretch	1, 2
		1482		
		1524		
	1626			
1634	<u>1636</u>	1634		
	1648			
	1676		C–H bond stretch, 1st OT of lignin ^a	1, 3
			Lignin (1698, 1723)	1, 4
1734		1734	Protein	4
	1756			
<u>1866</u>		1866		
<u>1894</u>	1902	<u>1894</u>		
	<u>1914</u>			
<u>1936</u>	<u>1940</u>	1938	Cellulose, water, O–H stretch, O–H bend	1, 2
	1982		Protein	1
	<u>2004</u>			
<u>2058</u>		<u>2050</u>	Lignin	4
			Lignin (2138)	4
	2228			
	2274		Cellulose, hardwood lignin (<u>2269</u>)	1, 4
	2286			
<u>2310</u>		<u>2310</u>		
	2312	<u>2318</u>		

^a Includes aromatic C–H bonds and overtones (OT) of lignin.

1, Barton *et al.* 1992; 2, Bassett *et al.* 1963; 3, Michell and Schimleck 1996; 4, Barton and Himmelsbach 1993.

summer 1996 and winter 1997 are shown in Figure 3a–c. The greatest positive and negative peaks of the regression coefficients correspond to the spectral features most important to the discrimination model. The regions of the spectrum that correspond to the most significant peaks in the regression coefficients displayed (Figure 3) are summarized in Table II, with assignments of chemical characteristics associated with these regions based on analyses of other plant species by previous researchers.

The greatest peaks generally are not within major regions identified in the referenced studies. One reason may be the limited analysis of woody tissue, specifically tree tissue, examined by previous researchers. Another reason may be that those studies primarily involved agricultural products that are unlike woody cypress tissue, and grow under supplemented water regimes.

Plots of cross-validated PLS–LDA scores generated from second derivative NIR reflectance spectra and shortwave NIR reflectance spectra of samples collected during the three sample periods are provided in Figure 4a–f, respectively. Although clustering of class 1 samples was evident for summer samples, there were more false positive and false negative identifications, resulting in lower percentages of correctly classified samples. Similar analysis of the samples using first derivative spectral transformations provided results that generally were similar, but with poorer model performance.

Class 2 and 3 samples collected prior to bud-break in the winter could not be discriminated using second derivative spectra transformations in the NIR region. Those samples, however, could be discriminated with 80–85% accuracy using second derivative spectra transformations in the shortwave NIR region, as summarized in Table III. Class 2 and 3 samples collected in the summer could not be discriminated using either spectral region.

Climatic conditions during the period prior to and during sampling in the winters of 1996 and 1997 and at sites in different USGS subregions during the same sample periods were not identical (Bacchus, 1999, appendix D). The robustness of the models, however, was demonstrated by using the winter 1996 model to predict the classes of the 1997 samples, and vice versa (Figure 5). Both models accurately predicted all class 1 samples. The only samples misclassified were two class 3 samples (BR site) from the winter 1997 data set (Figure 5a). Those trees failed to produce any leaves after the 1996 season, except from non-woody suckers that had sprouted from the boles of the trees, below the lowest remaining branches. Technically, those samples did not meet the sample requirements, but were included to avoid missing data points.

Drying the samples removed the influence of water from the spectral results. The NIR region primarily is related to the chemical composition of samples (vibration characteristics of chemical bonds), if differences resulting from moisture content and particle size are presumed to be controlled by drying and grinding, respectively (Barton *et al.*, 1992; Barton and Himmelsbach, 1993; Michell, 1994). Support for the former

Table III. Summarized cross-validation results for correlation of visual indicators of stress with shortwave NIR reflectance spectra (624–1092 nm) of dried, milled pond-cypress branch tips using PLS–LDA. Class 2 samples were compared with class 3 samples

Season	<i>n</i>	Class 2 versus Class 3						Percentage correctly classified
		Derivative	Components	True+	True–	False+	False–	
Winter 1996	44	1st	10	20	12	5	7	73
		2nd	5	22	13	3	6	80
Summer 1996	43	1st	1	—	—	—	—	—
		2nd	2	11	13	11	8	56
Winter 1997	47	1st	3	15	16	10	6	66
		2nd	6	22	18	3	4	85

Classes, samples, class sample size and classification performance were as described in Table I. True+ = class 2 identified as class 2; true– = class 3 identified as class 3; false– = class 2 identified as class 3; and false+ = class 2 identified as class 3.

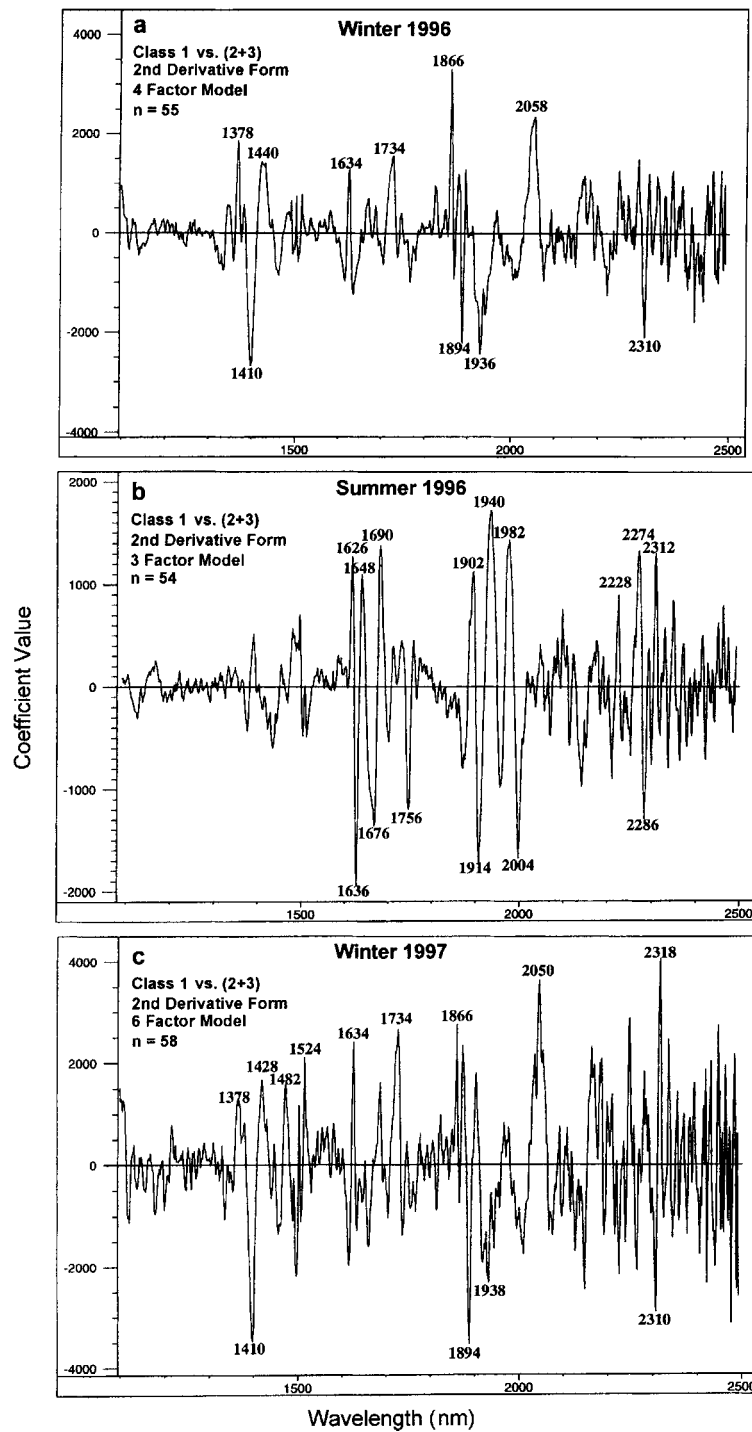


Figure 3. Regression coefficients for class 1 (unstressed) versus combined class 2 (moderately stressed) and class 3 (severely stressed) pond-cypress trees for second derivative NIR reflectance spectra (1112–2492 nm) of dried, milled branch tips collected in: (a) winter 1996, (b) summer 1996 and (c) winter 1997

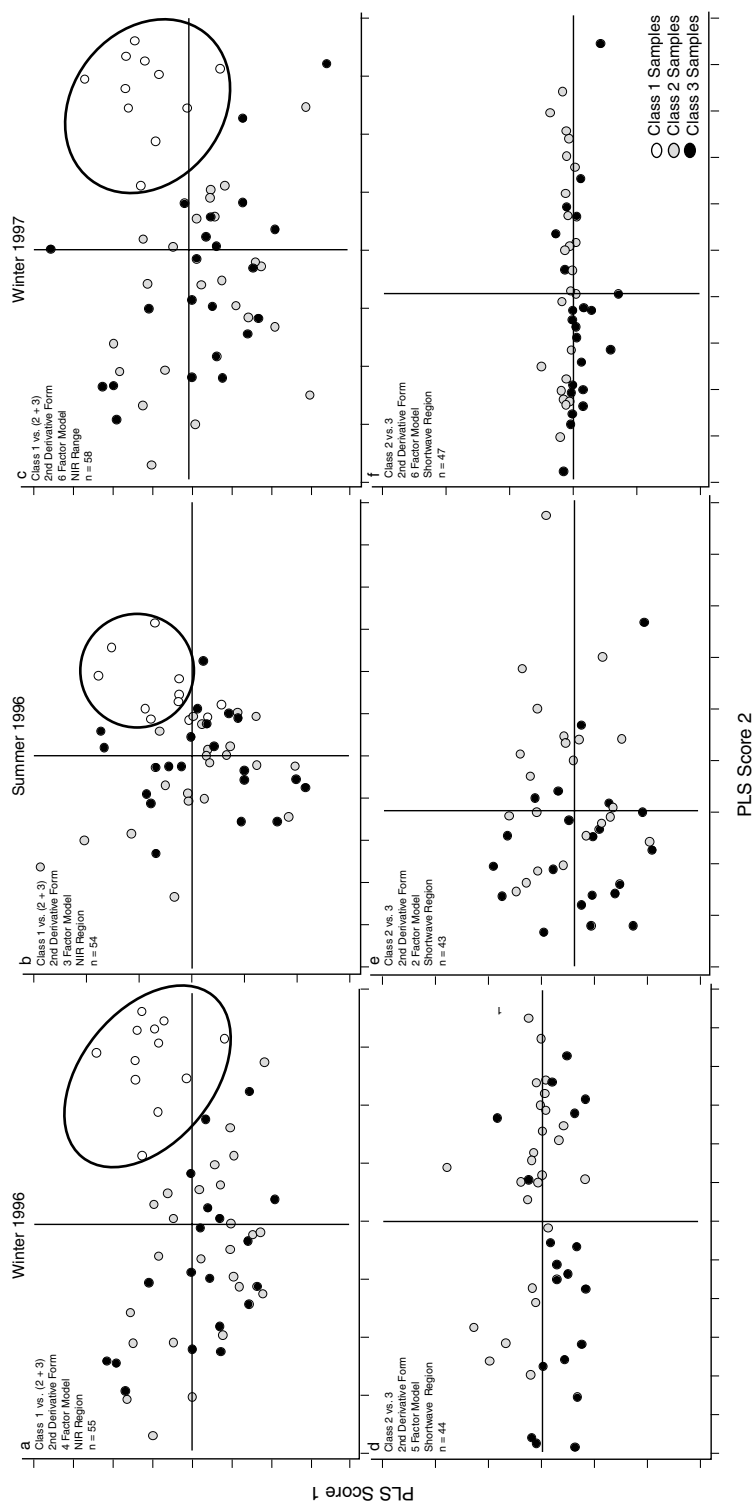


Figure 4. Cross-validated PLS-LDA scores generated from second derivative NIR reflectance spectra (1112–2492 nm) of dried, milled branch tips collected in winter 1996, summer 1996 and winter 1997 (a–c, respectively) from class 1 (unstressed) versus combined class 2 (moderately stressed) and class 3 (severely stressed) pond-cypress trees; and cross-validated PLS-LDA scores for class 2 versus class 3 generated from second derivative reflectance spectra in the shortwave NIR region (650–1100 nm) for the same tissue during the same seasons (d–f, respectively)

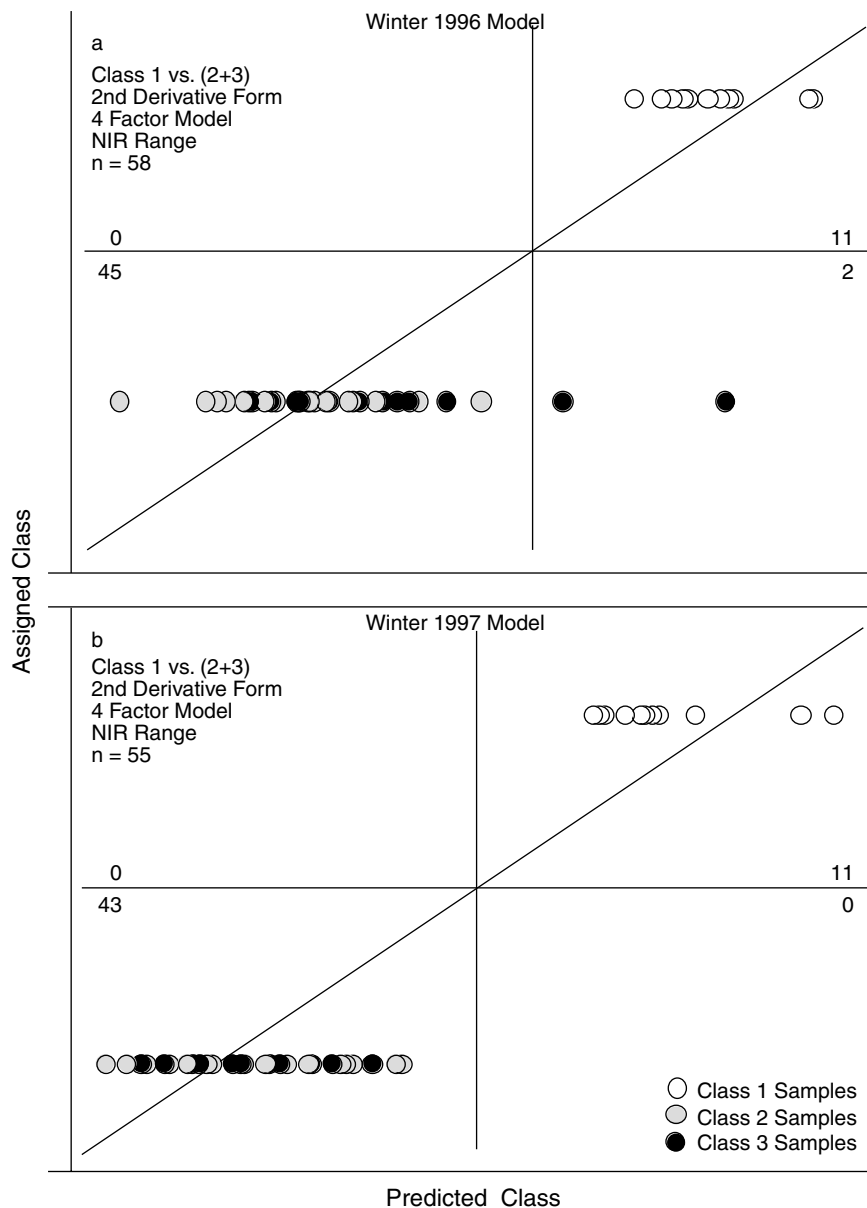


Figure 5. Predicted class versus assigned class discriminating class 1 versus combined class 2 and class 3 using: (a) model developed from winter 1997 data to predict classes of winter 1996 samples and (b) model developed from winter 1996 data to predict classes of winter 1997 samples. Samples were as described in Figure 2

assumption is provided by the fact that the most significant peaks of the regression coefficients are not located within the regions of the spectrum corresponding to water. Two forms of support are provided for the latter assumption. Indirect support is provided by the correct classification of various species of other trees, despite the characteristics of the wood chips analysed (Furumoto *et al.*, 1999). Direct support is provided by the exemplary prediction performance of the two models developed from the winter data sets in this study, despite the fact that the 1996 samples were processed fresh and analysed promptly after collection, whereas the

1997 samples were frozen after collection and stored approximately 2 years prior to analysis. Consequently, discrimination of class 1 samples from combined class 2 and 3 samples collected prior to leaf-out in the winter is presumed to be the result of chemical (vibration) differences and that those differences were not influenced significantly by the different storage conditions.

The inability of the models to discriminate between class 2 and class 3 samples may result from the use of multiple visual indicators of stress to assign trees to these two classes and the fact that some trees in the study exhibited indicators representing class 2, as well as indicators representing class 3. Another possible reason for the inability to discriminate these two classes of stress may be that irreversible damage to the trees occurs relatively soon after the onset of chronic hydroperiod perturbation and although the visual indicators may proceed along a continuum as the tree approaches death, the chemical state of the tree may be very similar once the tree begins exhibiting multiple visual symptoms of stress. Additional research is required to determine: (i) characteristics of the significant peaks associated with the spectrum; (ii) if the two stress classes can be discriminated chemically or by identifying individual symptoms of decline that are more diagnostic than the multiple symptoms of decline used in this study; and (iii) if the textural differences resulting in some of the milled samples in this study can be avoided or further evaluated in the future.

The most challenging and time-consuming aspect of the approach used in this study was the collection of branch-tip samples. Consideration was given to collection of wood samples from a more accessible location on the tree. The most logical alternative location is the butt of the tree, in the zone where basal decay ultimately occurs in chronically stressed trees. Schimleck and Michell (1998) used NIR spectroscopy to evaluate longitudinal and radial variation in wood from three groups of plantation-grown eucalyptus trees in Australia and found considerable variability along both axes. Those findings suggest that collecting wood samples from the bases of cypress trees could introduce considerably more error than the method used in this study, and that significantly more research would be required to evaluate a sampling approach using wood other than branch-tip samples. Another disadvantage of this alternative approach is that wounds are left in the trees, if increment borers are used to collect the wood samples as in the study by Schimleck and Michell. Although healthy trees rapidly form callus tissue that would seal the resultant wounds, sampling from chronically stressed trees may facilitate infection by pathogens and hasten irreversible damage.

Application of results

The results of this study suggest several important findings. First, there appears to be a high correlation between the spectral analysis results and visual symptoms of stress commonly used by forest pathologists as indicators of premature decline in trees (Foster and Wallis, 1974; Sinclair *et al.*, 1987; Hendrix Jr. and Campbell, 1990; Bertrand and Hadden, 1992; Manion and LaChance, 1992; Butin, 1995; Tainter and Baker, 1996). Thus, spectral analysis of pond-cypress appears to provide an objective, scientifically based means of early detection of stress. Because depressional wetlands throughout the south-eastern Coastal Plain are associated with fracture traces, and are underlain by relict sinkholes, they maintain a more direct hydrological connection with the underlying Floridan aquifer system than the surrounding uplands. Therefore, trees in these depressional wetlands are likely to be exposed to adverse impacts of groundwater mining and ASR activities before trees in surrounding uplands. The findings of this study suggest that mature pond-cypress trees in natural stands can be used as hydroecological indicators of areas where unsustainable yield from the Floridan aquifer system and adverse impacts of ASR activities have occurred. These findings also suggest that pond-cypress can be used in conjunction with hydrological monitoring and modelling to determine what the sustainable yield of the Floridan aquifer system is within each subregion.

Class 1 samples were collected from three of the six USGS Subregions of the Floridan aquifer system extending throughout the south-eastern Coastal Plain (Krause and Randolph, 1989). Consequently, the spectral characteristics of dried branch tips collected from unstressed mature pond-cypress trees prior to winter bud-break were similar from year to year, despite site-specific differences (e.g. soils, rainfall, temperature) throughout the portion of the south-eastern Coastal Plain region evaluated in this study. Therefore, pond-cypress reference wetlands (e.g. in areas not associated with groundwater mining) can be designated for

comparison with pond-cypress wetlands in areas of groundwater mining and other anthropogenic groundwater alterations, even across subregional boundaries.

This study also determined that NIR spectral characteristics of dried, milled pond-cypress branch tips vary seasonally. Higher correlation between visual indicators of stress and spectral characteristics occurring prior to winter bud-break than during full leaf-out in summer. Consequently, if the technique of spectral analysis is applied for monitoring purposes, collections of samples restricted to late winter, immediately prior to bud-break should improve results.

Finally, phenomena were observed by Bacchus (1999) that appear to be related to groundwater mining, and warrant more detailed research. These phenomena involve the potential 'parasitization' of declining trees by mycorrhizal fungi and blackgum (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.). Butin (1995) describes how the 'harmonious state of equilibrium' between trees and the mycorrhizal fungi associated with tree roots can shift with changing environmental conditions, 'triggering clearly aggressive or defensive reactions'. Examples of such mycorrhizal fungi provided by Butin include *Boletus* and *Lactarius*. The former was observed during several autumn seasons of this study, fruiting at various locations from the wood of declining pond-cypress trees. Collected fruiting bodies were identified as *B. bicolor* and archived by Richard T. Hanlin, University of Georgia (personal communication, 1998), who indicated that previous occurrence of fruiting bodies from this species had not been reported from wood of a standing tree. *Lactarius* also were observed several years during this study, fruiting from the soil around the bases of declining pond-cypress trees (Bacchus, unpublished data). Similarly, the first report of saprophyte, *Gymnopilus fulgens*, fruiting from the wood of a live tree (Miller and Bacchus, 1998) was observed on declining pond-cypress in a wetland that appears to exhibit evidence of subsurface hydroperiod perturbation. This fungus was observed fruiting during several winter seasons during this study. Unusual behaviour also was exhibited by blackgum roots that enveloped the knees or bases of declining pond-cypress (and pine) trees.

The unusual behaviour of those fungi and blackgum (see Bacchus, 1999, figure 13), in addition to the proliferation of Spanish moss and lichens on declining trees, may be linked to biochemical changes in macro- or micronutrients in the declining trees. Forest pathology literature does not appear to address the mechanism(s) responsible for proliferation of Spanish moss and lichens associated with premature decline in trees; however, increased light was determined not to be a primary factor for the sites in this study (Bacchus, unpublished data). Lichens have been shown to sequester essential minerals from rocks, whereas epiphytes, such as Spanish moss, are known to obtain necessary nutrients via rainwater flowing over the branches that support the epiphytes.

Depressional pond-cypress wetlands are extremely oligotrophic (nutrient-limited) systems, with naturally acidic water. The low pH further limits bioavailability of the nutrients. Therefore, nutrients that leach out of the slowly declining trees may trigger abnormal responses by neighbouring organisms to sequester these nutrients. In addition to initiating more extensive combined hydrological and ecological research, it is clear that more general ecological research is needed, with specific emphasis in the fields of fungal ecology, forest pathology, and tree physiology, to address the environmental problems associated with anthropogenic groundwater perturbations in the south-eastern Coastal Plain.

CONCLUSIONS

The NIRSystems™ 6500, a visible/NIR scanning monochromator, appears to be well-suited for discriminating branch-tip samples collected from pond-cypress trees exhibiting visual symptoms of stress (e.g. those from groundwater mining areas) from those collected from pond-cypress trees exhibiting no visual symptoms of stress (e.g. those not associated with groundwater mining areas). This type of spectral analysis requires limited sample preparation; detects reflected radiation in 2 nm intervals in the 400–2500 nm region and in the NIR (1100–2500 nm) region; and exhibits high correlation with the chemical composition (bond characteristics) of samples. Important advantages of NIRSystems™ 6500, when compared with other spectral analysis systems

such as satellite sensor-images or radiometer data include: (i) lack of seasonal and climate constraints for data analysis, (ii) reduced background noise, and (iii) the ability to conduct a multiplicity of analyses with a single operation.

The NIR spectral reflectance characteristics of dried, milled branch tips collected prior to winter bud-break from *in situ* mature pond-cypress trees assigned to class 1 (no visual indicators of stress), but growing in different USGS Subregions of the Floridan aquifer system (south-eastern Coastal Plain), were similar for 1996 and 1997. These samples were clustered, despite differing site-specific conditions (e.g. soils, rainfall, temperature). Thus, the primary NIR spectral characteristics on which the models were based appear to be more strongly influenced by stress-related factors than by site-related factors.

The similarity of NIR reflectance spectra for the two winter samples provided additional support for the influence of stress-related factors on the models developed in this study. The 1996 winter samples were analysed fresh, promptly after collection, whereas the 1997 winter samples were frozen and stored approximately 2 years prior to analysis. This observation, combined with findings of other researchers, suggest that the physical characteristics of the wood that are influenced by freezing are less important than other factors (e.g. chemical bond characteristics) in NIR reflectance discrimination of samples and in the identification of significant spectral regions by the models.

A high correlation was documented between visual stress indicators (used by forest pathologists and tree physiologists to identify premature decline in trees) and NIR spectral characteristics of dried, milled branch tips collected from mature pond-cypress trees *in situ*, prior to winter bud-break. This supports the conclusion that these visual indicators can be used for rapid, inexpensive, general assessments of unsustainable aquifer yield in the south-eastern Coastal Plain. The NIR spectral reflectance analysis also can provide a less expensive, more rapid, and precise means of differentiating stressed trees from non-stressed trees than wet-chemical analysis, as well as a more objective means of quantitatively assessing visual characteristics of stress and premature decline such as branch dieback, or reduction in the number or size of leaflets.

The data in this study suggest there are significant seasonal differences in the NIR spectral characteristics of pond-cypress branch tips, although only one set of summer samples was evaluated. Visual indicators of stress and NIR spectral characteristics of pond-cypress branch tips collected in late summer (after full leaf-out) exhibited poorer correlation compared with winter samples. This was consistent with predictions, based on reported optimal carbohydrate availability in branch tips of deciduous trees immediately prior to bud-break. Therefore, although visual indicators may be used throughout the year, samples for spectral (or chemical) analysis should be collected in the winter, prior to bud-break, for optimal results.

Class 2 and 3 samples collected during the winters of 1996 and 1997 could not be discriminated using second derivative spectral transformations in the NIR region. Those samples could be discriminated using second derivative spectra transformations in the shortwave NIR region, but with only 80–85% accuracy. Characteristics associated with the red-edge of the spectrum, and characteristics unrelated to chemical bonding may be important in discriminating these two classes. This study also suggests that chemical changes that occur in pond-cypress trees after initiation of chronic water stress may not change significantly over time, whereas visual indicators become more pronounced. Further development of this technique may provide a means of discerning various degrees of stress or may confirm that the stressed trees share chemical traits that lead to their death after a prolonged period, during which the visual indicators are magnified.

Results from this research, used in conjunction with hydrological monitoring and modelling, could provide a scientifically based mechanism for determining sustainable yield of the Floridan aquifer system. The hydroecological approach also could provide a means of adjusting groundwater withdrawals before irreversible structural damage to the aquifer and damage to private property occurs at new sites of withdrawals, or intensifies at sites of existing withdrawals. Likewise, this approach, used in conjunction with hydrological monitoring and modelling, also could provide a mechanism for monitoring ASR sites to prevent similar damage from associated groundwater perturbations.

More extensive research is needed to determine the specific impacts of aquifer withdrawals and injections on the aquifer matrix, the overlying surficial aquifer and surface water, and the intimately linked ecosystems.

Determining the biochemical mechanisms of water stress that may be involved in mediating differences in spectral responses, and at what point these changes become irreversible also requires additional investigation. The analyses in this study were conducted using dried plant tissue to eliminate interference by water bands, which also occur in the spectral region of interest. Additional research also is needed to determine if space-based spectroscopy (hyperspectral or ultraspectral remote sensing data) of natural stands can be used for baseline monitoring and detection of water-stressed trees (*in situ*) that are associated with areas of anthropogenic groundwater alterations. Complications of a space-based approach that must be overcome include the conversion/obscuring of the canopy, with increasing subcanopy and groundcover vegetation, and epiphytes, and the difficulty of maximizing data collection in relatively small areas (zones of depressional wetlands), in addition to predominance by water bands. Rapid advancement in the field of space-based remote sensing may be able to overcome these difficulties, or to identify spectral signatures based on the biochemical characteristics of the obscuring component in the near future.

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