An experimental study of the utility of adventitious roots for determining the hydroperiod in isolated wetlands

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Abstract: Accurate measurement of the hydroperiod in isolated wetlands currently relies upon the installation and frequent monitoring of devices such as piezometers and staff gauges. Observations of biological indicators of the hydroperiod can provide supplemental data to these devices and potentially replace them as a means of accurately determining this hydrologic interval. In this preliminary study, our objective is to determine whether adventitious root formation and maturation on buttonbush (Cephalanthus occidentalis) is a viable indicator of the hydroperiod in isolated wetlands. Buttonbush seedlings were flooded in a controlled environment over a three month period in the summer of 2011. During this time, the length and complexity of adventitious roots observed were recorded. We found a significant positive relationship between average lengths of primary adventitious roots and time of inundation. The sequential appearance of secondary, tertiary and quaternary roots also corresponded with the length of the hydroperiod. From these preliminary results, we see a strong potential of using adventitious roots on buttonbush to help determine the hydroperiods of isolated wetland systems. Ideally, future studies will extend the period of investigation beyond our three month interval and calibrate these findings with examples from natural wetlands.

Key words: Adventitious roots, biological indicator, buttonbush, Florida, hydroperiod, wetland.

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Introduction

Wetlands provide important ecological functions such as supplying habitats for both flora and fauna, providing aquifer recharge catchments, and removing pollutants from surface water (Hansson et al. 2005; Madhu et al. 2016; Mitsch & Gosselink 2000; Woodward & Wui 2001). Accurately monitoring the hydrology of wetlands is essential for ensuring that they continue to provide these important services to the human built and natural environments. Florida relies heavily on wetlands for 1) storing and cleansing of surface waters; and 2) recharging the state’s aquifers that are subsequently used for agricultural, industrial, and residential water supplies (Marella 1999; Marshall et al. 2003). Consequently, monitoring and assessment of wetlands is of great importance for Florida’s water resource managers.

Hydrological conditions in wetlands, such as seasonal high water elevation, normal pool elevation, and annual hydroperiod, can fluctuate due to climate change, extremes in seasonal droughts and flooding, and anthropogenic impacts (Kracauer-Hartig et al. 1997; Lee et al. 2006; Parolin et al. 2016; Zedler 2000). For this article...
we use the hydroperiod definition of Pechmann et al. (1989) as the length of time a site contains standing water during the year.

Specific to karst environments, such as in Florida, the formation of sinkholes and erosional depressions creates isolated wetlands within larger ecological landscapes (Tihansky 1999). The connectivity between wetland hydrology and the karst aquifer below can cause rapid changes in wetland hydrogeology (O’Driscoll 2003). Human activities such as ditching and groundwater withdrawal impact the natural state of wetlands (Brinson & Malvárez 2002; Faulkner 2004). Understanding the degree of change that has occurred from a wetland’s natural state to its current condition can provide the necessary information for creating suitable regulations and management practices to ensure that it continues to provide important ecological functions and ecosystem services (Flournoy 1997).

The determination of the hydroperiod for a particular wetland is important for understanding its overall function for the natural ecosystem within its catchment (Snodgrass et al. 2000). Both floral and faunal species utilizing a wetland are highly reliant on the temporal duration of wetland inundation (Bried et al. 2013; Pechmann et al. 1989; Wilcox et al. 2002). For example, ephemeral wetlands provide frogs with an aquatic habitat free of predatory fish that are unlikely to become established in these seasonally inundated systems (Gonzalez 2004). Likewise, hydrophytic vegetation is able to utilize those portions of an ephemeral wetland that would leave other plant species vulnerable to over-wetting and anaerobic soil conditions. Isolated wetlands, in particular, often flood with heavy rains, after which the flood waters slowly subside due to infiltration, percolation and evapotranspiration, creating unique hydrologic conditions that guide ecological development.

Experimental studies have investigated the influence of hydroperiods of varying length on plant growth. For example, using progressively longer periods of inundation, of up to 100 days, Slusher et al. (2014) found that Bald cypress (Taxodium distichum) and sweet bay (Magnolia virginiana) coped well with the extended hydroperiods, unlike Pond pine (Pinus serotina) and Swamp chestnut oak (Quercus michauxii). In a similar study, Day et al. (2006) determined that Black willow (Salix nigra) grew faster in peat soils with short hydroperiods, while the Bald cypress with the same soil substrate was less sensitive to fluctuating water levels. Observing hydroperiods of three, six, and twelve months, Correa-Araneda et al. (2012) noted that both tree height and the total number of species decreased in wetlands with the longer periods of inundation. A three year study conducted by Megonigal & Day (1992) measured Bald cypress growth for both periodic and continuous hydroperiods. After one year they found that root and shoot production was highest for periodically flooded plants. However, after three years of growth, there were no significant differences in total biomass between the two hydroperiods, unlike with root-to-shoot ratios.

The large number of wetlands in Florida makes their hydrological monitoring impractical for environmental resource managers using the standard technique of staff gauges and piezometers. Hydrologic indicators of periodically inundated biota are often relied upon by scientists to characterize seasonal high water and normal pool elevations within wetlands lacking conventional monitoring devices (Carr et al. 2006). Some of these indicators include: a) inflection points on tree buttresses; b) the lower extent of lichen lines and/or epiphytic bryophyte (moss) collars; c) the upper most extent of adventitious root formation; and d) the water-ward extent of saw palmetto (Serenoa repens) growth occurring at the wetland fringes. Of these indicators, elevations taken at inflection and the waterward extent of saw palmetto provide significant insight to the historic normal pooling elevation of these systems. Lichen and moss growth ceases upon their inundation, leaving distinct lines on trees and other vegetation within a wetland (Carr et al. 2006). In addition, the types of moss species found in a wetland can indicate whether it is ephemeral, perennial, or intermittent in hydrological permanence (Fritz et al. 2009).

Adventitious root formation by wetland plants is a response to anaerobic conditions in inundated soil (Clemens et al. 1978, Justin & Armstrong 1987). Under these conditions, plants then produce roots beginning just above the soil and continuing to the upper limits of the water column (Kozlowski 1984). Adventitious roots have been found to increase a plant’s capacity for water absorption (Hook & Scholtens 1978; Tsukahara & Kozlowski 1985), as well as its rhizosphere oxidation, while reducing toxicity from the soil substrate (Hook et al. 1970; Hook & Brown 1973) and helping supply the plant with essential hormones (Reid & Bradford
Plant height differences were minimal which randomly selected, healthy buttonbush seedlings. As stated above, the formation of adventitious roots by wetland plants is a response to anaerobic conditions found in inundated soils (Kozlowski 1984; Armstrong et al. 1991). We hypothesize that there is a clear relationship between adventitious root formation and the length of the hydroperiod. Specifically, the extent of maturation of individual adventitious roots can provide a quantifiable proxy of their time of inundation. This hypothesis will be tested in a controlled environment by measuring the adventitious root formation of the wetland species buttonbush (*Cephalanthus occidentalis*) during different periods of root inundation. The objectives of this study are to: 1) determine the type and length of root structures for each inundation period; and 2) investigate the effect on root growth of an interruption to inundation of the hydroperiod.

### Methods

#### Study design

This study will be investigating the applicability of a wetland species, buttonbush (*Cephalanthus occidentalis*), in a controlled setting (containers) to determine whether this particular plant can be used as a measure the length a hydroperiods in isolated wetlands. Buttonbush is an obligate wetland species that was selected for both its ability to grow in the deepest sections of many isolated wetland systems and its commonality in Florida (Wunderlin & Hansen 2003). Buttonbush is native to Florida, grows to 3 m in height and has a symmetrical rounded crown. It is a warm-season shrub or small tree, has elongated lenticels and swollen stem bases. Buttonbush has glossy dark green leaves that are whorled and lance-shaped. The most distinctive characteristic is the pincushion-like spherical flowers which are densely clustered and bloom in June through September (Wunderlin & Hansen 2003).

In order to test our hypothesis we planted, at an even spacing of 20 cm, five groups of six randomly selected, healthy buttonbush seedlings. Plant height differences were minimal which reduced interplant competition for light. Each group had a different inundation period ranging from one to four months for groups A to D respectively. Water was added to each plant container as needed to maintain a constant level of inundation required for each group predetermined hydroperiod. A control group (E) was watered in a manner to ensure that flooding did not take place but that the soil was moist so as not to limit growth (Fig. 1). This group was needed to ensure that adventitious root propagation was due to flooding and not as a response to some other environmental factor. All plants were located in the same environmental setting so that climatic conditions were identical for each group. As in its natural habitat, the plants experienced partial shade with total shade in the morning and after 4 pm and full sun exposure in between. Every two weeks the length of all primary, secondary, tertiary, and quaternary adventitious roots produced for each plant was measured. This study was undertaken at the University of South Florida Botanical Gardens in Hillsborough County (lat. 28°03’28’’N, long. 82°25’26’’W), Florida, from July to October 2011. The climate of the study site has an average annual total of 1180 mm precipitation and annual average temperature of 23 °C.

In this study, we attempted to mimic hydroperiod conditions in ephemeral wetlands by flooding the treatment groups using specific inundation periods within the natural wet season of west central Florida. Small isolated wetlands in Florida are typically ephemeral, containing surface water during the wet season, followed by the dry season with periods of no standing water. Plants in the four treatment groups were flooded in containers so that the base of each stem was submerged to a depth of 7.6 cm. Water levels were maintained at a constant depth by drilling overflow drains at a uniform level for all containers. Group A was flooded for four weeks. Group B was flooded for four weeks, drained and left dry for another four weeks before being flooded for an additional month. Group C was flooded for eight weeks and then left dry for four weeks, and Group D was flooded for 12 weeks. Specifically, group A was compared to group C to determine the effect of an extra four weeks of inundation. Group B was compared to group D to measure how a month of dry conditions in between eight weeks of wet conditions would compare with 12 weeks of wet conditions.

Every two weeks the length of all primary roots and representative secondary, tertiary, and
quaternary adventitious roots produced for each plant was measured to the nearest tenth of a centimeter. Photo-documentation of adventitious root propagation in the experimental groups was conducted throughout the study.

**Statistical Analyses**

We used box-whisker-box plots to provide descriptive statistical representations of the data. To determine whether the differences between the various groups were statistically significant, a 1-way ANOVA test conducted. Simple linear regression was used to establish any relationship between root propagation and the length of inundation. All analyses were conducted in Excel 2013.

**Results**

Within two weeks of inundation all four experimental groups produced primary adventitious roots with lengths and complexity of these roots varying greatly across the groups (Fig. 2, Suppemental information (SI) Table S1). Only the primary root lengths are depicted in the box/whisker plots as these are the root lengths that would be most likely measured in the field. Figure 3a shows the primary adventitious roots growing downward towards the soil. During this period, secondary adventitious root growth was observed on plants for all groups except Group B (SI Table S1). The control group did not produce any adventitious roots. Tertiary adventitious root growth was observed on plants in Groups B and C after four weeks (SI Table S2). At six weeks, median primary roots lengths for Groups C and D were 4.9 cm and 8.7 cm respectively (SI Table S3a). Eight weeks primary root growth reached maximum lengths of 11.2 cm (Group C) and 15.0 cm (Group D) (SI Table S3b). At this time, these roots could not be counted without damage: hence, the maximum root length decreases between 10 and 12 weeks of monitoring. After 10 weeks, Group D continued to produce primary, secondary and tertiary adventitious roots, as well as quaternary roots.

A major objective of this study was to investigate the effect of an interruption to inundation of the hydroperiod on root growth. Consequently, after four weeks of inundation for Groups A and B, flooding was ceased to observe the effect of dropping water levels on existing adventitious roots. As expected, the adventitious roots in these experimental groups quickly became desiccated and brittle. By eight weeks (SI Table S3b),
the desiccation of adventitious roots in Groups A and B continued with the disappearance (fell off the primary root structures) of all secondary roots and many primary roots. After 4 weeks without water, Group B plant stems were re-inundated. While many of the adventitious roots had died during the previous month, some primary and secondary roots survived and began to grow during the subsequent two weeks of inundation. The plants of Group A remained dry and adventitious roots continued to die and shed from the base of the plant stems. Group C plants were not flooded for the two weeks following their eight weeks of inundation, and the adventitious roots quickly desiccated (Fig. 3b and d).

After 10 weeks, the only adventitious roots remaining were in Groups B and D because of their continued inundation (Fig. 2e-f, SI Table S3c). Group D’s adventitious roots were longer than roots observed in group B after their longer period of inundation. With 12 weeks, Groups B and D continued to produce primary, secondary and tertiary adventitious roots. Quaternary roots were only observed on plants in experimental group D (SI Table S3d). Groups A and C remained dry and all roots had desiccated beyond the point of regrowth. Group B adventitious roots showed some recovery after being re-inundated for one month. The primary roots as measured in Group D (Fig. 4) show a clear trend of increasing length of the 12 weeks on continuous inundation.

After performing a 1-way ANOVA test, it is apparent that there is a clear statistically significant difference between the groups (columns–
periods of inundation) regarding primary root length and time of inundation ($F_{39} = 29.34; P < 0.001$). As this test found that the variance of length was greater between the groups than within the groups, we felt it justified to quantitatively correlate the averages of all primary roots observed in all plants in the experimental groups against time of inundation (Fig. 5). There is a clear positive linear relationship ($R^2 = 0.94, P < 0.001$) between the length of primary adventitious roots from the experimental groups and the variation in lengths of time the plants were inundated.

**Discussion**

In Florida, the multifaceted utilization of wetland ecosystem services requires natural resource managers to understand their ecological functions. Several biological indicators of hydrology discussed in this study are in current use to understand water level fluctuations, human induced impacts quantification, and the extent of wetland boundaries. The purpose of this study was to investigate the utility of adventitious root formation on wetland plants as a biological indicator of an isolated wetland’s hydronperiod. Our results demonstrate that the use of adventitious roots on buttonbush have the strong potential to be a valid indicator of wetland hydronperiod (Fig. 5). Consequently, an environmental resource manager could locate buttonbush in a wetland and collect data on the length of adventitious roots observed. An average primary adventitious root length could then be generated for that particular wetland. Using the relationship
established in this study as a calibration for the field observations, a manager could simply use the mean adventitious root length as an indicator of the number of weeks of inundation.

Studies conducted on adventitious root formation on other wetlands species such as Hypericum fasciculatum (Carr et al. 2006) and Salix caroliniana (David 1996), could be used in conjunction with data collected from buttonbush plants growing in a wetland. An environmental resource manager would then have three different species to use in determining hydroperiod for a given wetland. This would be helpful as there is a great deal of vegetative species variability among isolated wetlands. Slusher et al. (2014) demonstrated the potential benefits of using more than one species as not all species respond to changing water levels in the same manner.

Megonigal & Day (1992) investigated the influence of varying hydroperiods on Bald Cypress root structure and concluded that root-to-shoot ratios were indicative of the length these plants were flooded. However, to determine this required the excavation of the soils which is time consuming and could potentially disturb plant growth. Our approach, while not providing the level of detail achieved by Megonigal & Day (1992), is more time efficient and has minimal disturbance. Soil type was an important factor for how different plants coped with changing hydroperiods (Day et al. 2006). For example, they found that Bald cypress in peaty soils was not sensitive to fluctuating hydroperiods. We did not consider the influence of soil type on root production of the Buttonbush, but as all plants was nursery-bred, they had the same peaty soil which we concluded was used to maximize plant growth. With the same soil type, the impact of different soil compositions for the various groups was removed as a potential complicating factor.

Several limitations were identified during our experiments. One such limitation was the rapid desiccation of exposed young adventitious roots: such a scenario could occur in isolated wetlands where fluctuations in water levels are rapid. Short adventitious roots may form and then be shed quickly. If periods of rapid inundation are separated by several months, the adventitious roots developed in response to these events may be small or nonexistent during a survey of the wetland. Consequently, during periods of large deviations from the mean precipitation levels for the particular wetland, the measurement of the hydroperiod using buttonbush adventitious roots must be used with caution. A final limitation of using buttonbush adventitious root lengths is when primary adventitious roots enter the soil after achieving a certain size. Not only are the roots fragile, and their extraction difficult, but the roots may change their rate of growth once in the soil. Consequently, the total length of primary adventitious roots that have entered the soil is difficult to ascertain with accuracy.

**Conclusions**

The results of this preliminary study highlight the possibility of determining the hydroperiod of a wetland without intense monitoring using standard instrumentation. Experimental data collected over a twelve week period found that the length of adventitious roots propagated from the stems of

Fig. 4. Box-Whisker Plots Depicting Group D Primary Adventitious Root Growth over 12 week Period.

Fig. 5. Average primary root length over time. Only plants that were inundated at each time interval were included in the calculations.
wetland buttonbush plants strongly correlated with defined periods of inundation. Taking these results, we found that the relationship derived from our study could be used to accurately estimate the hydroperiod of a natural wetland. However, we feel it is important in the future to canvas additional reference wetlands that have hydroperiods between three and 12 months to further establish whether our findings can accurately predict wetland hydroperiods. If successful, then this novel technique can be used by wetland managers to increase the number of wetlands they monitor without an increase in manpower or instrumentation.

References


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**Supporting Information**

Additional Supporting information may be found in the online version of this article.

**Table S1.** Adventitious Root Growth after 2 Weeks Inundation: Please see Figure 1 for details of groups A-D. Abbreviations: PR (Primary Root), SR (Secondary Root), TR (Tertiary Root) and QR (Quaternary Root). All units are centimeters.

**Table S2.** Adventitious root growth after 4 weeks inundation.

**Table S3.** Adventitious root growth after inundation for a) 6 weeks; b) 8 weeks; c) 10 weeks; and d) 12 weeks.