Use of Adventitious Roots For the Determination of Hydroperiod in Isolated Wetlands

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Use of Adventitious Roots

For the Determination of Hydroperiod in Isolated Wetlands

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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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 may be able to supplement data collected from these devices and could potentially replace them as a means of accurately determining this hydrologic interval. The study objective was to determine whether adventitious root formation and maturation on buttonbush (*Cephalanthus occidentalis*) could be used as 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. When average lengths of primary roots were regressed against time of inundation, a linear regression \((r^2)\) of 0.94 was calculated. The structure of these roots was then compared to adventitious roots observed in a natural wetland with a hydroperiod of 12 months. This was undertaken to allow a comparison of the observed lengths and complexity of adventitious roots in the controlled experiment with roots in the natural environment. The regression of both sets of observations yielded an \(r^2\) value of 0.99. Consequently, the results of this study found that the length of adventitious roots on buttonbush can help determine the hydroperiods of isolated wetland systems.
Introduction

Wetlands are extremely important habitats for flora and fauna. They also function as recharge areas for aquifers and as natural filters of water inputs. Wetland health is therefore extremely important for the environment as a whole. Due to the high density of wetlands occurring in Florida and because of the functions they provide, wetland impacts are an issue of special concern. Understanding the historical and present state of a wetland’s health can provide necessary information for suitable regulations and management practices for a particular wetland. Hydrological conditions, such as seasonal high water elevation, normal pool elevation, and hydroperiod, can fluctuate due to climate change, seasonal drought or flooding extremes, geological changes (formation of sinkholes as an example), and human induced impacts such as wetland ditching or groundwater withdrawal.

A significant measure when investigating a wetland is its hydroperiod which can be defined as the length of time that a wetland is inundated with water (Foster, 2007) and its determination is extremely important for understanding the overall environmental condition of that wetland. Both floral and faunal species utilizing a wetland are heavily influenced by the amount of time that a wetland remains inundated. For example, ephemeral wetlands provide frogs with an aquatic habitat free of predator fish who have not yet established themselves in the seasonally inundated system (Gonzalez, 2004). It is therefore important to know the hydroperiod of any given wetland when determining the overall function of that wetland.
Currently, the hydroperiod of a wetland is determined using staff gauges and piezometers that are read continuously over the length of a Water Year (Tampa Bay Water defines a Water Year as beginning in October and ending in September with October through May being the Dry Season and June through September being the Wet Season).

Elevations of wetland bottom are established and recorded in National Geodetic Vertical Datum (NGVD) and can be defined simply as the lowest elevation observed within a wetland. This elevation is identified by canvassing a wetland using a rod and level to determine where the lowest elevation occurs. By making observations of water levels on a regular basis, researchers are able to create a hydroperiod of a wetland by reading the fluctuation of water levels above and below the wetland bottom. For example, if a wetland is monitored bimonthly for hydroperiod and the staff gauge reads 0.35 feet above wetland bottom at the beginning of a month but two weeks later has gone dry, that wetland has a hydroperiod of 0.5 for that month. A wetland that is inundated for an entire year (a lake for example) would have a hydroperiod of 12.0. It is important to note that the hydroperiod does not place emphasis on depth of inundation but focuses strictly on whether or not water is pooling above the ground surface within the confines of the wetland (Sonenshein, 1996).

Wetlands that have not been monitored for their hydrology using the staff gauge and piezometer technique pose a difficult problem for environmental resource managers. The functional role of these wetlands must be based upon current observations of hydrology which may not be indicative of long term changes that have occurred within these systems. Biological indicators of hydrology are often relied upon by scientists to
characterize seasonal high water and normal pool elevations within wetlands lacking conventional monitoring devices (Rochow and Carr, 2004). Some of these biological indicators include: a) inflection points on tree buttresses; b) the lower extent of lichen lines and/or epiphytic bryophyte (moss) collars growing on wetland vegetation; c) the upper most extent of adventitious root formation on wetland vegetation: and d) the water-ward extent of saw palmetto (*Serenoa repens*) growth occurring on wetland fringes. Of these indicators, elevations taken at inflection points on tree buttresses occurring within forested wetland systems and elevations taken at the water-ward extent of saw palmetto growing along the edge of herbaceous wetland systems provide significant insight to the historic normal pooling elevation of these systems. Lichen lines, moss collars and adventitious roots provide information on seasonal high water elevations. Lichen and moss both begin to die off when inundated and can create very clear indicators of the greatest extent of seasonally high water elevations in that they create a line on trees and other vegetation within a wetland where their growth has abruptly stopped or been inhibited by inundation (Rochow and Carr, 2004). The types of moss species observed in a wetland can indicate whether the wetland is ephemeral, perennial, or intermittent in hydrological permanence (Fritz et al., 2009). Adventitious root formation by wetland plants, as a response to anaerobic conditions in inundated soil, produce roots beginning just above the soil and continuing all the way to the upper limits of the water column (Kozlowski, 1984). Currently, none of these biological indicators are being used to characterize the hydroperiod within wetlands. This is due in part to the fact that these indicators provide information on surface water elevation within wetlands, but are not utilized for the determination of how long surface water has pooled within a wetland.
Objective of this study

The purpose of this study is to investigate the utility of adventitious root formation on wetland plants as a biological indicator of isolated wetland hydroperiod. Ultimately, the goal will be to provide a methodology for accurately determining the hydroperiod by measuring the extent of adventitious root growth observed within a given isolated wetland system. As stated above, the formation of adventitious roots by wetland plants is a response to anaerobic conditions found in inundated soils (Kozlowski, 1984). By definition, hydroperiod is the length of time a wetland is inundated, meaning that there should be a relationship between adventitious root formation and the hydroperiod. Specifically, the extent of maturation of individual adventitious roots should provide information on their time of inundation. By taking measurements of length and complexity of adventitious roots, the aim is to demonstrate that their growth is a physiological record of the length of time the plant has been inundated. The hypothesis of this thesis is that buttonbush (*Cephalanthus occidentalis*) adventitious root formation will increase with the length of inundation for this plant species.

Literature Review

Pertinent concepts to this study include; the use of biological indicators in wetlands as a viable indicator of wetland hydrology, physiological plant responses to flooding, adventitious root propagation and adventitious root cellular anatomy.

Biological indicators are extremely useful for depicting hydrological trends in wetlands. Discussing the concept of “normal pool”, Carr and Rochow (2004) examined several biological indicators of long-term historical water levels that can be observed
within isolated wetland systems. Elevations of six different biological indicators were taken from wetlands with known hydrology. Results of this study indicated that inflection points on cypress buttresses offered the most accurate depiction of historical normal pool elevations within the wetland systems studied. This was due to the fact that these inflections points were formed prior to potential hydrological impacts and offer the best representation of historic normal pool. In a study by Tiner (1991), the use of wetland vegetation as a means of identifying wetland boundaries showed that morphological adaptations observed in hydrophytes enabled these plants to occur in wetland areas. Some of these indicators include tree trunk buttressing, aerenchymous stems, and adventitious root formation. The article offers a strong argument for the importance of biological indicators in the determination of wetland hydrology. In his book entitled, “Wetland Indicators: A Guide to Wetland Identification, Delineation, Classification and Mapping”, Tiner (1999) offered an overview of the current biological indicators used in the science of wetland determination. By showing how wetland plants reacted to the hydrological conditions found within wetlands, the author provided a foundation for interpreting wetland hydrology trends using these indicators. In a study by Southerland et al., (2007), researchers in Maryland realized the importance of improving biological indicators to better assess stream conditions. Development of new fish and macroinvertebrate Indices of Biotic Integrity (IBIs) were developed for smaller streams. It was found that these new assessment techniques were better able to accurately classify the biological condition of smaller streams than older methods that had been employed in the past. In a study by Miller et al., (2006), researches stressed the value of utilizing plants as an indicator of human caused wetland impact. 50 components of plant community were identified during
the beginning of the study. Of these, eight attributes showed a high reaction to disturbance and were used in wetland assessments. By isolating these eight components from the 50 originally identified, researchers were able to efficiently assess overall conditions in wetlands.

Next, literature providing information on the formation of adventitious roots in response to flooding was reviewed. Mapping the extent of this physiological response is central to determining whether adventitious roots can accurately provide information about wetland hydroperiod. In an article by Gill (1975) results of a study on a plant species (*Alnus glutinosa*) that is known to be flood tolerant showed that as adventitious roots were removed, plant survivorship decreased. Like the Gill experiment, Tsukahara and Kozlowski (1985) flooded plants (*Platanus occidentalis*) long enough to cause the formation of adventitious roots. During these experiments, significant decreases in sapling growth were observed after adventitious roots were removed, suggesting that these roots were central to the survivorship of the plant during the flood event. Jackson and Drew (1984) examined what affects the formation of adventitious roots had on various inundated plant species. Most notably, several species having original root systems that were able to tolerate a brief flooding event did not produce adventitious roots. Observations of shoot re-growth and leaf emergence in inundated species began to occur only after the formation of adventitious roots. Intolerance to flooding by other species seems to coincide with these species inability to produce adventitious roots.

Lastly, literature providing information on adventitious root formation at the cellular level was reviewed. Understanding how these roots form, the plant hormones involved with their formation, and how they differ from the original root structure is an
important component to understanding if adventitious roots can accurately depict length of inundation. In the article by Angeles, Evert and Kozlowski (1986), the authors discussed hypertrophied lenticels or oversized pores that were observed forming on the submerged portion of stems. These pores are significant in the understanding of adventitious root formation in that many of these roots emerge from the lenticels. The authors suggested that adventitious roots may provide the flooded plant with the ability to absorb water and nutrients not able to occur otherwise due to sub-soil root system death.

Hook and Scholtens (1978) provided a comparison of aerated versus inundated root morphology. Tupelo roots found in well aerated soils were compared with roots growing in inundated soils for morphology, epidermal and endodermal characteristics, formation of adventitious roots, intercellular space found within their relative cortexes, and rhizosphere oxidation. It was found that roots growing in inundated soils had increased amounts of intercellular space when compared to roots growing in well aerated soils.

Kozlowski (1884) provided some causes of flooding injury, specifically focusing on phytotoxic compounds that are known to accumulate in saturated soils and inundated roots. The article explains that deleterious chemicals found in the soil can be oxidized by the plant releasing oxygen through its root system. This diffusion of oxygen requires that the root structure have aerenchyma tissue to provide the airspace needed for diffusion. Kozlowski then explains how ethylene, a known plant hormone, is produced by anaerobic means during soil inundation and that ethylene promotes the development of aerenchyma tissue and ultimately, the diffusion of oxygen.
Research Design

Currently, a methodology for determining hydroperiod with the use of biological indicators does not exist. Staff gauges and/or piezometers are required to document surface water elevations occurring in wetlands. While these devices offer exact information on water level height, they must be checked at a high frequency to offer information on surface water level durations. As an example, the eleven fresh water wellfields located in the greater Tampa Bay area, under the jurisdiction of Tampa Bay Water, require bimonthly monitoring for water levels. Each of these wellfields has many wetlands that require monitoring (As an example, Morris Bridge Wellfield contains 28 wetlands). For a scientist trying to determine the hydroperiod of one wetland within these wellfields, they must visit that wetland 24 four times in a year. Even though this can be a very labor intensive process, the resulting hydroperiods are still generated for each wetland based on an extremely small dataset consisting of only 24 entries for the entire year.

The goal of this project was to create a methodological technique for utilizing adventitious roots as a means of depicting surface water duration within isolated wetland systems. If this new technique can be created, and more importantly, validated by field observations, scientists would be able to make assumptions about a wetland’s Dry and Wet Season hydroperiods based on one or two visits to that wetland throughout the Water Year. This methodology would improve efficiency and could provide valuable data on unmonitored wetlands.

As discussed above, adventitious roots are a natural response of plants to flooding. Essentially, these roots are a record of the inundation that has occurred within a
wetland. If these roots enable a plant to continue metabolic activities during periods of inundation, it can be assumed they, along with the rest of the plant, will continue to grow. A wetland scientist could enter into a wetland at the beginning of the Dry Season and choose representative wetland plants growing in the deepest portion of the wetland. The scientist could remove any pre-existing adventitious roots and make baseline observations of these plants. At the end of the year, the scientist could then return to the wetland and make observations on the adventitious roots that have formed. By measuring the maturation of these adventitious roots, including their length, overall numbers, and complexity, a scientist would be able to make assumptions about how long that plant, and therefore the wetland supporting that plant, was inundated. In wetlands monitored by use of staff gauges and/or piezometers, the validity of this technique could be tested and if successful, it could then be employed in wetlands that do not have water level reading devices or when constant year-long wetland monitoring is not feasible.

Methods

This study investigated the propagation of adventitious roots on buttonbush, a species selected for its ability to grow in the deepest sections of many isolated wetland systems and the fact that it is quite common in Florida. Thirty buttonbush seedlings were divided into five treatment groups each with different flooding durations (Figure 1). There were six buttonbush seedlings per group. A control group contained plants grown without inundated soils to ensure that adventitious root propagation was due to flooding and not a response to some other factor. Plants in the other four treatment groups were flooded in containers so that the base of each stem was submerged to a depth of three
inches. Water levels were maintained at a constant depth by drilling overflow drains at a uniform level for all containers. The control group was watered in a manner to ensure that flooding did not take place. Soils in this group were kept moist. Watering was conducted to ensure that soils were not inundated and that no water pooled in the bin where control groups plants were contained. The experiment took place at the University of South Florida Botanical Gardens and observations were made every two weeks from July 1st to October 1st 2011 Water was added to each container as needed to maintain a constant level of inundation.

Group 1 was flooded for one month. Group 1/3 was flooded for one month, drained and left dry for one month before being flooded for an additional month. Group 2 was flooded for two months and then left dry for one month, and Group 3 was flooded for three months. Group 4 was the control group and not flooded. Isolated wetlands often flood after a heavy rain and then slowly begin to dry up. Conditions of adventitious roots after varying durations of inundation were recorded. Specifically, group 1 was compared to group 2 to see what effect an extra month of inundation would have. Group 1/3 was compared to group 3 to see how a month of dry conditions in between two months of wet conditions would compare with three months of wet conditions.

This study attempted to mimic hydroperiod conditions by flooding the treatment groups within this time frame. Small isolated wetlands in Florida are typically ephemeral, containing surface water during the Wet Season and going dry during the Dry Season. Every two weeks the length of all primary, secondary, tertiary, and quaternary adventitious roots produced for each plant was recorded.
In addition, adventitious roots were harvested from a mature, fully grown buttonbush plant growing in a wetland with a 12 month hydroperiod. Lengths of primary, secondary, tertiary, and quaternary adventitious roots were recorded from these specimens. Photo-documentation of adventitious root propagation in the experimental groups was conducted throughout the experiment. Pictures were also taken of the control group and of the roots harvested from the reference wetland. Data on length of adventitious roots observed for each experimental group were plotted against time of inundation. Statistical analysis of the results was conducted using linear regression and a correlation coefficient was generated to determine whether there was a significant relationship between the period of inundation and root propagation.

Figure 1. Experimental Design
Results

Experimental and control groups were set up on 1 July 2011 at the Botanical Gardens located on the Tampa campus of the University of South Florida. Six buttonbush seedlings, each in individual pots were placed in five different containers and were inundated up to three inches above the interface between stems and soils. Observations of the subsequent adventitious root growth that occurred were made every two weeks for a period of three months. The following section discusses the data collected during this time.

After two weeks of inundation all four experimental groups reacted to flooding by producing adventitious roots (Table A1). The shortest primary adventitious roots observed were on plants in experimental group 2 and 1/3 at 0.9 cm. The longest primary adventitious root observed after two weeks of inundation was located on a plant in experimental group 3 at 8.0 cm. Median primary adventitious root length ranged from a low of 2.5 observed in experimental group 1 to a high of 3.1 in experimental group 3.

Secondary adventitious root growth was observed on plants in experimental groups 1, 2 and 3. The length of secondary adventitious roots ranged from a minimum of 0.1 cm to a maximum of 0.2 cm within these experimental groups. No secondary adventitious roots were observed on plants in experimental group 1/3.

While maximum primary root length was highly variable, minimum root length was fairly constant at 1.0 cm among the 1, 2, and 1/3 experimental groups. The control group did not produce adventitious roots.

Figures 2-5 show adventitious roots emerging from buttonbush plants after being inundated for two weeks. Beginning at the point of emersion, as shown by the water
stain, adventitious roots are visible growing downward towards the soil. While all 4 experimental groups produced adventitious roots, lengths and complexity differed as shown by the box and whisker plots of experimental groups after two weeks inundation seen in Figure 6.

Box and whisker plots depict the median of the dataset, with the upper quartile showing the median of all data larger than the median value and the lower quartile showing the median of all data smaller than the median value. The mean is represented by a point within each box and whisker plot. Whiskers depict the maximum and minimum values observed in each dataset.

Tables have been included as Appendix A. Minimum, maximum, and median values recorded during each two week observation are included for each experimental group. The standard deviation and percent of the dataset for each experimental group that were more than two standard deviations from the mean are also reported for each two week observation.

Figure 2. Close-up of Group 1 Adventitious Roots Emerging after Two Weeks Inundation
Figure 3. Group 1/3 Adventitious Roots Emerging after Two Weeks Inundation

Figure 4. Group 2 Adventitious Roots Emerging after Two Weeks Inundation
Figure 5. Group 3 Adventitious Roots Emerging after Two Weeks Inundation

Figure 6. Primary Root Growth after Two Weeks Inundation
After 1.0 month of inundation, all 4 experimental groups reacted to flooding by producing both primary and secondary adventitious roots (Table A2). Existing adventitious roots continued to increase in length. Primary adventitious root length ranged from 0.9 cm to 11.1 cm.

Secondary adventitious root growth was observed on plants in all experimental groups after 1.0 month of inundation. The length of secondary adventitious roots ranged from 0.1 cm to 5.0 cm.

Tertiary adventitious root growth was observed on plants in experimental groups 2 and 1/3 after 1.0 month inundation. The length of tertiary adventitious roots ranged from 0.1 cm to 0.2 cm within these experimental groups. No quaternary roots were observed in any experimental group after 1.0 month of inundation.

Figures 7-10 show adventitious roots emerging from buttonbush plants after being inundated for 1.0 month. Primary and secondary adventitious roots are evident on plants within all experimental groups. Groups 2 and 1/3 exhibit tertiary root growth stemming from secondary roots. Figure 11 shows that differences between the experimental groups are less pronounced than they were at the end of 2 weeks of inundation. While all four experimental groups produced adventitious roots, tertiary roots were only observed in experimental groups 2 and 1/3.
Figure 7. Group 1 Adventitious Roots after 1.0 Month Inundation

Figure 8. Group 2 Adventitious Roots after 1.0 Month Inundation
Figure 9. Group 1/3 Adventitious Roots after 1.0 Month Inundation

Figure 10. Group 3 Adventitious Roots after 1.0 Month Inundation
After 1.5 months of inundation, experimental groups 2 and 3 reacted to flooding by continuing to produce primary, secondary and tertiary adventitious roots (Table A3). Groups 1 and 1/3 were not inundated during this time period in order to observe how a drop in water level affects existing adventitious roots. As seen in Figures 12 and 13, adventitious roots in these experimental groups quickly became desiccated and brittle. Measurements of desiccated roots caused them to break off the plant stem and no measurements of length were taken. As seen in Figures 14 and 15, existing adventitious roots on plants in experimental groups 2 and 3 continued to increase in length.
Primary adventitious root length after 1.5 months of inundation ranged from 0.7 cm. to 17.1 cm. Secondary adventitious root growth continued to increase in length in experimental groups 2 and 3. Lengths ranged from a minimum of 0.1 cm to 7.1 cm. Tertiary adventitious root lengths ranged from 0.1 cm to 1.0 cm. No quaternary roots were observed in any experimental group after 1.5 months of inundation.

Figure 16 shows the relationship between experimental groups 2 and 3 after 1.5 months of inundation. Group 3 adventitious roots were much longer than group 2 even though they have been inundated for the same length of time.
Figure 13. Group 1/3 Adventitious Roots after Two Weeks Dry

Figure 14. Group 2 Adventitious Roots after 1.5 Months Inundation
Figure 15. Group 3 Adventitious Roots after 1.5 Months Inundation

Figure 16. Primary Root Growth after 1.5 Months Inundation
After 2.0 months of inundation, experimental groups 2 and 3 reacted to flooding by continuing to produce primary, secondary and tertiary adventitious roots (Table A4). Groups 1 and 1/3 continued to stay dry during this time period. As seen in Figures 17 and 18, adventitious roots in these experimental groups began to shed away from the plant stems. Secondary roots were almost non-existent and many primary roots also died. As seen in Figures 19 and 20, existing adventitious roots on plants in experimental groups 2 and 3 continued to increase in length.

Primary adventitious root length after 2.0 months of inundation ranged from 1.1 cm to 15.0 cm. Some roots originating from the stem and longer than 15.0 cm had grown in sufficient length that they were beginning to enter into the soil. These roots could not be counted without damaging them. This is why maximum root length decreases between the 1.5 and 2.0 month monitoring events. Secondary adventitious root growth continued to increase in length in experimental groups 2 and 3 after 2.0 months of inundation. Lengths ranged from 0.1 cm to 6.3 cm. Tertiary adventitious root growth continued to increase in length in experimental groups 2 and 3 after 2.0 months of inundation. Lengths ranged from 0.1 cm to 1.1 cm. No quaternary roots were observed in any experimental group after 2.0 months of inundation.

Figure 21 shows the relationship between experimental groups 2 and 3 after 2.0 months of inundation. Group 3 adventitious roots continued to be longer than group 2 roots even though they have been inundated for the same length of time.
Figure 17. Group 1 Adventitious Roots after 1.0 Month Dry

Figure 18. Group 1/3 Adventitious Roots after 1.0 Month Dry
Figure 19. Group 2 Adventitious Roots after 2.0 Months Inundation

Figure 20. Group 3 Adventitious Roots after 2.0 Months Inundation
After 2.5 months of inundation, experimental group 3 reacted to flooding by continuing to produce primary, secondary and tertiary adventitious roots. Quaternary roots were observed for the first time on plants in experimental group 3. Only group 3 and group 1/3 adventitious roots were able to be measured after 2.5 months of inundation (Table A5). Group 1 plants remained dry and adventitious roots continued to die and shed away from the base of plant stems (Figure 22). Group 1/3 plant stems were re-inundated after being dry for one month. While many of the adventitious roots died during the month of dry conditions, some primary and secondary roots survived and began to function during the subsequent two weeks of inundation (Figure 23). Group 2 plants were not flooded for the two weeks following their two months of inundation. Adventitious roots quickly desiccated and became too brittle to measure (Figure 24). Existing adventitious roots growing on group 3 plants continued to increase in length (Figure 25).
Primary adventitious root lengths after 2.5 months of inundation ranged from 0.8 cm to 14.9 cm. Secondary adventitious root growth was observed on plants in both experimental group 1/3 and 3 after 2.5 months of inundation. Lengths ranged from 0.1 cm to 2.2 cm. Tertiary adventitious root growth was only observed on plants in experimental group 3. Group 1/3 tertiary roots had become desiccated and were no longer viable during the 2.5 month observation, although the remains were still visible. The length of tertiary adventitious roots ranged from 0.1 cm to 2.8 cm. Quaternary adventitious root growth was observed on plants in experimental group 3 after 2.5 months of inundation. Lengths ranged from 0.1 cm to 0.2 cm.

Figure 26 shows the relationship between experimental groups 1/3 and 3 after 2.5 months of inundation. Group 3 adventitious roots are longer than roots observed in group 1/3 due to their prolonged inundation period.
Figure 23 Group 1/3 Adventitious Roots after Two Weeks Re-Inundated

Figure 24. Group 2 Adventitious Roots after Two Weeks Dry
Some group 1/3 roots survived the month of dry conditions and became turgid. These roots, along with new adventitious roots were able to be counted and compared.
with the 3 month experimental group. As expected, these roots were much shorter than
the 3 month experimental group.

After 3.0 months of inundation, experimental groups 1/3 and 3 reacted to flooding
by continuing to produce primary, secondary and tertiary adventitious roots. Quaternary
roots continued to be observed on plants in experimental group 3 (Table A6). Group 1
plants remained dry and adventitious roots were almost nonexistent (Figure 27). Group
1/3 adventitious roots showed some recovery after being re-inundated for one month
(Figure 28). Group 2 adventitious roots continued to desiccate and were very fragile
(Figure 29). Existing adventitious roots growing on group 3 plants continued to increase
in length (Figure 30).

Primary adventitious root lengths after 3.0 months of inundation ranged from 1.7
cm to 15.0 cm. Secondary adventitious root growth was observed on plants in both
experimental groups 1/3 and 3. Lengths ranged from 0.1 cm to 8.5 cm. Tertiary
adventitious root growth was observed on plants in experimental group 1/3 for the first
time since they were initially inundated for 1.0 month. At the end of 3.0 months of
inundation, lengths ranged from 0.1 cm to 1.3 cm. The length of quaternary adventitious
roots in group 3 ranged from 0.1 cm to 1.3 cm. Figure 31 shows the relationship between
experimental groups 1/3 and 3 after 3.0 months of inundation. Group 3 adventitious roots
are longer than roots observed in group 1/3 due to their prolonged inundation period.
Figure 27. Group 1 Adventitious Roots after 2.0 Months Dry

Figure 28. Group 1/3 Adventitious Roots after 1.0 Month Re-Inundated
Figure 29. Group 2 Adventitious Roots after 1 Month Dry

Figure 30. Group 3 Adventitious Roots after 3.0 Months Inundation
Due to new adventitious root growth on the 1/3 experimental group, median root length decreased for this group while the maximum root length continued to increase. Group 3 adventitious roots also continued to increase in length.

As stated earlier, primary adventitious roots were observed entering into the soil at 1.5 months of inundation. These roots would break if an attempt was made to pull them from the soil and could not be measured for the remainder of the study. Figure 32 shows primary adventitious root length increasing through 1.5 months of inundation and then decreasing at 2.0 months. From then on, primary adventitious root lengths appear to stay constant. This trend is due to the fact that longer adventitious roots were entering into the soil and therefore, were no longer counted. Median primary adventitious root length continued to increase from 2.0 months of inundation through 3.0 months of inundation. This shows that shorter roots continued to grow in length.
The above graph depicts primary root growth in experimental group 3 as it was observed every two weeks throughout the three month experiment. Note the overall decline in root length after the 1.5 month observation. This was due to adventitious roots entering into the soil. Once subterranean, these roots could no longer be measured without being damaged in the process. Therefore, maximum root length leveled off while median root length continued to increase.

Figure 33 shows the emergence of primary adventitious roots in groups 1, 2, 1/3 and 3 at two weeks of inundation. In addition, secondary roots were observed on plants in groups 1, 2, and 3. After 1.0 month inundation, all four experimental groups were observed producing primary and secondary adventitious roots. Groups 2 and 1/3 also displayed tertiary adventitious roots during this observation. After 1.5 months of inundation for plants in groups 2 and 3, primary, secondary and tertiary roots are well
established on plants. This trend continues through 2.0 months of inundation. At 2.5 months, group 1/3 has been re-inundated for 2 weeks. While primary and secondary adventitious roots are able to be observed, all tertiary roots observed at the end of 1.0 month of inundation were no longer present. After 2.5 months of inundation, group 3 plants are producing all root types. The quaternary roots were not observed on any plant prior to the 2.5 month observation. At 3.0 months of inundation, group 1/3 plants are growing primary, secondary, and new tertiary roots. Group 3 plants are continuing to produce primary, secondary, tertiary, and quaternary adventitious roots.
The histogram above shows well defined primary roots and the emergence of secondary roots within two weeks of inundation. Tertiary root emergence is evident after 1 month of inundation. Primary, secondary, and tertiary root growth continues through 2 months of inundation. Quaternary root emergence is evident by 2.5 months of inundation in experimental group 3.
Adventitious roots were harvested from a mature buttonbush plant growing in a reference wetland with a known hydroperiod of 12.0 months. These roots were observed emerging from a low branch growing parallel with the water (Figure 34). While most of the adventitious root bundle was exposed above the water column, the roots were long enough to reach into the water. As seen in Figure 35, root masses were highly complex, and composed of primary, secondary, tertiary and quaternary roots. No further root branching beyond the quaternary root was observed.

Primary adventitious root lengths observed on a plant in the reference group ranged from 23.9 cm to 30.4 cm (Table A7). Secondary adventitious roots ranged from 4.2 cm to 8.2 cm. The length of tertiary adventitious roots observed on plants in the reference wetland ranged from 0.7 cm to 4.4 cm. Quaternary adventitious roots ranged from 0.2 cm to 0.5 cm. Figure 36 shows the varying lengths of primary adventitious roots observed on samples collected from the reference wetland.
Figure 34. Reference Wetland Adventitious Roots
Figure 35. Close-Up of Reference Wetland Adventitious Root
The averages of all primary roots observed in all plants in the experimental groups were plotted against time of inundation (Figure 37). The confidence interval of the relationship was $r^2=0.94$ (Figure 30). The confidence interval offers the proportion of the variation in the length of adventitious roots that is based on variations in the length of inundation (Gotelli, Ellison, 2004). Therefore, 94 percent of the variation observed in the length of primary adventitious roots from the experimental groups can be attributed to the variation in lengths of time the plants were inundated.
Figure 37. Average Primary Root Length over Time. Only plants that were inundated at each time interval were included in the calculations.

The average length of primary adventitious roots observed on plants in the reference wetland was then determined and a second scatter plot was created where all the averages were plotted against time of inundation (Figure 38). A regression line was then generated for the data. The resulting confidence interval was $r^2=0.99$. This shows that the reference data fit into the trend established in the experimental data.
Figure 38. Average Primary Root Length over Time with Reference Wetland Data

Discussion

In Florida, governmental regulations, industry, development and tourism have made wetland ecological function an important issue for natural resource managers. Biological indicators are effective tools for these managers trying to understand the ecological conditions of their subject areas. Several biological indicators discussed in this study are currently in use to help in this understanding such as water level elevation, 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 isolated wetland hydroperiod.

The results of this study demonstrate that the use of adventitious roots on buttonbush is a valid indicator of wetland hydroperiod. Figure 33 highlights some of the key trends observed in the formation of adventitious root bundles on buttonbush. As seen
in Figures 37 and 38, average length of primary adventitious roots on buttonbush were highly correlated with length of inundation.

In all experimental groups, primary adventitious root emergence occurred within two weeks of plants being inundated. Secondary adventitious root growth was observed in all groups after one month of inundation and tertiary roots within one and a half months of inundation. This latter observation coincided with the first observation of primary roots entering into the soil. Finally, after two and a half months of inundation, quaternary roots were observed for the first time. If additional experiments show adventitious root bundles forming in this manner, at these time intervals, an environmental resource manager could use observations of buttonbush adventitious root bundles to determine how long water pooled in a wetland. For example, if only primary and secondary adventitious roots were observed, it could be concluded that hydroperiod within the wetland is no longer than 1 month. Likewise, if root bundles contain primary, secondary, tertiary, and quaternary adventitious roots, it could be concluded that the wetland has been inundated for at least two and a half to three months. Additional research must be conducted to record all trends associated with length of inundation and adventitious root propagation over a twelve month period. If additional growth trends could be identified, an environmental resource manager could utilize them to accurately identify length of inundation within a wetland.

Average adventitious root length was highly correlated with length of inundation in this study. An environmental resource manager could canvass a wetland for buttonbush 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 results from 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. For example, a mean adventitious roots length close to 4.50 cm would suggest a hydroperiod of 1.0 month. For another example, a mean primary adventitious root length near 9.50 cm would suggest a hydroperiod of 3.0 months for the subject wetland. As additional research is conducted, the relationship between length of adventitious roots and length of inundation will be more firmly established. Based on this research, the regression equations provided in this study could provide an environmental resource manager with an adequate tool for determining hydroperiod in wetlands containing buttonbush but with no piezometers or staff gauges.

In conjunction with the buttonbush, other species common in isolated systems in Florida such as peelbark St. Johnswort (*Hypericum fasciculatum*) and Carolina willow (*Salix caroliniana*), could possibly be used to determine hydroperiod based on their ability to produce adventitious roots. In particular, Carolina willow is often found in isolated wetlands and readily produces adventitious roots. While buttonbush readily occurs in marshes, they are typically absent in herbaceous systems. Likewise, peelbark St. Johnswort is common in herbaceous systems but would be absent from forested systems. Carolina willow can be found in marsh and herbaceous systems that do not contain a dense canopy. Studies done on adventitious root formation on these plants in response to flooding could supplement the 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 high amount of variability among isolated wetlands.
While data collected during this study suggest that adventitious roots growing on buttonbush could be used as an accurate measurement of surface water duration, the study must be reproduced with other species, more individuals and over longer time periods before any definitive conclusion could be made. During this experiment, several limitations were identified of using only buttonbush adventitious roots as the sole biological indicator of hydroperiod. One such limitation was the rapid desiccation of exposed young adventitious roots; a scenario that could occur in isolated wetlands where fluctuations in water levels are rapid and short adventitious roots may form and then be shed very quickly. If periods of fast 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. For example, assuming a dry Wet Season, if rains fell in June but July and August were relatively dry, adventitious roots may be entirely shed from a plant by September. If rains then re-inundated the wetland in March, at the time of a hypothetical wetland survey, it may seem to a resource manager that no Wet Season inundation had occurred.

Likewise, if a wetland has a 12 month hydroperiod, it may be that adventitious root lengths observed by an environmental resource manager are the result of several years of growth. In this case, well developed roots may be able to withstand a dry year with no wetland inundation. If an environmental resource manager was to collect data on an impacted wetland with a shorter than normal hydroperiod, well established adventitious roots able to withstand the initial impact may show historical conditions. If hydroperiod was determined based on these adventitious roots, the wetland impact could be missed. Conversely, this may be the strength of well-established adventitious roots as
a biological indicator of hydroperiod. If an environmental resource manager was to canvass a wetland for adventitious roots and observed primary root lengths suggesting long periods of inundation, it should be expected that given normal rainfall in the area, that the wetland should not be dry and that these roots should be immersed in the water column and functioning. If the wetland is dry for a prolonged period of time, inconsistent with the length of primary adventitious roots observed, it may be that the wetland is not functioning as it had historically. Consequently, when large deviations from the mean precipitation levels for the particular wetland being investigated have occurred, then the measurement of the hydroperiod using buttonbush adventitious roots must be considered tentative.

Another limitation of using buttonbush adventitious roots was the entering of primary adventitious roots into the soil after growing to a certain length. These roots are fragile, and pulling them from the soil often results in breaking of the root at the soil line. The total length of primary adventitious roots that have entered the soil is therefore difficult to accurately measure. In cases where all or most of the primary adventitious roots have entered the soil, the use of these roots as a biological indicator is highly compromised.

**Conclusion**

Data on adventitious roots need to be collected from wetlands monitored for hydroperiod, which also contain buttonbush. By then comparing average root length observed with known hydroperiod, the results of this study can be further validated. It is important to canvass wetlands that have hydroperiods between three and 12 months to
show whether or not the regressed relationships found in this study are accurate for determining hydroperiod from average primary adventitious roots length. If repeated experiments continue to show adventitious root growth is highly correlated to time of inundation, environmental resource managers can begin using this physiological response as a biological indicator of hydroperiod.
References


Table A1. Adventitious Root Growth after 2.0 Weeks Inundation

<table>
<thead>
<tr>
<th>2.0 Weeks of Inundation</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 1</th>
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<tr>
<td></td>
<td>Primary Root (cm)</td>
<td>Secondary Root (cm)</td>
<td>Tertiary Root (cm)</td>
<td>Quaternary Root (cm)</td>
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<tr>
<td>Minimum</td>
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<td>NA</td>
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<td>Maximum</td>
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<td>Maximum</td>
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Table A2. Adventitious Root Growth after 1.0 Month Inundation

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<th>Group 1</th>
<th>Group 2</th>
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### Table A3. Adventitious Root Growth after 1.5 Months Inundation

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### Table A4. Adventitious Root Growth after 2.0 Months Inundation

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### Table A5. Adventitious Root Growth after 2.5 Months Inundation

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Table A6. Adventitious Root Growth after 3.0 Months Inundation

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Table A7. Adventitious Roots Observed in Wetland with 12 Month Hydroperiod

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