PATTERNS OF INORGANIC PHOSPHATE AND CARBOHYDRATE ALLOCATION IN SAWGRASS (*CLADIUM JAMAICENSE* CRANTZ) AND SOUTHERN CATTAIL (*TYPHA DOMINGENSIS* PERS.) GROWN AT LOW AND HIGH PHOSPHATE LEVELS.

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Xing-Hai Zhang, Department of Biological Sciences, and has been approved by the members of his supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Brian Thomas Hill, son of Dr. George Robert Hill and Wilma Jean (Johnson) Hill, was born on November 21, 1981 in Martin County, FL. He grew up in St. Lucie County, FL and later graduated from Fort Pierce Westwood High School in 2000 while concurrently achieving the rank of Eagle Scout as a member of the Boy Scouts of America Organization. While working part-time, he continued his education on a full-time basis at Indian River Community College in Fort Pierce, Florida where he received his Associate of Arts degree in 2003. He then went on to Florida State University in Tallahassee, Florida where he graduated with a Bachelor of Science degree in Biology in May 2006. After working in the field of wetland restoration (2005-2007), he entered Graduate School at Florida Atlantic University, in August 2007. He married Santina Irene Kneuer the daughter of Mr. Donald Francis Kneuer Jr. and Josephine Lynn (Stevens) Kneuer, on May 2, 2009. Brian and his wife are anticipating the birth of their first child in March 2010.

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ABSTRACT

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Title: Patterns of Inorganic Phosphate and Carbohydrate Allocation in Sawgrass (*Cladium jamaicense* Crantz) and Southern Cattail (*Typha domingensis* Pers.) Grown at Low and High Phosphate Levels.

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In recent history, *C. jamaicense* has been displaced by another native monocot, *T. domingensis,* predominantly resulting from increased phosphorous enrichment in the Everglades. This study aimed to elucidate these two species responses to low and high [P_i] in terms of allocation, photosynthate partitioning and growth. *C. jamaicense* growth was independent of P_i, while *T. domingensis* growth increased with [P_i]. Under high [P_i], allocation to younger *T. domingensis* shoots occurred, while *C. jamaicense* shoots retained more [P_i], while low [P_i] resulted in homogeneous allocation patterns for both species. Additionally, P_i deficiencies induced carbohydrate levels in older shoots of *T. domingensis*, while [P_i] had no effect on photosynthate partitioning patterns in *C. jamaicense*. ACP activity was induced by P_i deficiency in all *T. domingensis* shoots and increased with shoot age, while no effect was observed in *C. jamaicense.* Results indicate these two species differ in allocation strategies when [P_i] is altered.

DEDICATION

This manuscript is dedicated to the individuals who have most enriched my life. First, to my best friend and wife, Santina, who has been my well of joy, inspiration, perseverance and forever shall be. Second, to my mother, who has offered endless guidance and wisdom to which there is no equal in this world. Finally, to my late father, who never saw my dreams achieved although he wished them dearly.

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LIST OF ABBREVIATIONS

3-PGA	3-phosphoglycerate
ACP	acid phosphatase
ANOVA	analysis of variance
ATP	adenosine triphosphate
BSA	bovine serum albumin
EXS	ERD1/XPR1/SYG1
FW	fresh weight
ha	hectares
HP	high phosphate treatment group
LP	low phosphate treatment group
M	middle shoot sample
O	old shoot sample
P	phosphorous
P_i	inorganic phosphate
Pers.	Persoon
PHO1	Phosphate 1
PHO2	Phosphate 2
PHR1	v-myb myeloblastosis viral oncogene homolog
Pht1	phosphate transporter family 1
Pht2	phosphate transporter family 2
p-NPP	*p*-nitrophenylphosphate
R:S	root to shoot ratio
RSA	root structure architecture
SE_{Mean}	standard error of the mean
TCA	trichloroacetic acid
TIC	total insoluble carbohydrate
TP	triose phosphate
TPC	total protein content
TPT	triose phosphate/phosphate translocators
TSC	total soluble carbohydrate
UBC24	ubiquitin conjugating enzyme 24
WCA	Water Conservation Area
Y	young shoot sample

INTRODUCTION

PHOSPHATE AS AN ESSENTIAL NUTRIENT

Inorganic phosphate, or P_i, is essential to plant nutrition and is widely considered one of the most limiting nutrients to plant growth both in agriculture and in the natural world, typical concentrations being <10μM, often <2μM in many environments (Barber et al., 1963; Bieleski, 1973; Raghothama, 2000; Bucher, 2007; Wissuwa, 2003; Mimura, 1995; Mudge et al., 2002). Phosphate is considered to be essential due to its importance in a variety of metabolic functions such as signaling cascades, photosynthesis, cellular respiration, protein synthesis and regulation as well as synthesis of nucleic acids and other macromolecules (Bucher, 2007; Raghothama, 2000). Thus phosphate acquisition and maintenance is an extremely regulatory process in higher plants, involving a variety of metabolic pathways (Ticconi et al., 2001). Additionally, phosphorous is one of the primary elements required for cellular plasma membrane construction (Schachtman et al., 1998; Rausch and Bucher, 2002). These selectively permeable membranes distinguish the cell boundaries, function to protect and give the cell shape. Furthermore, the existence of a phospholipid bilayer is also an integral part of many intracellular organelles and as well as the independent functions they yield. (Hopkins and Hüner, 2004). It is for these reasons, as well as others not discussed that

phosphorous, specifically P_i, has been included in the list of essential nutrient elements of higher plants. Current estimations of the internal P_i requirements of plants suggest a concentration of approximately 5-10mM in cells and 400μM within the xylem are necessary for normal growth and development (Theodorou and Plaxton, 1993; Fang et al., 2009). It is easy to see that without a sufficient quantity of P_i availability, plants are unable to carry the most basic of cellular functions necessary for their survival.

On a global scale, it is predicted that more than 2 billion hectares on Earth are deficient in phosphorous (Nanamori et al., 2004). Historically, crops selected for propagation were often subjected to deficient conditions resulting in reduced crop production potentials and thus limitations in distribution and availability. Both historical and modern repeated agricultural practices have resulted in the depletion of essential nutrients from the soil, most notably N and P_i. This depletion can occur in a relatively few number of growing seasons and thus will ultimately require an alternative approach in order to overcome these effects. The direct application of P_i enriched fertilizers is one of the most common practices in many developed countries to replenish limited soil nutrients and enhance the level of crop production (Myers, 1990; Schachtman et al., 1998). On the other hand, many third world countries are denied access to the availability of these commercial grade fertilizers, often due to political, financial and geographic constraints. In these regions, extreme nutrient depleted soils are a continuous battle for individuals as well as state and local governments. These areas are often forced to cope with these effects through the use of more primitive methods resulting, ultimately, in lower crop yields.

Significant increases in annual crop production, as a result of the use of P_i containing fertilizers, has intensified the rate of application to commercially grown products and subsequently has become a necessary practice for modern agricultural entities. In more recent history, many technological advances have benefited the way in which fertilizers are applied to crops through the use of satellites and global positioning systems. These technologies target the precision of application and proper concentrations thereby reducing the total amount of fertilizer used as well as reducing the amount of potential pollution created in surrounding environments. The financial investments associated with many of these advanced systems are often exceedingly high and thus can deter both large companies and small agricultural firms alike and can further delay transition. Consequently, broad application of P_i containing fertilizers remains consistent in agricultural and thus P_i is currently being harvested at such an alarming rate worldwide, that availability of the commercially ubiquitous mineral may become severely limited within the next 100 years (Raghothama, 1999). Furthermore, anticipated population growth suggests the human population is likely to continue increasing at an exponential rate in the future. As a result, the worldwide demand for food and other resources is also anticipated to increase and therefore it is likely that the demand for additional agricultural production may partially be dependent on this finite mineral supply.

At the root/soil interface, plants absorb P_i most readily in the orthophosphate form of $H_2PO_4^-$, by means of an active H^+/ P_i co-transporter. It is of note that soil pH has

a dramatically influential role in which form of P_i is most predominant within the soil. A pH at or below pH 6.8, the most commonly found form of phosphorous is $H_2PO_4^-$, from pH 6.8 through pH 7.2 the most commonly found form is HPO_4^{2-} and above a pH of 7.2, the predominant form is PO_4^{3-} (Hopkins and Hüner, 2004). P_i can be accessible to a plant from any given source via one, or any combination thereof, of three different means. First, by stimulating growth of the taproot, adventitious roots and root hairs towards the mineral source, second, by the mass-flow of water through the soil carrying P_i towards the absorption zone of the root, and third by direct diffusion of P_i through the soil (Ullrich-Eberius et al., 1981; Barber et al., 1963). Furthermore, soil P_i availability becomes increasingly more and more limited as soluble pools of inorganic phosphate are depleted further as a result of complex ion interactions in the soil, soil chemistry and P_i mineralization, competition effects from soil microbes as well as competition from other individual plants (Raghothama, 1999). Figure 1 summarizes the interactions between plants and the environment with respect to P_i availability.

There are over 170 different varieties of phosphorous containing minerals known in existence (Holford, 1997). These P_i containing minerals can be formed by introducing P_i with ions such as Fe^{2+} and Al^{3+}, similarly with Ca^{2+} and Mg^{2+} to form precipitates and a variety of other insoluble compounds, commonly found in many acidic and alkaline/neutral soils respectively, throughout the world (Holford, 1997; Rausch and Bucher, 2002; Raghothama, 1999; Ho-Hsu, 1968). Additionally, conversion of P_i to organic forms via soil microorganisms can comprise up to 50% of the total soil

phosphorous in some cases, and can also lower the availability of soil P_i to plants (Jungk, et al, 1993). These interactions and other various properties can in effect immobilize up to 80% of available P_i, continually making phosphate one of the most limiting nutrients required for plant growth and development (Raghothama, 2000).

PHOSPHATE AND PHOTOSYNTHESIS

P_i concentration in leaves has been shown to decrease from ≥5mM down to 0.2mM or less during times of excessive P_i starvation (Rausch and Bucher, 2002). Interestingly, when external concentrations of P_i are increased up to 1mM, net photosynthesis also increases, while starch production decreases within the chloroplast (Rausch and Bucher, 2002). Similarly, when intracellular P_i concentrations are low, there is an overall net reduction in photosynthesis, although the accumulation of starch increases (Heldt et al., 1977; Foyer and Spencer, 1986). Starch is identified as the predominant polysaccharide polymer product of photosynthesis, composed largely of monomeric glucose arranged in polymers to form of amylopectins (Smith et al., 2005). Starch is produced within the stroma of the chloroplast during the day, where it is stored until it can be consumed during the night. Starch polymers are considered to be relatively large and immobile, thus catabolic degradation is necessary to achieve smaller, more manageable molecules for transport to the cytosol. Degradation of starch primarily occurs via several forms of amylase as well as a variety of other de-branching enzymes. The primary products formed upon degradation of starch granules include

monomeric glucose as well as maltose. Once in the cytosol, these molecules can be utilized for further biosynthesis of sucrose or otherwise consumed in the glycolytic and cellular respiration pathways (Smith et al., 2005; Flügge et al., 1999). Additionally, the newly formed sucrose molecules can be transported to the phloem via plasmodesmata (symplastic) or apoplastic transport and further translocated to other portions of the plant when necessary (Figure 2).

The relationship between P_i depletion in the cytosol and the subsequent increase in starch synthesis results from the strict transport activity of the triose-phosphate/phosphate translocators (TPT). TPT is a passive anti-transporter located on the inner membrane of the chloroplast, responsible for export of triose phosphate (TP) and 3-phosphoglycerate (3-PGA) from the chloroplast and simultaneous import of P_i from the cytosol in a strict 1:1 ratio (Rao and Terry, 1989). These exported photosynthetic products (TP/3-PGA) are consumed in the synthesis of sucrose as well as amino acids in the cytosol. Any P_i liberated during these reactions assimilates into the cytosolic pool of P_i and can be reabsorbed by the chloroplast (via TPT) or consumed elsewhere within the cell (Smith et al., 2005; Rausch and Bucher, 2002). When cellular P_i diminishes, the transport activity of TPT is reduced resulting in an accumulation of TP in the stroma. The resulting accumulation of starch is attributed to this TP accumulation while sucrose synthesis in the cytosol is reduced (Flügge et al., 1999). Contrastingly, when cytosolic P_i is not limited, the transport activity of the TPT increases and thus reduces the amount of TP available for starch production within the stroma while

increasing the TP available for the synthesis of sucrose in the cytosol (Smith et al., 2005) (Figure 2).

Previously it was discussed that P_i is functionally important to plants both structurally and biochemically. In general, as the supply of P_i is reduced and becomes more limiting, starch accumulation levels will increase and the senesce activity in older shoots also increases. Interestingly, many plants also have the capacity to synthesize photosynthates in one tissue (source) and translocate to another tissue (sink) via vascular transport system. This source/sink activity occurs normally in such processes as grain filling and in flower production but can also occur in normal senescing procedures in older shoot tissues (Hopkins and Hüner, 2004). The sink/source role of shoot tissues is dependent on age whereby a younger shoot may act as a sink, while as the shoot ages its role becomes more that of a source tissue (Hopkins and Hüner, 2004). Sink tissues generally include developing young shoots, meristematic tissue, organs designed for storage as well as root tissues.

The rate at which starch and sucrose can be solubilized and exported is determined by complex network of factors. Most importantly, the strength of a sink seems to preferentially influence the rate of translocation of photosynthates whereby sink strength is defined as being the product of the size and the activity of the sink (Pieters et al., 2001; Hopkins and Hüner, 2004). Relative sink proximity to source tissues also can determine the preference of one sink over another in receiving allocated resources (Hopkins and Hüner, 2004). Additionally, the activity and size of a sink tissue

can be influenced by limited P_i availability specifically in terms of the rate of growth (Pieters et al., 2001). In general, photosynthates can be transported in any direction depending on the location of the sink. Generally, translocation from older shoots is preferentially directed to the roots and has been observed in *Solanum lycopersicum, Glycine max* and *Ricinus communis* L. (Heuwinkel et al., 1992; Fredeen et al., 1989; Rufty et al., 1993; Jeschke et al., 1997; Ticconi and Abel, 2004).

P_i ACQUISITION, TRANSPORT AND HOMEOSTASIS

Several different molecular, morphological and physiological mechanisms have been developed and are employed by plants to aid in the acquisition of P_i and to cope with low P_i availability. Furthermore, the degree to which these strategies are employed is highly differential among higher plant species (Jungk et al., 1993). These mechanisms can include secretion of organic acids, such as citrate and malate, into the root/soil interface to liberate immobile P_i precipitates, secretion of acid phosphatase to liberate, via hydrolysis, organic P forms, other secreted exudates from the roots, which function to liberate other forms of normally immobile precipitates, altered root structure architecture, increased root to shoot ratio, proliferation of root hair and lateral root growth, alterations in glycolysis, photosynthesis and other metabolic processes, as well as remobilization and/or recycling of internal P_i (Tadano et al., 1993; Duff et al., 1994; Green, 1994; Jones, 1998; Rao et al., 1999; Raghothama, 1999; Rausch and Bucher, 2002; Smith et al., 2003; Nanamori et al., 2004; Hammond et al., 2004;

Föhse et al., 1991). At the root system level, alterations in root structure architecture (RSA) have been shown to induce primary root inhibition while stimulating lateral root and root hair formation that would ultimately lead to a significant increase in the surface area of the root, allowing for increased absorption of nutrients (Fang et al., 2009). Another source of P_i for plants includes organic phosphate produced by soil microorganisms, which is an available source of P_i by way of hydrolysis via acid phosphatases and other organic acids (Seeling and Zasoski, 1993). These phosphatases can be secreted from the roots and induction is strongly correlated with increased levels of P_i starvation (Rausch and Bucher, 2002; Sharma et al., 2002; Kuhn et al., 2005). Furthermore, symbiotic relationships formed with arbuscular mycorrhizae have been found to increase the P_i acquisition capabilities of a wide range of terrestrial plant species (Lynch, 1995; Harrison, 1999; Hause and Fester, 2004). In addition to these physiological mechanisms for coping with P_i deficiency, evidence has led to the theory of high and low affinity phosphate transporters, which may be influenced by P_i status or be constitutively expressed, respectively (Epstein et al., 1963; Rausch and Bucher, 2002; Smith et al., 2000). The high affinity phosphate transporter family, Pht1, is primarily responsible for P_i uptake at the root/soil interface as well as P_i loading into the xylem (Rausch et al., 2004). The low affinity P_i transporter family, Pht2, were first thought to be localized in the plasma membrane (Daram et al., 1999), but were later isolated in plastidic membranes as well (Rausch et al., 2004). There is still some debate as to the

importance and precise physiological function of low affinity transporters and what significance they might have in P_i acquisition.

Initially, P_i enters the plant from the soil into the root epidermal (cortical) cells, via a high affinity phosphate transporter, *Pht1*, in *Arabidopsis thaliana* (Rausch and Bucher, 2002). From these cortical cells, P_i can then be translocated via symplastic transport pathways for further downstream loading into the xylem (Smith et al., 2003). *PHO1*, a gene identified as being involved in the loading of P_i into the xylem as well as the possible involvement in signal transduction (due to the presence of an EXS domain in the C-terminal), co-facilitates loading of P_i into the xylem (Poirier et al., 1991; Bari et al., 2006). The mechanistic basis of how *PHO1* plays a crucial cooperative role in the precise loading of P_i into the xylem is still under further investigation (Hamburger et al., 2002).

Once P_i is loaded into the xylem, it is destined to be transported to the most actively growing sink tissues where it is then released from the xylem via an unknown mechanism (Smith et al., 2003; Marschner, 1995). P_i may be deposited into sink tissues and be distributed to several different pools including the cytosol, for general metabolic functions, vacuoles, for storage, apoplastic pools destined for various biosynthetic pathways, back into the phloem, to be transported to the other aerial parts and in times of P_i abundant conditions, deposited in a P_i -efflux pathway in order to prevent shoot toxicity (Jeschke et al., 1997; Rausch and Bucher, 2002; Raghothama and Karthikeyan, 2005). At the shoot cellular level, P_i is brought into the cells via a co-transport system

along with H^+ with the function of a phosphate transporter located in the plasma membrane (Mimura, 1995). These cellular deposits can immediately replenish cytosolic concentrations of P_i, but primarily are deposited into the vacuoles. Vacuolar storages of P_i have been shown to be the main regulators of cytosolic concentration levels as well as maintaining, at least in part, the apoplastic levels of P_i (Mimura, 1995).

During times of limited P_i availability however, many other genes have also been shown to be differentially regulated and subsequently expressed based on uptake rates and the internal P_i concentrations at the cellular level (Ticconi and Abel, 2004). Several studies have shown that in the *pho2* mutant of *Arabidopsis thaliana*, accumulation of P_i occurs within the shoot tissue, indicating a possible deficiency in either translocation from shoots down to the roots, P_i loading into the phloem or alternatively in other general allocation processes (Delhaize and Randall, 1995; Bari et al., 2006; Lin et al., 2009). Furthermore, the *pho2* characteristic accumulation of P_i in the shoots was shown to influence architecture at the root system level (Williamson et al., 2001). Further characterization of this *pho2* mutant phenotype has led to the identification of a potential P_i regulatory pathway. This pathway involves PHR1, a MYB-like transcription factor has also been shown to regulate expression of miRNA399, a 21bp microRNA that in turn regulates the activity of *PHO2* (Bari et al., 2006; Grennan, 2008). Several labs have concurrently identified that *PHO2* is *UBC24*, a previously identified ubiquitin-conjugating E2 enzyme of *Arabidopsis thaliana* (Grennan, 2008). This pathway is a novel mechanism which Pi starvation initiates responses and results in subsequent

downstream regulation of targeted protein levels involved in homeostatic loading, transport and allocation of inorganic phosphate. This set of genes will ultimately prove to be important in the dissection of P_i nutrition mechanisms within higher plants.

P_i levels throughout the plant are thought to be maintained on two different levels, whereby extracellular P_i concentrations are monitored and adjusted via phosphate acquired from the soil as well as from senesced tissues, while intercellular concentrations are considered to be maintained by the vacuolar system (Mimura, 1995; Martinoia et al., 2007; Fang et al., 2009). Vacuolar P_i deposits can comprise between 85-90% of the total cellular stores and can become exhausted during times of prolonged P_i starvation (Fang et al., 2009). Homeostatic control of the cytoplasmic P_i levels requires a phosphate transporter embedded in the membrane of the vacuole, dedicated to import/export of the mineral. Preliminary evidence has identified an ATP-dependent transporter dedicated to this function, however precise characterization of this putative transporter remains elusive (Rausch and Bucher, 2002; Martinoia et al., 2007).

Remobilization of P_i from old or senescing leaves to younger ones has been shown to occur in *Arabidopsis thaliana* in order to cope with limited P_i availability and during times of P_i stress (Raghothama and Karthikeyan, 2005). The specificity of remobilizing P_i to the younger shoots is as a result of the increased sensitivity and susceptibility of the tissues to desiccation due to decreasing nutrient levels. Also of note, remobilization of P_i from these senescent tissues is also basally critical in the recycling of nutrients under normal circumstances (Smith et al., 2003; Fang et al., 2009).

Studies have shown that hydrolases, such as acid phosphatases and ribonucleases, are also expected to have a role in P_i remobilization (Fang et al., 2009). Interestingly, acid phosphatases are expected to contribute to the overall P_i status of the plant in a similar manner that occurs in the rhizosphere, by degrading organic forms of phosphate and liberating the otherwise inaccessible P_i in old and/or senescent tissues. Additionally, ribonucleases may also contribute to the whole plant P_i pools by degradation of RNA during times of low P_i availability as shown in *A. thaliana* (Fang et al., 2009). Further research is necessary to further the dissection of the P_i starvation response pathway(s) and to determine the full spectrum of gene expression and regulation when P_i is deficient. Although complex and not fully understood, these regulatory processes are necessary for maintaining the balance of cytosolic and organellar P_i concentration levels and broad scale P_i homeostasis of the plant.

Furthermore, the ability to remobilize phosphate from otherwise inaccessible sources is critical for plant survival during prolonged times of extreme nutrient stress, when leaves are prematurely senesced. P_i recycling has been shown to be effective in the succession of particular species in environments where nutrients are extremely limited (Nanmori et al., 2004). In certain cases, plants can increase leaf longevity as well as induce early leaf senesce in order to cope with reduced nutrient levels in the rhizosphere and thus reduce the overall nutrient requirements (Hopkins and Hüner, 2004). This type of strategy has been employed by the pteridophyte *Pleopeltis polypodioides* (Polypodiacieae), which can survive prolonged periods of excessive

drought by limiting water loss and reducing or altering metabolic pathways. Specifically, the *Brassica* hybrid cultivar Mulato has been shown to possess a higher tolerance of P_i deficiencies by increasing recycling measures as well as altering photosynthate partitioning as compared to that of *Oryza sativa* L. cultivar Kitaake (Nanamori et al., 2004).

EUTROPHICATION AND THE FLORIDA EVERGLADES

Historically, the Florida Everglades spanned continuously from Lake Okeechobee southward to Florida Bay encompassing an approximate area of 10,000km^2 or greater than 1.2 million ha (Davis, 1943; Steward and Ornes, 1975; Davis et al., 1994). As a result of increased rates of agriculture, development and other anthropogenic factors over the past several decades, the Florida Everglades is ever being degraded, destroyed and further fragmented into an ecosystem with previously undocumented dilemmas and historically unrecognizable vegetative, hydrological, and environmental changes (Newman et al., 1996). Within the current Everglades ecosystem, many different habitat community types persist including, but not limited to, sawgrass marshes, wet prairies, hydric pine flatwoods, pine rocklands, strand swamps and tropical hammocks as well as tree islands and deepwater sloughs. Some of these community types are endemic to the United States, Florida, while even some are endemic to the Everglades region specifically. Perhaps one of the most well characterized, well-publicized and defining communities of the Florida Everglades is the vast sawgrass (*Cladium jamaicense*

Crantz) marshes, accounting for 60% to 70% of the historic Everglades ecosystem (McPherson et al., 1976). *C. jamaicense*, a C_3 monocot also known as Jamaica swamp sawgrass of the Order Poales, Family Cyperaceae, is most well known based on its distinctly defined saw-toothed leaf margins (Wang et al., 2002) (Figure 3). Due to its relatively slow rate of growth and capacity for leaf retention, *C. jamaicense* is thought to have evolved the capacity to thrive in an extreme nutrient poor, specifically P_i, environment, such as the historic condition of the Florida Everglades (Steward and Ornes, 1975). Receiving much of its annual phosphorous input from rainfall most of the phosphorous found in these soils is either in an organic form or otherwise immobile due to interactions with calcium (Davis, 1994; Kuhn 2002). These oligotrophic, sawgrass marshes host an important array of functions to the Everglades ecosystem such as a foraging grounds and habitat to a variety of fish, reptiles, amphibians, wading birds and other microorganisms. Among these species, the sawgrass marshes are home to several endangered/endemic animal species including the American Alligator (*Alligator mississippiensis*), Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*), Snail kite (*Rostramus sociablis*) and the Florida panther (*Puma concolor coryi*) (McCormick et al., 1998). Additionally, the Everglades are also home to many other endemic plant taxa comprising 5-10% of the total 850 plant species (Gunderson, 1994). Among these roughly 70 endangered and/or endemic species includes the Miami Lead Plant, *Amorpha crenulata* (Fabaceae), Beach clustervine, *Jacquemontia reclinata*

(Convolvulaceae) as well as members of the Asteraceae, Cyperaceae, Orchidaceae and Euphorbiaceae families.

In recent history, the displacement of sawgrass by another C_3 monocot species, southern cattail *Typha domingensis*, also of the Order Poales, Family Typhaceae, has occurred mainly in the northern areas of the Everglades (Davis et al., 1994; Wang et al., 2002). *Typha domingensis* Pers. is a native Florida plant, with a characteristic tube-shaped inflorescence, whose success was limited to isolated stands that previous conditions of the historical Everglades system would allow (Miao and Sklar, 1998). According to McCormick et al. (1998), *T. domingensis* naturally occupies and is distributed within areas of disturbance, like that of the alligator holes found in the Everglades. Other sources have identified that individuals of *T. domingensis* were historically smaller in stature (Newman et al., 1996) and suggested to be adapted to growing in areas of high nutrient content and disturbance (Davis, 1994a). The vegetative community shift is, at least in part, as a result of changes in the nutrient input and composition of the region due to agricultural runoff from the boundless use of fertilizers in the effort to increase crop production and yield (Miao and Sklar, 1998). Furthermore, changes in the hydrology of the Everglades region have also been identified as a factor in the dominating presence of *T. domingensis* over *C. jamaicense* (Newman et al., 1996). Reports have indicated that in areas where *C. jamaicense* historically dominated, water flow was consistently flowing similar to that of a broad sheet, whereas in more recent history in those areas where *T. domingensis* has

dominated, that broad sheet has been interrupted to create areas with an increased seasonal hydroperiod, while other areas have a reduced amount of annual inundation (Newman et al., 1996).

A nutrient gradient spans from the northern border of Water Conservation Area 2A (WCA 2A) approximately 8km south with varying concentration levels of P_i (top 30 cm soil; 450 mg P_i kg^{-1} peat DW to 1300 mg Pi kg^{-1} peat DW) that have allowed succession of *T. domingensis* in areas with high P_i concentration while displacing *C. jamaicense* (Lorenzen et al., 2001). Additionally, another study correlated the extent of the nutrient gradient to the increased proliferation of *T. domingensis*, whereby the closer to the source of nutrient enrichment, there was an increased content of phosphorous in *T. domingensis* leaves over that of *C. jamaicense* at similar nutrient concentrations (Koch and Reddy, 1992).

In low P_i containing areas along the concentration gradient, *C. jamaicense* remains ubiquitous, with *T. domingensis* occurring as isolated patches throughout the region (Miao et al., 2008). According to the 2008 South Florida Environmental Report, inorganic phosphate concentrations within Water Conservation Area 2A ranged between averages of 4 to approximately 200 µg P_i L^{-1} for the previous calendar year. One study has indicated that along this north to south nutrient gradient, *C. jamaicense* biomass allocation to leaves, roots, shoot bases and rhizomes were similar in areas with varying levels of P_i concentration, while increased root and decreased leaf biomass were observed for *T. domingensis* in areas with relatively low P_i concentrations (Miao and

Sklar, 1998). Similarly, no changes have been observed in *C. jamaicense* root to shoot ratios, nor relative growth rates for low and high P_i levels (Lorenzen et al., 2001). At low P_i, *C. jamaicense* and *T. domingensis* have similar P_i use efficiencies, while at higher P_i concentration levels; *T. domingensis* has a 3-13 fold higher capacity for P_i uptake (Lorenzen et al., 2001). Interestingly, both species showed a marked increase in root P_i content at the elevated nutrient levels over the control, indicating an increased capacity for uptake while differing in ability to utilize excess P_i (Koch and Reddy, 1992).

In another field study, *T. domingensis* has been shown to accumulate on average twice the concentration of phosphorous (P) in healthy leaves when compared with those of *C. jamaicense*, while in dead leaves, P concentrations were similar between the two species (Davis, 1991). Similarly, allocation of P to *T. domingensis* leaves was on average 2.7 times higher than that of *C. jamaicense* and senescent leaves of *T. domingensis* showed evidence of higher amounts of remobilization of P than did *C. jamaicense* upon desiccation (Davis, 1991). It is also interesting to note that while *C. jamaicense* maintains a relatively consistent number of leaves and senescing very few leaves, *T. domingensis* has been shown to have higher rates of leaf turnover indicating a major difference in the strategy for growth and survival between these two species (Davis et al., 1994).

These studies all indicate that *C. jamaicense* and *T. domingensis* have adapted different life history strategies based on the nutrient composition of the environments in which it historically occupied. *C. jamaicense* is considered to be more tolerant to low

nutrient conditions which has allowed it to succeed in those types of conditions while *T. domingensis* is considered to be more adapted to eutrophic sites with increased levels of disturbance activity. Previous reports have identified differences in the adaptations accumulated by each species in its natural environment (Newman et al., 1996; Newman et al., 1998; Davis et al., 1994; Davis, 1994; Davis, 1991). It is therefore the objective of our study to determine the significance of those adaptations by determining the profiles of the pattern of allocation of specifically, inorganic phosphate and photosynthate products. To the best of our knowledge, this is the first study of its kind regarding *C. jamaicense* and *T. domingensis* where patterns of allocation of P_i and carbohydrates have been examined at the individual shoot level and correlated to the conclusions previously reported at the whole shoot or root levels.

MATERIALS AND METHODS

SEED GERMINATION

Seed stocks were obtained from Water Conservation Area 2 (WCA2), due east of Boca Raton/Delray Beach located in Palm Beach County, Florida and transported to Florida Atlantic University where they were kept in cold storage (4˚C) until ready for use. Prior to sowing, seeds were sterilized in a 10% bleach solution for 20 minutes and then rinsed with approximately 200mL of sterile autoclaved water three separate times to ensure removal of all traces of the sterilization solution. *T. domingensis* seeds were germinated within Magenta® Boxes under sterile conditions on complete, modified Hoagland's solution media with Phytagel™, for approximately 3 weeks in an artificial (light/dark) growth chamber (16hr light, 24°C/ 8hr dark, 18°C). As for *C. jamaicense* seeds were germinated in a similar manner, however the lapsed time between sowing and significant germination was approximately 6-7 weeks. Each seedling was then collected, rinsed to remove any residual solid media and transferred to a 375mL hydroponic container system containing complete media (1mM P_i), where they were given approximately 2-3 weeks to acclimate to the shift in media type. Plants used in this experiment were sown concurrently to ensure similar developmental conditions between both the treatment groups as well as between individual replicates. In the

case of *T. domingensis* an intermediate measure was needed to ensure a gradual step down of the relative humidity otherwise sample desiccation occurred. Surrounding the replicates in the hydroponic containers with a plastic bag and gradually opening the bag each day until the relative humidity of the growth room equated that inside the plastic container. This intermediate weaning step was concluded within the 2-3 week acclimation period. As for *C. jamaicense*, no intermediate weaning step was required in the transition from the solid media system to the hydroponic media system.

HYDROPONIC MEDIA

Two hydroponic media representing 0μM P_i and 1000μM P_i were prepared and titrated to pH6.5 with 1N KOH based on a modified Murashige and Skoog (MS) solution formula (Table 1). To ensure consistency among the treatments 3712.5mL of 0mM and 37.5mL of 1mM was measured out and mixed together to provide 3750mL of 10μM (LP) hydroponic media that was needed for 6 replicates. (For 100μM (HP) media, 3375mL of 0mM and 375mL of 1mM were used to make a total of 3750mL of 100μM.) Both media were made fresh each week and the appropriate proportions mixed together in a 4L-graduated cylinder and dispensed to the appropriate treatment containers. Plastic tubing was engineered with a 1" slit at one end, cut to the exact height and were used to give support to the treatment replicates and keep to plants at the relative same height in relation to the media level in the hydroponic containers (Figure 5).

WEEKLY MEASUREMENTS

For weekly fresh weight and P_i measurement, plant samples were removed from the media, gently rinsed off using dH_2O and blotted dry with dry paper towels three times to remove any remaining liquid. Normalized growth was determined for each of the species by the FW (g) of the current week divided by the FW (g) of the initial starting weight. While the plant removed, media was returned to a volume of 375mL, mixed by pouring into a 1L beaker and a sample of media was taken for analysis of P_i content. Due to differences in treatment P_i concentrations and the scale of which P_i content was to be measured on, a volume of 1.0mL or 200μL was taken for the low and high P_i treatments, respectively. Samples were concentrated to remove all liquid, then resuspended in 300μL of sterile H_2O and analyzed according to the method in the "P_i measurement". At the beginning of the week, similar samples of fresh hydroponic media from each treatment batch had also been collected. Both of the hydroponic media samples that had been taken were analyzed concurrently for P_i content. While empty, hydroponic containers were rinsed with dH_2O to remove any media residue on a weekly basis and replenished with the appropriate concentration of the freshly made hydroponic media. Additionally, any visible algae growth on interior surface of container was removed on a weekly basis via a light brushing/rinsing procedure. Plants were placed back in their containers and returned to the growth chamber. Replicates were monitored on a daily basis to ensure minimal loss of H_2O to evaporation as well as

absorption. Volume of the hydroponic containers was maintained by the addition of dH_2O.

SAMPLE PREPARATION

To begin harvesting each of the treatment replicates, a final fresh weight was collected and the plant was dissected according to root and shoots and then according to the individual shoot age. For *T. domingensis*, there was 2-3 senesced shoots that were considered to be completely desiccated and thus were removed from the replicates once the final FW (g) had been taken. The most superficial living shoot was deemed the eldest shoot tissue, while the deepest shoot was deemed as the youngest shoot tissue. All shoots were aligned from the youngest basal shoot to ensure proper comparison between the treatments. Each of these wholly dissected plants was photographed using a 6.1 mega-pixel Kodak EasyShare® DX7630 digital camera, followed by each shoot being quickly weighed and categorized, then frozen in liquid N_2 and stored at -80˚C. Specific tissue samples were later made into aliquots by retrieving from the -80˚C freezer and grinding the specified tissue to flour in liquid N_2 using a mortar and pestle. 30-50mg aliquots were quickly taken from the ground tissue flour and stored in 1.5mL microcentrifuge tubes. Each aliquot mass was recorded and the sample was immediately returned to the N_2. Aliquots were stored at -80˚C until analyses could be performed.

P_i MEASUREMENT

Total inorganic phosphate, P_i, was quantified via the method adapted from Ames et al. (1966) and later modified by Fujji et al. (2005). All equipment was thoroughly cleaned and free from all sources of inorganic phosphate as recommended. For P_i measurement in tissue, 3mL/$FW_{(g)}$ of P_i Buffer, prepared fresh daily, was added to 1.5mL tube containing tissue aliquot. P_i Buffer solution containing 100mM NaCl, 10mM Tris-HCl, 1mM EDTA, and 14.1mM β-mercaptoethanol was prepared and mixed by inversion. The calculated volume of P_i Buffer was added to each sample and the sample was homogenized using a combination of micro-pestle and vortexing techniques. For the subsequent analysis of total protein content (TPC), a sample was taken (10μL) as well as a sample for the measurement of acid phosphatase (ACP) (15μL) prior to proceeding. A volume of 1% glacial acetic acid equivalent to 10x the calculated volume of P_i buffer used was added to the sample and incubated with shaking at 42˚C for 20 minutes. Samples were centrifuged at 4000x g for 10 minutes to pellet. A volume of supernatant less than or equal to 300μL to yield a final P_i concentration of 0ng-40ng was removed from each sample and placed in a fresh 1.5mL microcentrifuge tube. All volumes were brought up to 300μL with dH_2O and a volume of 600μL 0.42% NH_4-molybdate tetrahydrate and 100μL 10% ascorbic acid was added to each sample. Final volume was equal to 1.0mL and was incubated for 30 minutes at 45˚C. The absorbance values were measured at a wavelength of 820nm using a spectrophotometer (Jenway) using 1.0mL

disposable cuvettes, against a concurrently prepared standard curve. Color development was stable for several hours.

TOTAL PROTEIN MEASUREMENT

Total protein content (TPC) was measured based on the method adapted from Bradford (1973). Total protein was measured using the Bio-Rad Protein Assay® and measured against a BSA standard curve. Previously, 10μL sample was taken from the procedure in "P_i measurement" and was diluted in 90μL P_i Buffer in a fresh 600μL microcentrifuge tube for a total volume of 100μL. Samples were centrifuged at 4000x g for 10 minutes to pellet. 80μL of the supernatant was carefully removed without disturbing the pellet and added to a fresh 1.5mL microcentrifuge tube. 720μL H_2O was added to bring the volume up to 800μL, as well as 200μL Bio-Rad Reagent was added for a final volume of 1.0mL. Samples were mixed by inversion several times and incubated at room temperature for 5 minutes. Absorbance values were determined spectrophotometrically at a wavelength of 595nm, using disposable 1.0mL cuvettes, against a concurrently prepared standard curve (0, 2, 4, 6, and 8μg BSA protein). Additionally, in cases where the protein concentration was relatively high in the tissue sample, it was necessary to also include a 1:10 dilution of the protein containing supernatant solution whereby allocation of an additional 8μL of the supernatant to a fresh 600μL microcentrifuge tube and the addition of 792μL dH_2O and 200μL of Bio-Rad Reagent.

ACID PHOSPHATASE MEASUREMENT

Acid phosphatase activity was quantified using an adapted version of the p-nitrophenyl phosphate colorimetric assay method developed initially by Tabatabai and Bremner (1969) where acid phosphatase activity will be estimated by measuring the release of *p*-nitrophenol from *p*-nitrophenylphosphate. 15μL sample taken from procedure in "P_I Measurement" was diluted in 135μL P_i Buffer for a final volume of 150μL (1:10 dilution). Samples were centrifuged at 4000x g for 10 minutes. 50μL supernatant was taken and combined with an additional 50μL Pi Buffer for a final volume of 100μL (1:20). The remaining of the original 100μL (1:10) of supernatant was stored at -80˚C for later use. To the (1:20) dilution, 200μL dH_2O, 100μL .9M CH_3COONa-$3H_2O$, and 100μL .015M p-nitrophenyl phosphate (*p*-NPP) were added for a total volume of 500μL. Samples were incubated at room temperature for 30 minutes. The reaction was terminated by the addition of 500μL 10% TCA prepared fresh daily and 500μL saturated Na_2CO_3. Absorbance values were determined with the use of a spectrophotometer, using 1.0mL disposable cuvettes, at a wavelength of 405nm. One unit of acid phosphatase activity is defined as the activity that cleaves 1μmol of *p*-nitrophenylphosphate per minute (Nanamori et al., 2004). Activity was reported relative to each of the species that were examined.

CARBOHYDRATE MEASUREMENT

Total Soluble Carbohydrate (TSC) content was measured using the anthrone-sulfuric acid based method adapted from Viles, 1949; McCready, 1950 and Morris, 1948. A previously prepared aliquot of 30-50mg of tissue sample was homogenized in 80% (v/v) ethanol and incubated at 80˚C for 20 minutes. The samples were centrifuged at 13000x g for 10 minutes at which point the now soluble carbohydrate containing ethanol was transferred to a fresh 1.5mL microcentrifuge tube. The tissue samples were washed two more times to yield a final volume of 300μL. This volume was completely dried under vacuum to remove all traces of ethanol and then resuspended in 50μL H_2O. Resuspension solution was assayed for total soluble carbohydrate using a volume corresponding to a final concentration of 0-20μg/mL (10μL) of the resuspended solution added to 90μL H_2O in a screw-capped, microcentrifuge tube for a total volume of 100μL. 1.0mL of 0.14% anthrone (Sigma) in 100% H_2SO_4 (Fischer Scientific) was added for a final volume of 1.1mL. The samples were incubated in boiling H_2O for 20 minutes, cooled down and measured spectrophotometrically at an absorbance of 620nm within one hour. Samples were measured using glucose as a standard (0, 5, 10, 15, 20$\mu g_{glucose}$/mL). The 0.14% anthrone in 100% H_2SO_4 was prepared fresh daily.

The insoluble material remaining from the previous ethanol washes was dried under vacuum to remove all traces of liquid and then resuspended in 200μL H_2O. Samples were autoclaved in a screw-capped, microcentrifuge tube at approximately 121˚C for 3 hours to gelatinize and reduce the number of any localized structures that

may be present in the polymers (Figure 6). As a result of autoclaving, a portion of the volume of H_2O was lost; samples were subsequently dried under vacuum to remove all traces of liquids, and then resuspended in 200μL H_2O. The pH was adjusted to 25mM Na-Citrate pH4.8. 1U of amyloglucosidase (Sigma-Aldrich) and 3U of α-amylase from porcine pancreas (Sigma-Aldrich) were added to samples and were incubated for 18 hours at 37˚C. Samples were centrifuged at 13000x g for 5 minutes. The supernatant was assayed for total insoluble carbohydrate in the same manner as the measurement of total soluble carbohydrate. Insoluble carbohydrates fractions include all carbohydrates not soluble in an aqueous ethanol solution, including starch as well as proportions of cellulose.

STATISTICAL ANALYSIS

Results were analyzed using an one-way analysis of variance (ANOVA) at a significance level where $p < 0.05$. SPSS (Version 17), statistical software used was utilized for the ANOVA, licensed to Florida Atlantic University. Mean, standard deviation and SE_{Mean} were determined using Microsoft Excel® 2004.

RESULTS

PHENOTYPIC DIFFERENCES AT LOW AND HIGH P_I

Cladium jamaicense (Crantz) and *Typha domingensis* (Pers.) were grown at low (LP) and high P_i (HP) concentrations as described in the MATERIALS AND METHODS section. Treatment groups were established and comprised of individuals sown concurrently on equivalent nutrient containing media and subsequently selected for the experiment based on similarities in whole plant fresh weight to ensure maximum homogeneity. There were no observed phenotypic changes in either of the treatment groups nor in whole plant fresh weight (FW) at any given time point for *C. jamaicense* for the duration of the 13-week experiment (n=6) (Figure 7 and 8). A significant difference in FW was found between the LP and HP treatments of *T. domingensis* beginning at the end of the fourth week (t=4) ($p<0.05$; n=6) (Figure 7). Furthermore, these significant differences remained between the LP and HP treatments of *T. domingensis* for the remainder of the experimental treatment period of 8 weeks (t=8). Prior to week 4, (t=4), no significant differences were found between the LP and HP treatments of *T. domingensis*. At the end of 8 weeks, the HP treatment of *T. domingensis* exhibited a mass gain of approximately 3.3 times more than that of the LP treatment indicating growth is dependent of P_i availability (Figure 7). Time points for

harvesting the two species were determined by weekly estimations of P_i depletion from the media as well as estimations of mean relative growth rate.

Samples were subsequently dissected based on root and shoot and further into individual shoot tissues according to position within the sheath arrangement (see MATERIALS AND METHODS) (Figure 9). There were no observable differences in total shoot number among the two treatment levels for *C. jamaicense*, however *T. domingensis* generated approximately twice the number of shoots at the HP level than when grown at the LP level (Table 2). No differences in FW were detectable between the treatment groups for *C. jamaicense* at each of the individual shoot levels (Figure 10a). In addition, no significant differences in FW were detectable for root tissue in response to P_i concentration for *C. jamaicense* (Figure 10b). In contrast, *T. domingensis* individual shoots exhibited significant FW differences between treatment groups in all except the youngest individual shoots (Figure 10c). Additionally, root FW within the HP treatment level of *T. domingensis* was approximately 85% higher than that in the LP treatment (Figure 10d) ($p < 0.05$; n=6). There was no significant difference found between the root to shoot ratios (R:S), for either treatment level in *C. jamaicense* (Table 3), however R:S for *T. domingensis* at the LP treatment level were approximately 1.5 times greater than that of the HP treatment group. Results indicate P_i concentration greatly influences growth of *T. domingensis* while *C. jamaicense* was not at all influenced by P_i status.

Subsequent selection of shoot tissues for further molecular and physiological analyses was accomplished by selecting three shoots that would represent the full age spectrum of all shoots in both *C. jamaicense* and *T. domingensis*. These three samples were denoted young (Y), middle (M) and old (O), corresponding to the relative age of the shoot (Figures 9, 10a,c). For *C. jamaicense*, shoot numbers 3, 6 and 10 were selected for the Y, M and O representative shoot samples while in *T. domingensis*, shoots 1, 3 and 5 were selected (Figure 11).

ACID PHOSPHATASE (ACP) ACTIVITY IN RESPONSE TO P_I DEFICIENCY.

Results indicate that P_i treatment had no impact on shoot ACP relative activity in *C. jamaicense* (Figure 12a). Likewise, decreased P_i availability had little to no impact on relative ACP activity levels in root when compared to that of the HP in *C. jamaicense* (Figure 12a). Notably, the relative ACP activity detected in the O shoot tissue was significantly higher than those activity levels in either the Y or M shoot tissues for *C. jamaicense* (Figure 12a). Relative ACP activity of O shoots in *C. jamaicense* was approximately 1.9 times higher than that of the Y shoots and 2.3 times higher than that of the M shoots at the HP treatment level (Figure 12a), suggesting a normally induced, but relatively low level of remobilization in older senescing shoot tissues.

In *T. domingensis*, on the other hand, reduced P_i concentrations of the LP treatment group were sufficient to significantly induce the relative level of ACP activity in all shoot tissues (Y, M and O), suggesting compartmentalized changes in metabolic

activity within individual shoots in order to cope with a P_i deficiency at the whole plant level (Figure 10b) ($p < 0.05$, n=3). In the LP treatment, relative ACP activity levels in O shoots of *T. domingensis* were approximately 4.5 times higher than that of those measured in Y shoots and 1.9 times higher than that of the M shoot tissues at the same corresponding treatment level (Figure 12b). P_i availability had no detectable influence on the relative ACP activity at the root tissue level within *T. domingensis* at either of the treatment levels (Figure 12b). Furthermore, relative ACP activity levels were increasingly induced as shoot age increased in the LP treatment while activity levels were similar among the HP treatment tissues in *T. domingensis* (Figure 12b). This data also provides further evidence that the corresponding concentrations of P_i used in this study were most likely sufficient to induce or suppress P_i starvation at the cellular level.

CARBOHYDRATE PARTITIONING IN RESPONSE TO P_I DEFICIENCY

TOTAL SOLUBLE CARBOHYDRATE CONTENT (TSC) CONTENT

Results indicate that total soluble carbohydrate (TSC) content between the LP and HP treatment levels were similar for *C. jamaicense* at all individual shoot tissue levels (Figure 13a). Additionally, TSC contents were similar between LP and HP treatment groups in *C. jamaicense* root tissue (Figure 13a). No significant differences in TSC content were found among individual shoot tissues at the LP treatment level in *C. jamaicense* (Figure 13a). However, a significantly higher TSC content was found in the

M shoot tissue at the HP treatment level as compared with that of the Y shoot tissue of the same treatment group (HP) of *C. jamaicense*, while Y and O shoot tissue TSC contents were similar, as was the case for TSC contents of M and O shoot tissues (Figure 13a). M shoot tissue at the HP treatment level contained a TSC content 70% higher than that of the Y shoot TSC content at the same treatment level in *C. jamaicense*.

In *T. domingensis*, P_i deficiencies resulted in significantly higher TSC contents in O shoot tissue as compared with that of the Y shoot tissue, while in the M shoots, TSC content was intermediate to that of Y and the O shoots (Figure 13b). TSC content in the O shoot tissue was estimated to be 70% higher than that of the Y shoot tissue in the LP treatment level for *T. domingensis*. TSC contents were similar in all *T. domingensis* shoots when grown at the HP treatment level. However, in *T. domingensis* root tissue the TSC contents in the LP treatment level were found to be 50% higher than that of the HP treatment level (Figure 13b).

TOTAL INSOLUBLE CARBOHYDRATE (TIC) CONTENT

In *C. jamaicense*, results indicate that TIC content among all shoot tissues was similar (Figure 14a). Likewise, TIC content of root tissue was similar at both LP and HP treatment levels in *C. jamaicense*. Similarly, TIC content was similar in all shoots belonging to *T. domingensis* at both LP and HP treatment levels (Figure 14b). Significantly higher TIC content was detected in M shoots at both treatment levels as well as in the O shoot tissue of *T.* domingensis (Figure 14b). In the M shoots of *T.*

domingensis, TIC content of the LP treatment was 90% higher than that of the HP treatment level. Similarly, the O shoot tissue of the LP treatment level in *T. domingensis* was 70% higher than that of the TIC content in the HP treatment level (Figure 14b). These results indicate that P_i deficiencies induce the level of TIC production (Figure 14c and d) and these estimated whole plant increases are as a result of variable increases at the individual shoot level (Figure 14a and b).

P_I ALLOCATION

Results indicate that there was a significant degree of difference between the LP and HP treatments for the Y and M shoot tissues in *C. jamaicense*, but not in the O shoot tissue (Figure 15a). Among these tissue samples, there were approximately 2 and 5 times the accumulation of P_i in the HP treatment compared with that of the LP treatment group in the Y and M shoot tissues, respectively. Notably, P_i content was also found to be statistically higher within root tissue of *C. jamaicense* grown at HP than when compared with that of the LP treatment. On average, the *C. jamaicense* root tissue of the HP treatment had 5.4 times more P_i accumulated in the LP treatment group (Figure 15a).

In *C. jamaicense*, Y, M and O shoot tissues, showed similar levels of P_i content at the LP treatment level (Figure 15a). At the HP treatment level however, M and O shoot tissues accumulated twice the P_i than in Y shoots of the same HP treatment level in *C. jamaicense* suggesting P_i is accumulated as the shoot ages (Figure 15a). In *T.*

domingensis, both the Y and M shoot tissues accumulated more P_i when grown at the HP treatment level as compared with the LP treatment level (Figure 15b). In Y and M shoot tissues of *T. domingensis* there was approximately 3.3 times and 8.8 times more accumulation of P_i at the HP treatment level than that of the LP treatment level. O shoot tissue accumulated similar levels of P_i at each of the treatment levels (Figure 15b). Similarly, LP and HP treatment levels showed similar levels of P_i content in the root tissue of *T. domingensis* (Figure 15b). Among the Y, M and O shoot tissues of *T. domingensis*, the amount of P_i accumulation was similar when grown at the LP treatment level (Figure 15b). At the HP treatment level however, *T. domingensis* Y and M shoot tissues also accumulated similar concentrations of P_i, while the O shoot tissue accumulated 1.8 times less P_i than that of either the Y or M shoot tissues suggesting a preferential allocation of P_i resources to the younger, more actively growing tissues of the plant (Figure 15b).

TOTAL PROTEIN CONTENT

In *C. jamaicense*, total protein content (TPC) was similar between root tissues of both the LP and HP treatment levels (Figure 16a). Additionally, *C. jamaicense* shoot tissues contained similar levels of TPC at both treatment levels (Figure 16a). Furthermore, Y, M and O shoot tissues of *C. jamaicense* contained similar levels of TPC at the LP treatment level (Figure 16a). Additionally, TPC of *C. jamaicense* Y, M and O shoots was similar at the HP treatment as well (Figure 16a).

In *T. domingensis* all shoots at the HP treatment level accumulated higher levels of TPC when compared with those of the LP treatment level (Figure 16b). In *T. domingensis*, the Y shoot tissues contained 70% more TPC at the HP treatment than that of the LP treatment level. M and O shoot tissues of *T. domingensis* when grown at the HP treatment level contained approximately 1.7 times and 3.1 times more TPC of the LP treatment level, respectively (Figure 16b).

Among the LP treatment of *T. domingensis*, Y shoots had on average, 1.3 times and 3.3 times more TPC of either the M or the O shoots, respectively (Figure 16b). At the HP treatment level, the Y shoot tissue accumulated approximately 50% and 80% more TPC of either the M or O shoots within the same treatment, respectively (Figure 16b). TPC data further exemplifies the notion that *C. jamaicense* is less influenced by low P_i availability, while *T. domingensis* is more susceptible to limitations in P_i availability.

DISCUSSION

Results for the distribution of fresh weight among *C. jamaicense* indicated that a reduced P_i availability had no impact on the relative growth rate compared to that of the high P_i condition (Figure 7). This result is consistent with observations made in other studies, which implicates *C. jamaicense* as being specifically evolved to be more tolerant of highly oligotrophic systems such as the historic condition of the Florida Everglades (Newman et al., 1996; Koch and Reddy, 1992; Davis, 1994a). Plant life history strategy alterations have been shown to occur in environments where resources may be limited and conditions may be harsh, such as increased ability to conserve water, high temperature tolerances as well as rapid life cycles found in ephemeral plant species occurring in desert environments (Hopkins and Hüner, 2004). Examples also include those where adaptations have been made to cope with high salinity tolerances and low temperature regimes such as those species thriving on islands and in tundra environments, respectively (Hopkins and Hüner, 2004). Furthermore, specific life history characteristic traits commonly found in those species that thrive in oligotrophic environments include: slow growth rates and limited ability to respond opportunistically to fluctuations in seasonal nutrient concentrations, extended leaf longevity that translates to a slower leaf turnover rate and reduced amounts of total seed production,

although seeds are usually larger in size. These traits have also been identified in *C. jamaicense* by several other studies (Koch and Reddy, 1992; Davis, 1994a; Davis et al., 1994; Lorenzen et al., 2001).

The overall success of any given plant species survivability lies in its capacity to respond and subsequently evolve adaptations to the circumstances in the environment, which allow it to obtain resources more effectively than others in competition for that same resource (Krebs, 2001). For *C. jamaicense*, adaptations have accounted for the rigidity in growth as seen in response to eutrophication. This rigidity has reflected positively on its endurance and survival capabilities within the historical Florida Everglades ecosystem, however has also limited the competitiveness of *C. jamaicense* with more opportunistic species such as, *T. domingensis* with increased nutrient concentrations seen in more modern times. Previous and current evidence elucidate that *C. jamaicense*, has not responded to the low nutrient environment by mere avoidance of the conditions nor by becoming more opportunistic, but has accumulated specific changes that have conferred tolerance to prolonged nutrient limitations, specifically P_i, and have allowed for subsequent survival. In our study, root to shoot ratios (Table 3) as well as total shoot number (Table 2) in *C. jamaicense* were independent of P_i availability, suggesting that the capacity for growth of *C. jamaicense* is restricted although the more favorable conditions may allow it.

Contrastingly, qualities of *T. domingensis* indicate that it is specifically adapted to thriving in nutrient enriched environments (Newman et al., 1996). In addition to having

the higher capacity for an increase in fresh weight over a given period of time than that of *C. jamaicense* at similar P_i levels (Figure 7), *T. domingensis* also has been identified as having a increased capacity for nutrient uptake as P_i availability increases, as well as an increased capacity for P_i remobilization from senescing leaves (Figure 15) and a reduced leaf longevity (Table 2) which results in a higher leaf turnover rate. In our study, total protein levels were more variable in shoots of *T. domingensis* than that of *C. jamaicense* (Figure 16). This pattern of protein accumulation in *T. domingensis* further suggests that this species employs a strategy whereby resource allocation is strategically targeted in order to better sustain growth of the most rapidly growing, younger portions of the plant. Furthermore, it was observed throughout the experiment, that *T. domingensis* was able to remove a higher proportion of available P_i from the media, indicating its accumulation rate was higher than that of *C. jamaicense* when P_i was in excess.

The relative levels of acid phosphatase activity were similar in all shoots of *C. jamaicense* at both of the treatment levels (Figure 12a), consistent with the finding that the capacities for the remobilization of P_i from the older shoots of *C. jamaicense* is proportionally less than that of *T. domingensis* (Davis, 1991). A significantly higher level of acid phosphatase activity was seen in the eldest *C. jamaicense* shoot sampled of the HP treatment level, (Figure 12a) suggesting that acid phosphatase also plays a role in the normal senescing of leaves. Additionally, levels of acid phosphatase were relatively higher among older shoots of *T. domingensis* than when compared with younger shoots, (Figure 12b) suggesting that an increased level of P_i had been liberated from older

shoots. This increase in activity would allow the liberation of an organic source of P_i necessary to partially alleviate the deficit between P_i required for maintaining growth of younger shoots and the amount of available P_i. Elevated levels of relative acid phosphatase activity were not seen in the HP treatment of *T. domingensis* most likely due to a sufficient supply of P_i. Previous studies have indicated that there is a marked increase in the activity of acid phosphatase in roots of both *C. jamaicense* and *T. domingensis* at low P_i levels, compared with those activity levels at high P_i levels (Kuhn et al., 2002). In our study however, low P_i concentrations did not seem to impact the relative acid phosphatase activity levels in root tissue of either *C. jamaicense* or *T. domingensis* when compared to the HP treatment level (Figure 12). As mentioned earlier, the level of acid phosphatase activity increases as the cellular levels of P_i decrease (Rausch and Bucher, 2002). The similar levels of relative acid phosphatase activities between the two treatment levels in *T. domingensis* corresponded with the similar quantities of P_i as measured in those same tissues, however, not in the roots of *C. jamaicense* (Figures 12 and 15). The relative acid phosphatase activity levels for root tissue of *C. jamaicense* were similar between the two treatments (Figure 12a) although *C. jamaicense* root tissue accumulated on average 6.4 times more P_i at the HP treatment level than that of the LP treatment level (Figure 15a). Fresh weight data revealed that this increased uptake of P_i did not translate into any fluctuations in root FW, (Figure 10b) consistent with the findings of previous studies (Davis, 1991; Davis et al., 1994). This observed outcome in *C. jamaicense* roots may be as a result of the nature of the

hydroponic system, based on the notion that prolonged periods of flooding have resulted in an anoxic environment for roots and thus can induce regulatory reductions of the metabolic activity (Sharma et al., 2005). However, Kuhn et al. (2002) indicated that prolonged inundation (approximately 5cm above soil surface) of *C. jamaicense* and *T. domingensis* had no measurable effect on the level of acid phosphatase activity in roots at either 10μM, 80μM and 500μM P_i, although flooding did seem to allow for increased P_i uptake. Furthermore, Kuhn et al. (2002) illustrated 80μM P_i was sufficient to induce a significant increase in acid phosphatase activity of the roots when compared with 500μM P_i, while the 10μM and 80μM treatment groups exhibited similar levels of acid phosphatase activity. In our study, the P_i treatment concentrations for the LP and HP treatment levels were 10μM and 100μM, respectively. Taken together, these results suggest that the P_i concentration threshold sufficient to induce a change in acid phosphatase activity, specifically in roots, is presumably at or above the upper limits of our study (≥100μM) while the aqueous nature of the hydroponic system had little impact on the root FW (Figure 10b) as well as the relative ACP activity levels (Figure 12a). Additionally, ACP activity in the root tissue of *C. jamaicense* may already be at a maximum level at the HP treatment level and thus no difference would be detectable between the treatment levels, consistent with previous observations that little differences in ACP activity occurred between 10μM and 80μM (Kuhn et al., 2002).

In Kuhn et al., 2002, results also show *C. jamaicense* is capable of accumulating more P_i in root tissue than *T. domingensis*, also consistent with our findings (Figure 15).

Results here support the hypothesis that the internal P_i requirement of *C. jamaicense* is reduced (Davis, 1994a) and thus excess P_i that is absorbed accumulates in the root, rather than being utilized for growth, as is the case in *T. domingensis* (Figure 7). This type of mechanism would allow *C. jamaicense* to increase the chances of survivability in a low nutrient environment by taking in stores of P_i when possible and extending the benefits of those storage products for a longer period of time. It is unclear what influence, if any, seasonal fluctuations in precipitation and hydrology may have played in the evolution of mechanism of this type. One could imagine that the nature of the wet/dry seasons as seen in sub-tropical South Florida supplies much of the annual phosphorous input to the Florida Everglades in a few short months, while the majority of the year relatively low amounts of precipitation, and thus P_i, are added to the system. Such species that could conserve those highly limited resources most effectively would ultimately prove to be the best competitors under the conditions of the historic Everglades system. Once the annual P input is offset by increased agricultural activity in the region, natural selection begins to favor species that can utilize the increased P concentrations most quickly, such as in *T. domingensis*.

P_i allocation patterns among individual shoot tissues of *C. jamaicense* display a similar profile to that of *T. domingensis* when P_i is in short supply (Figure 15). Interestingly, the allocation pattern observed in *C. jamaicense* when P_i is in sufficient supply suggests that more accumulation, i.e. less remobilization, of nutrients is occurring in the mid to old shoots. This data is in agreement with the idea that *C.*

jamaicense has a lower nutrient requirement, relatively longer leaf longevity and slower leaf turnover rate than that of *T. domingensis* (Davis et al., 1994; Davis, 1994a; Newman et al., 1998), which would result in an accumulation of P_i as shoot tissues aged and may partially account for the persistence of *C. jamaicense* in the historical vegetation community of the Florida Everglades.

Allocation patterns in shoots of *T. domingensis* (Figure 15b) indicate that when P_i status is sufficient, P_i is distributed in the regions of the plant that are, or recently were, the most actively growing and are depleted from older, senescing shoots. However when P_i availability is deficient, distribution indicates a more homogenous pattern (Figure 15b) within a relatively fewer number of shoots (Table 2) when compared with the HP treatment level. This result is in agreement with field observations (Lorenzen et al. (2001) that *T. domingensis* has a limited capacity for survival and competition in P_i deficient conditions and that the higher nutrient requirements of the species have historically limited distribution of relatively small individuals in ruderal, eutrophic areas. The homogenous P_i distribution seen in the P_i deficient *T. domingensis* suggests that a minimum concentration of P_i must be maintained within each tissue and under severely depleted conditions older shoots are senesced and nutrients remobilized in attempts to maintain that minimum threshold. Furthermore, differences in the order of magnitude of P_i content between *C. jamaicense* and *T. domingensis* may suggest another strategy utilized by *T. domingensis* whereby conversion of a higher proportion of absorbed P_i to organic forms and thus would not be detectable using the method of Ames (1966).

Nanamori et al. (2004) report P_i:Total P ratios were higher in *Brassica* hybrid, a monocot extremely tolerant of nutrient deficient conditions, than in *Oryza sativa*, indicating a larger proportion of free P_i which may suggest the plant may accumulate more than it needs. Additionally, this increased conversion may be as a result of the elevated requirement of organophosphorous compounds, e.g. DNA and RNA, necessary to sustain the increases in the rate of cellular division for *T. domingensis* and subsequent growth increases (Figure 7). On the other hand Ames, 1966 also suggest that excess amounts of labile organic phosphate can superficially increase the estimations of P_i content. It is unclear which of these possibilities may explain the observed magnitude difference in P_i concentrations and thus further investigation is required to fully elucidate total phosphorous allocation in *C. jamaicense* and *T. domingensis*.

Carbohydrate analyses revealed a somewhat linear increase in the TSC content of *T. domingensis* when P_i was deficient, while in P_i sufficient conditions no differences in allocation were observed (Figure 13b). Previous studies have shown that during periods of senescence, solubilization of insoluble carbohydrates can occur for the purpose of remobilization to other parts of the plant, whereby the principle transport molecule is known to be sucrose (Cherbuy et al., 2001; Champigny, 1985). Furthermore, studies have shown that under short term P_i deficiency, the level of starch increases relative to that of sucrose (Rao and Terry, 1995; Qiu and Israel, 1992; Walters et al., 2004; Flügge, 1999; Rausch and Bucher, 2002; Raghothama and Karthikeyan, 2005). However, Rao and Terry (1995) also show that in *Beta vulgaris* L. long term P_i

deprivation, results in increases in both starch and sucrose, while the level of sugar phosphates decreased. This reduction of sugar phosphates is most likely attributed to increases in the acid phosphatase activity in response to P_i deficiency (Rao and Terry, 1995). Our results indicate an overall increase in the level of both TSC, TIC as well as acid phosphatase activity within *T. domingensis* after 8 weeks of growth in P_i deficient conditions which suggests a similar response to long-term P_i deficiency as also exhibited by *Beta vulgaris* L. (Nanamori et al., 2004; Rao et al., 1990; Rao and Terry, 1995).

While, our results indicate a significant increase in TIC of specific *T. domingensis* shoots, summation of data yielded a significant difference similar to those conclusions previously reported regarding P_i deficiency and starch accumulation at the whole plant level (Figure 14d). A similar pattern was also seemingly present in *C. jamaicense*, however without strong statistical support (Figure 14c). This lack of TIC accumulation in response to P_i deprivation may be attributed to additional TIC accumulation in the non-sampled shoot tissues, however it is more likely that the degree of TIC variability in the older shoot tissue samples of *C. jamaicense* led to this result.

Under extended periods of limited P_i availability, intentional sacrifice of older leaves (i.e. increased leaf senescence activity) can increase the overall P_i available to the plant by reclaiming phosphate groups from otherwise unavailable and immobile sources. Specifically, older shoots that are being senesced are fulfilling a role as a source for the remainder of the younger, more viable tissues (Figure 1). The overall reduction in the number of active tissues and subsequent increased availability of

limited cellular resources (remobilization) is one mechanism by which plants can tolerate periods of prolonged nutrient limitation (Figure 1) (Hopkins and Hüner, 2004). In addition to P_i, other resources have the potential to be recycled from tissues that are senescing in order to diminish inefficient resource allocation as well as to further reduce the rate at which carbon is being lost. Included among these additional recycled resources are quantities of carbon, nitrogen as well as carbohydrates, which can be solubilized for ease of transport (Pieters et al., 2001).

Regarding carbohydrates, it is known that moderate P_i limitations can reduce the cytosolic concentration of P_i and thus lead to an increase in the level of insoluble carbohydrates relative to that of sucrose levels (Flügge et al., 1999) (Figure 2). This mechanism allows for the continuation of photosynthesis production, despite short-term variations in surrounding P_i levels, however if the P_i limitation is prolonged, senescence activity can increase to liberate pools that can be utilized elsewhere in younger tissues. These insoluble carbohydrates located in the oldest shoots are solubilized and then translocated to other portions of the plant (Hopkins and Hüner, 2004). Furthermore, our results show relative ACP activity is greatest in the eldest shoots indicating the possibility that phosphate stress is at its highest level in these tissues and thus direct sucrose production should be at a relative minimum (Figure 12).

Limited P_i availability has generally been shown to have negative impacts on the overall growth rate of many plant species and thus its importance in the natural world as well as agriculture (Raghothama, 1999). In our study, we conclude that P_i availability

dramatically influences the growth rate of *T. domingensis* over *C. jamaicense* (Figures 7, 8 and 10). Additionally, reductions in growth rate have been shown to reduce relative sink strength in species that are influenced by P_i availability (Pieters et al., 2001). The primary factor determining the rate of solubilization and translocation of these carbohydrates has been largely attributed to relative sink strength, i.e. increased sink strength demands increased solubilization and export from source leaves (Hopkins and Hüner, 2004). Therefore, our hypothesis regarding the observed TSC levels in *T. domingensis* (Figure 13) indicates that carbohydrates have been solubilized in accordance with prescribed senescence activity brought on by prolonged periods of limited P_i availability. However, due to the prolonged P_i deprivation and the resulting reduction in the growth rate of *T. domingensis* (Figure 7), we believe that the sink strength of the youngest shoot has been reduced and thus the demand from the source tissues is also reduced resulting in an accumulation of soluble carbohydrates in the older shoot tissues (Figure 13). Figure 17 summarizes this hypothesis depicting the observed and the hypothesized soluble carbohydrate contents of the older leaves in relation to sink strength as influenced by relative P_i availability.

As for the case of *C. jamaicense*, our results detected relatively little change in the soluble carbohydrate content between the two treatment groups of the various shoot and root tissues indicating that P_i may not be limited in these tissues. One theory regarding *C. jamaicense* suggests the ability to tolerate prolonged periods of limited P_i availability due to potentially more effective acquisition by and accumulation of P_i in the

root tissue (Figure 15a) as well as a hypothesized overall lower cellular requirement for P_i (Kuhn et al., 2002). These two mechanisms may have increased the capacity of *C. jamaicense* to thrive in the historical Everglades ecosystem while preventing the succession of other, more P_i-dependent species such as *T. domingensis*. Additionally, the restricted consumption of P_i would result in the ability to maintain an exceedingly consistent level of P_i in the cytosol although P_i availability in the environment is variable. This consistency would allow for the observed, unaltered production levels of both insoluble and soluble carbohydrate in relation to P_i concentration (Figures 13 and 14).

Rao et al. (1990) suggest that P_i deficiency can alter carbon allocation patterns to structural carbohydrates such as hemicelluloses and cellulose. Rao and Terry (1990) also suggest that increases in leaf sucrose concentration under P_i deprivation is predominantly as a result of the reduced exportation of sucrose rather than increased synthesis or catabolism. While other shoots of *T. domingensis* showed similar content of TSC and TIC, independent of P_i treatment, concurrent increased levels of TSC and TIC in the older shoots of *T. domingensis* under P_i deficiency, potentially provides further evidence for differences in carbon allocation under short-term versus long-term P_i stress conditions (Figure 13b and 14b). Rao et al. (1990) suggest that the relationship between P_i deficiency and photosynthate partitioning is more complex than previously discussed and is most likely controlled by the enzymes associated with photosynthate metabolism.

Consistent with the previous findings, (Davis et al., 1994; Davis, 1994a; Newman et al., 1996) our observations indicate that *T. domingensis* may employ a more

opportunistic approach towards growth and survival while *C. jamaicense* is more tolerant of fluctuations in environmental conditions, specifically P_i. The concept of phenotypic plasticity states that genetically identical individuals who are separated and raised under different conditions elicit different physiological, morphological or behavioral responses (Krebs, 2001). In conclusion, the data in our study suggests that the phenotypic plasticity of *C. jamaicense* is markedly less than that of *T. domingensis* and this difference has influenced the historical and more modern interactions between the two species. Figure 18 summarizes the relative impact of each of the factors influencing the observed dominance of *T. domingensis* over the displaced *C. jamaicense* as identified in our study as well as compiled from Davis (1991); Davis et al. (1994) and Davis (1994a).

The severity to which *T. domingensis* has become dominant in certain areas of the Florida Everglades system is an issue that has developed as a result of several different factors, which collectively have led to displacement of *C. jamaicense* (Figure 18). Primarily, this domination has occurred as a result of nutrient enrichment, however in order for one species to displace another, a reduction in the number of physical individuals is necessary. Displacement can be accomplished through a variety of means, both physical and non-physical, including competition effects, increases in disturbance frequency and/or dramatic alteration of environmental regimes. In addition to nutrient enrichment, altered disturbance frequencies such as flooding and fire can physically stress or remove current species allowing for the more opportunistic species to take

hold (Davis, 1994a). Additionally, other factors have been and could possibly play a role in the dominance of *T. domingensis*, including increased foraging and seed dispersal, allelopathic effects as well as possible secondary effects of nutrient eutrophication on soil microbes (Gallardo et al., 1998). In certain cases, eutrophication has been shown to alter the number of native bacterial, fungal and also certain macro and microinvertebrates in the soil thereby conferring or eliminating potential interactions (Davis, 1994a).

Darwinian evolutionary theory suggests that survival of an organism is not by random chance, but rather it is determined by the ability to adapt or loose via natural selection, any attribute or ability in which would give an advantage, compared to that of another in the same or similar category, under a particular set of circumstances (Darwin, 1859). Natural selection is defined as the process by which adaptations are produced from interactions of genes and the environment (Freeman and Herron, 2004). Thus populations that accumulate an adaptation have the capacity to do so based on genetic variation. In other words, genetic variation among a population allows for the possibility of different adaptations to be accumulated. Evolutionary theory tells us that naturally selected adaptations will become more common over time and may even be ubiquitous within the population.

Our study supports the notion that *C. jamaicense* has accumulated such specific changes to allow a higher degree of success in environments where nutrient resources are extremely low and thus has led to a higher degree of tolerance to P_i deprivation

than the more phenotypically plastic, opportunistic, responses seen in *T. domingensis*. Further examination into what specific metabolic gains, losses or alterations have allowed for this development, would ultimately provide a better understanding of species specific interactions and responses, not only as an example in an ecologically, restorative context, but also in the broader context of general plant nutrition, stress tolerance and agriculturally important issues.

TABLES

Table 1. Nutrient composition of + P_i and – P_i hydroponic media.

	1000µM (1L)	0µM (1L)
0.5M $Ca(NO_3)_2$-$4H_2O$	10mL	10mL
0.5M KNO_3	10mL	10mL
0.1M KH_2PO_4	10mL	-
0.1M KCl	-	10mL
0.2M $MgSO_4$-$7H_2O$	10mL	10mL
1000x FeNaEDTA	1mL	1mL
Trace Minerals*	10mL	10mL

*Trace minerals contain 10mM H_3BO_3, 0.01mM $CoCl_2$-$6H_2O$, 0.01mM $CuSO_4$-$5H_2O$, 10mM $MnCl_2$-$4H_2O$, 0.5mM KI, 0.1mM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.03mM $ZnSO_4$-$7H_2O$.

Table 2. Mean shoot number upon dissection of *C. jamaicense* and *T. domingensis*.

	LP	HP	Sig.
C. jamaicense	12.833 ± 0.307	12.667 ± 0.333	-
T. domingensis	4.500 ± 0.224	8.500 ± 0.342	+

Mean shoot number determined for samples after being grown at low and high P_i concentrations ($p < 0.05$, n=6). R:S ± SE_{Mean}

Table 3. Mean R:S ratios in *C. jamaicense* and *T. domingensis.*

	LP	**HP**	***Sig.***
C. jamaicense	.354 ±.031	.311 ±.014	-
T. domingensis	.506 ±.060	.206 ±.015	+

R:S determined for samples grown at low and high P_i concentrations ($p < 0.05$, n=6). R:S ± SE_{Mean}

FIGURES

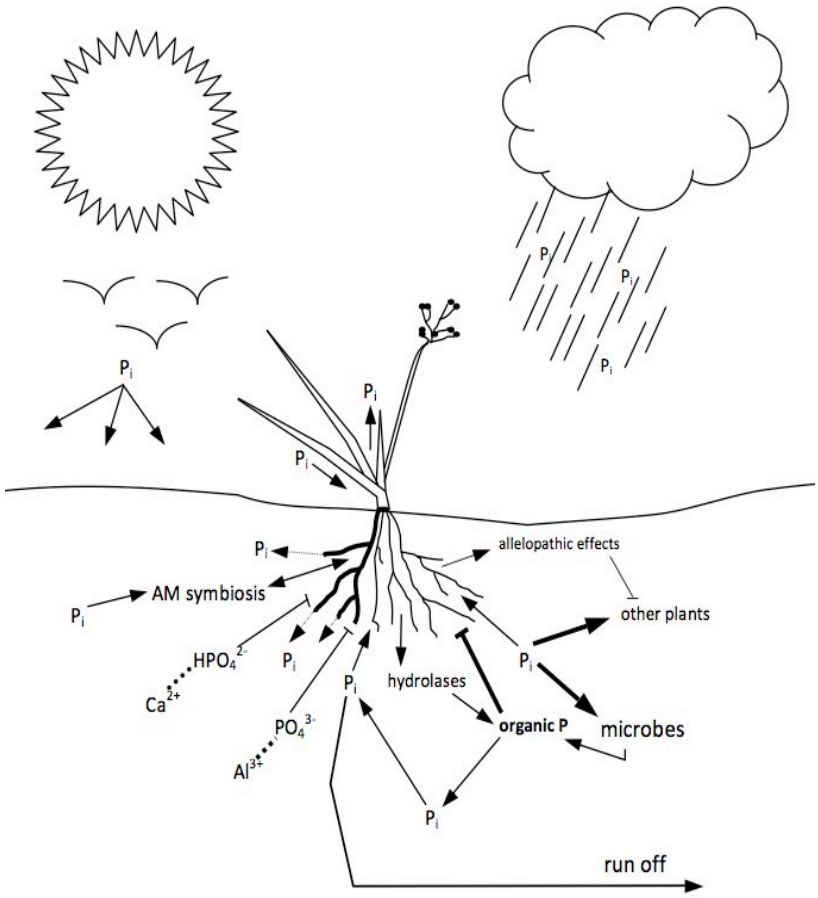


Figure 1. Theoretical model summarizing factors influencing P_i supply availability and acquisition from the surrounding environment in a typical monocot species. Bold arrows indicate increased competition. Dashed arrows represent increased exploration for additional P_i resources in the rhizosphere via adventitious root formation while bolded roots indicate increased root surface area by means of increased root diameter and root hair proliferation.

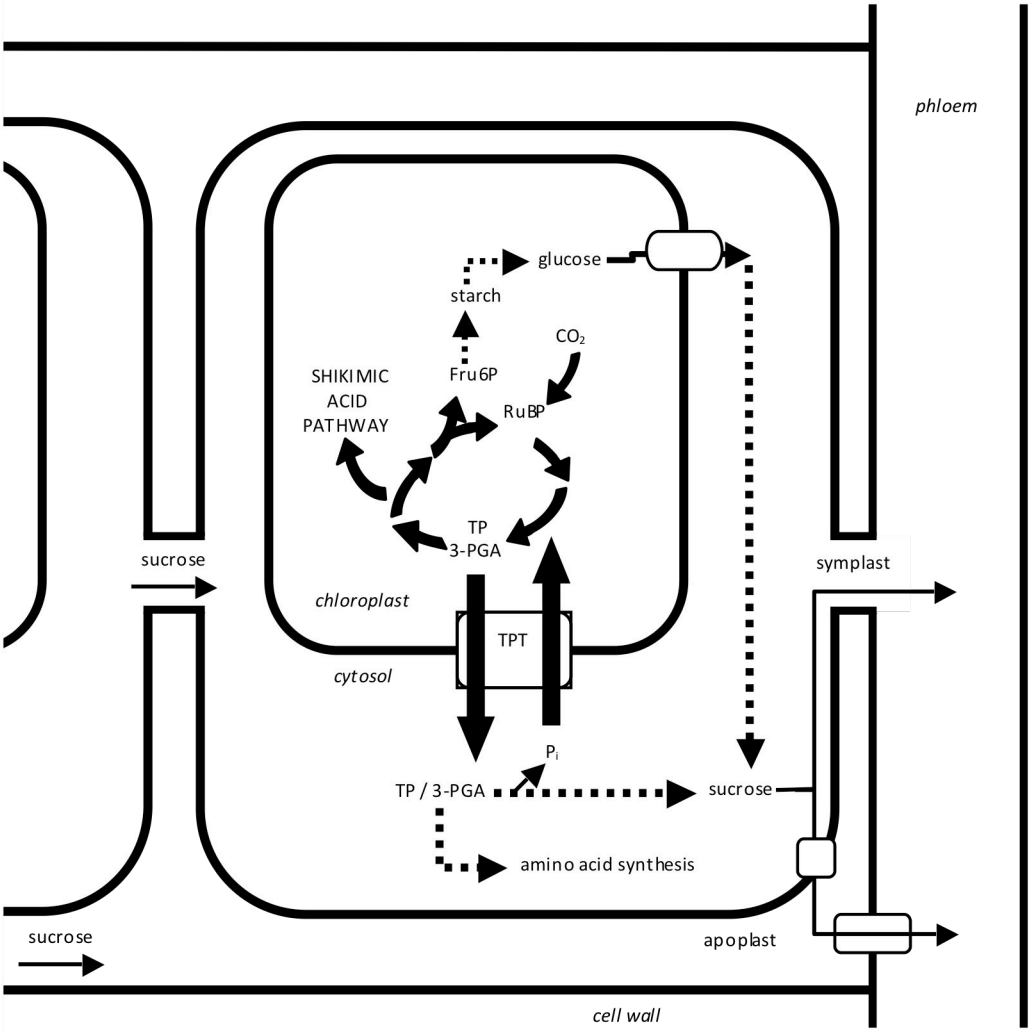


Figure 2. Transport and the influence of P_i availability on the synthesis of carbohydrates in plants. Triose phosphate (TP) and 3-phosphoglycerate produced by the fixation of CO_2 by Rubisco (RuBP) is exchanged for cytosolic inorganic phosphate (P_i) via the triose phosphate/ phosphate translocator (TPT). The activity of the TPT is reduced when P_i availability is limited thus a net increase in the level of starch synthesis occurs. Additionally, starch can be solubilized for transport to other sink tissues by degradation into glucose monomers and formed into transportable sugars such as sucrose.

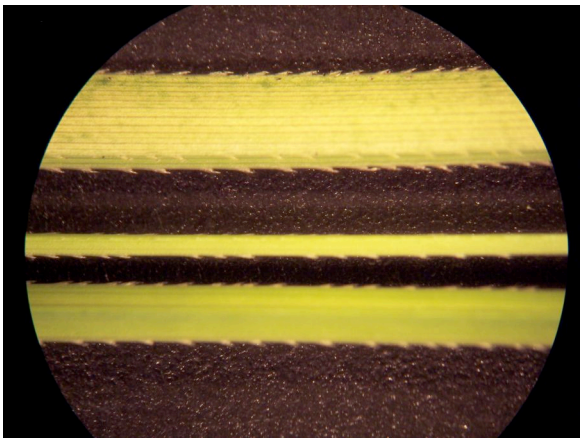


Figure 3. Photograph of the characteristic saw-toothed leaf margins found on *Cladium jamaicense*, or sawgrass.

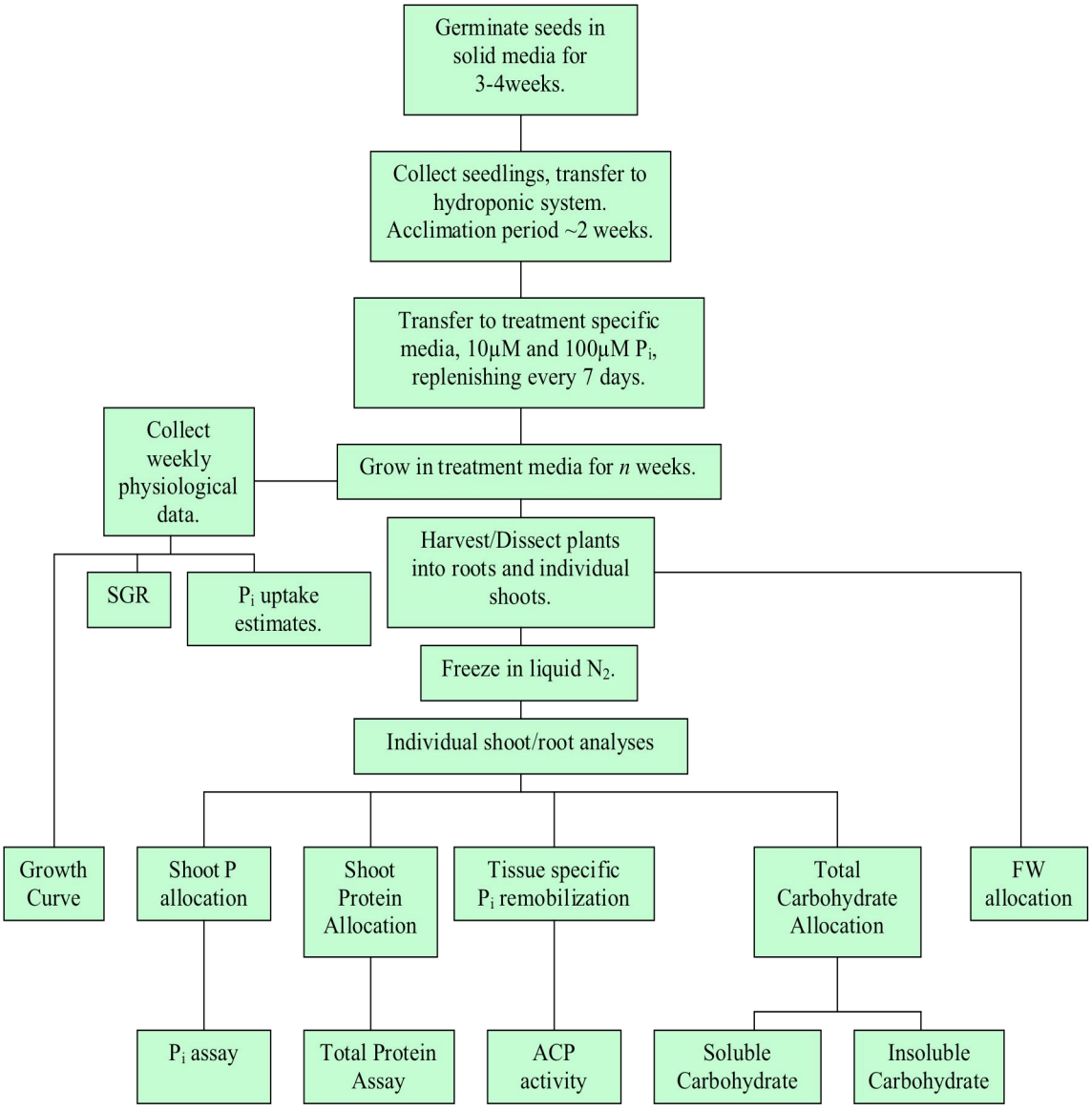
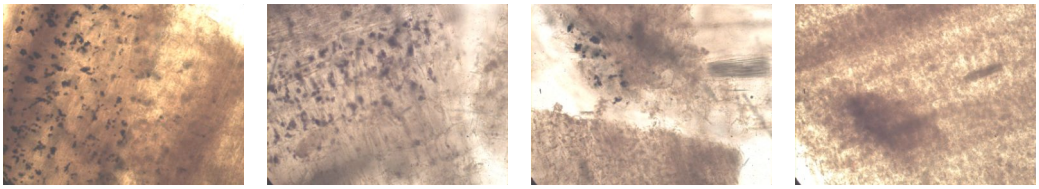


Figure 4. Flowchart outlining the experimental strategy used.

Figure 5. Example of hydroponic system design. Plastic tubing supports (not visible) were engineered and implemented to provide a consistent height of sample replicates in relation to the hydroponic media as well as within the container.



C B 1 3

Figure 6. Example of the relative effectiveness of mechanical starch liberation and gelatinization treatments of shoot tissue samples via boiling 10 min. (B) or autoclaving for 1 or 3 hours (1 and 3 respectively), compared to that of the control.

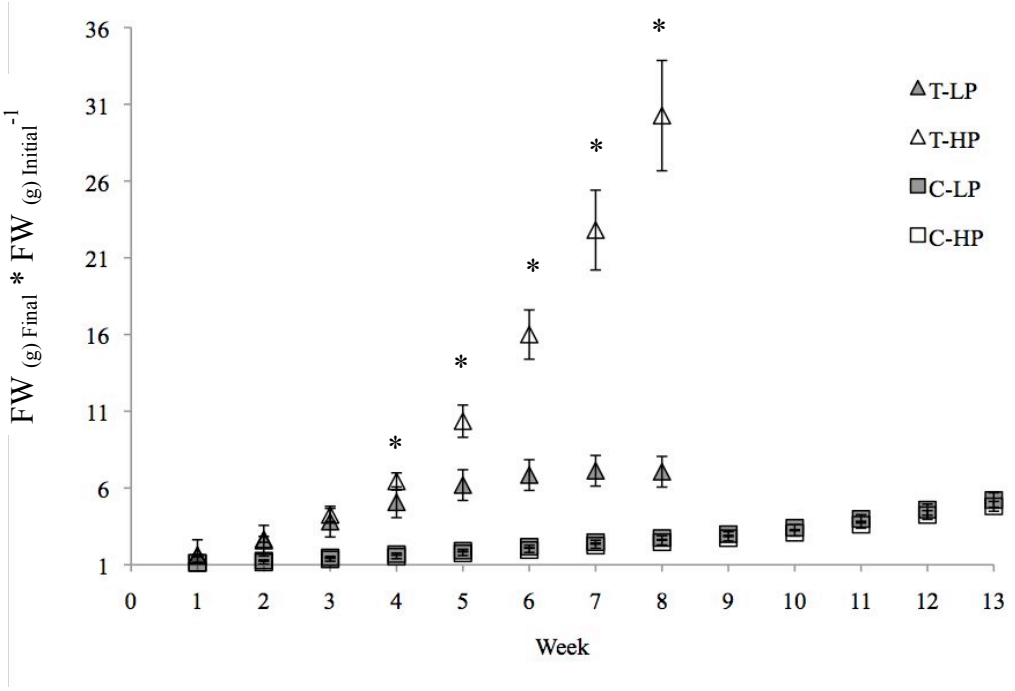


Figure 7. Normalized growth per week of *C. jamaicense*, squares, and *T. domingensis*, triangles, when grown at HP, open, and LP, shaded. (*) Indicates significance between treatment groups. ($p < 0.05$; $n = 6$) Values ± SE_{Mean}.

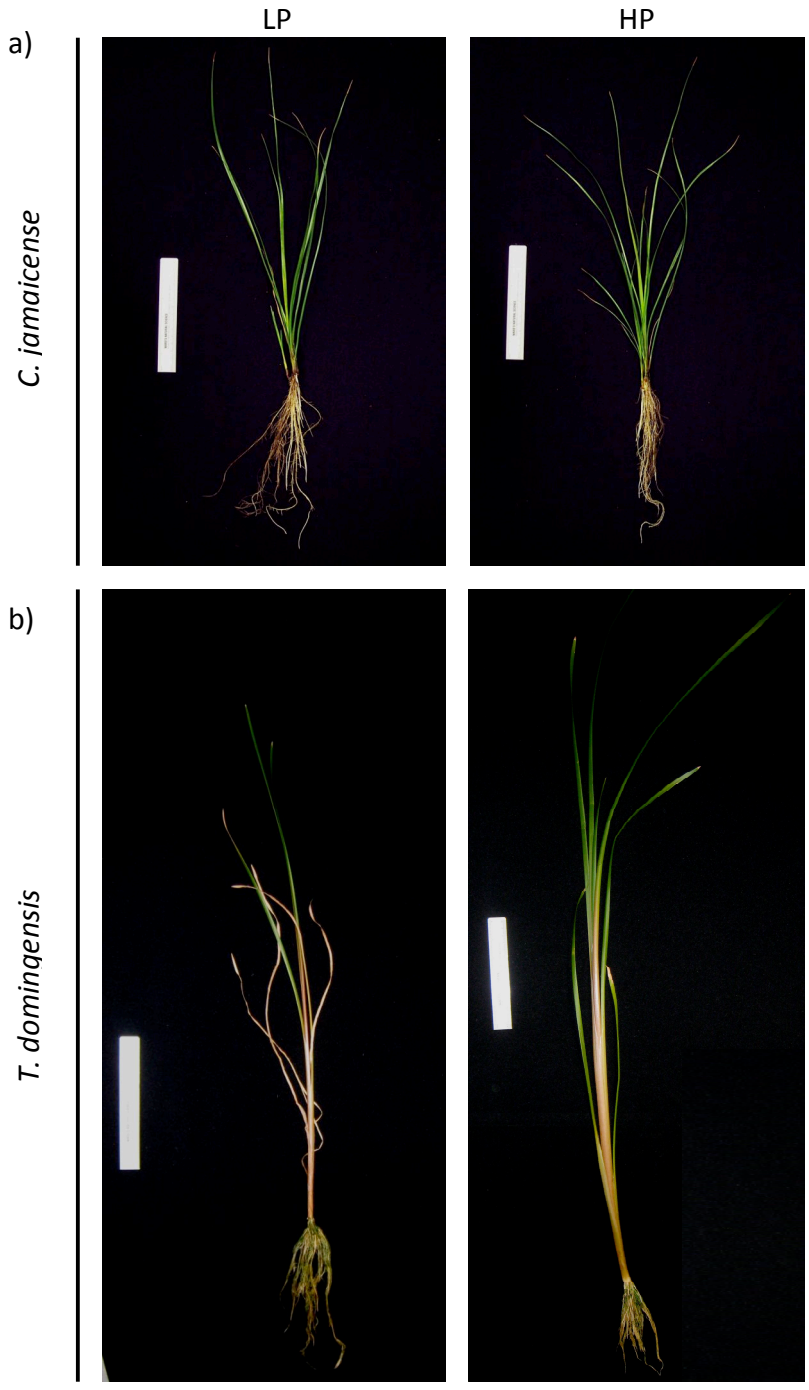


Figure 8. Phenotypic comparison of *C. jamaicense,* a) and *T. domingensis,* b) at the LP and HP treatment levels. Scale is equivalent to 15cm in length.

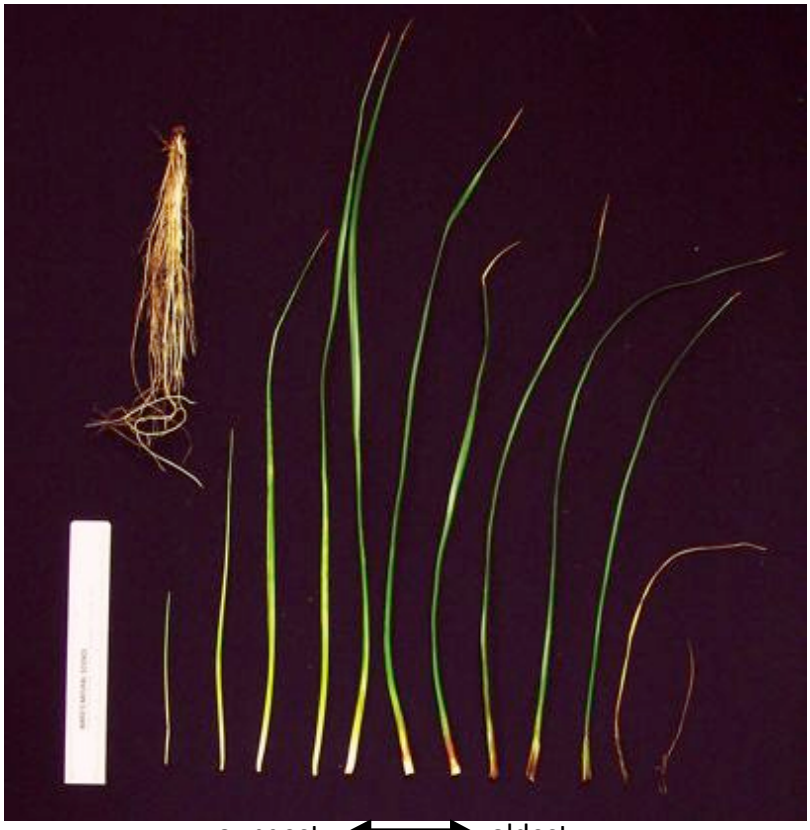


Figure 9. Example of a dissected sample replicate into root and individual shoot tissues. Shoot tissue dissected based on arrangement, increasing in age from left to right. Scale is equivalent to 15cm in length.

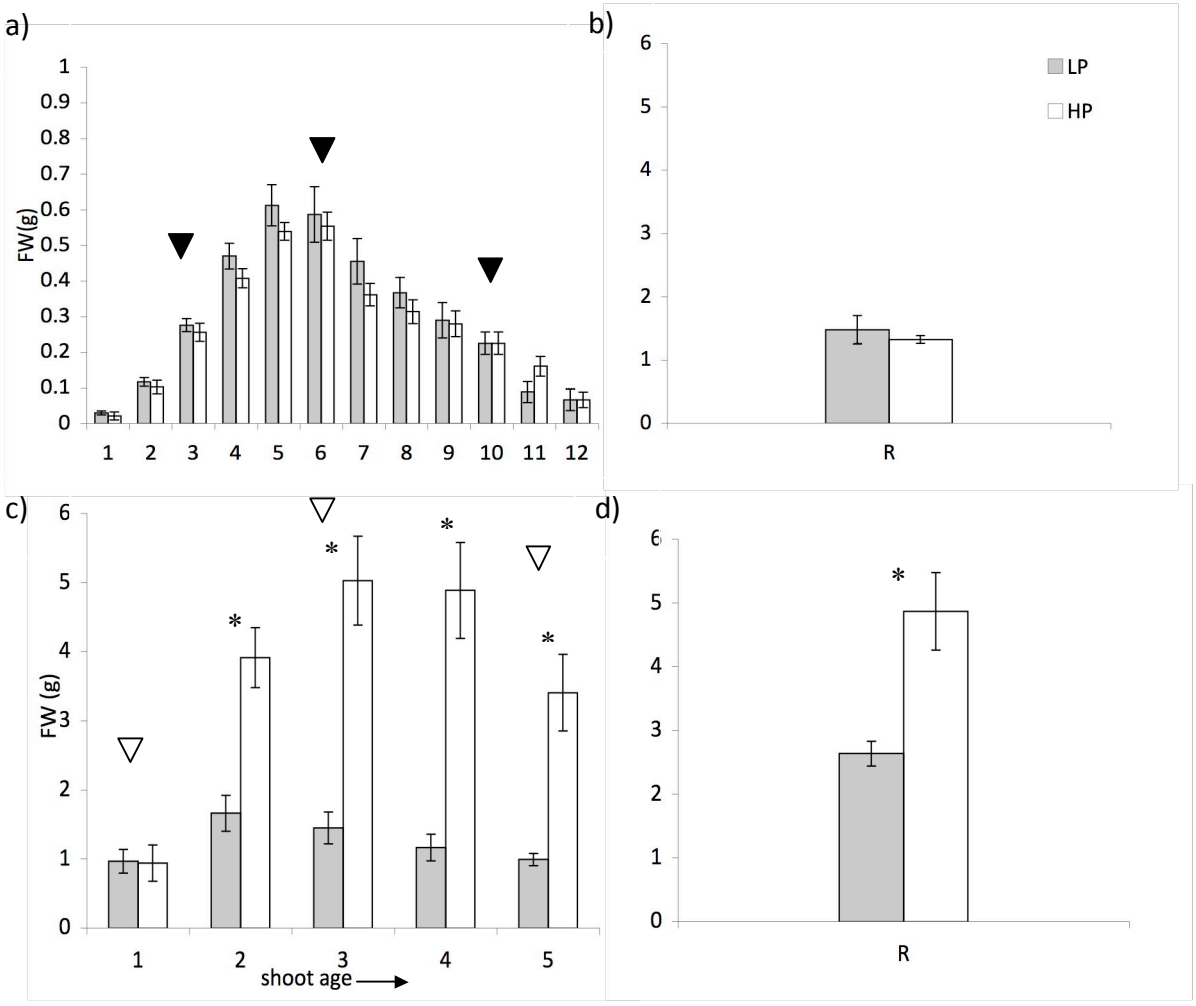


Figure 10. Fresh weight (g) allocation of *C. jamaicense* (a and b) and *T. domingensis* (c and d) root tissues (b and d) and individual shoot tissues (a and c) when grown at high and low phosphate conditions. For *C. jamaicense*, shoot numbers 3, 6 and 10 (darkened arrowheads) correspond to Y, M and O while for *T. domingensis*, shoot numbers 1, 3 and 5 (open arrowheads) correspond to Y, M and O. (*) Indicates significance between treatment groups. ($p < 0.05$, n=6) Values ± SE_{Mean}.

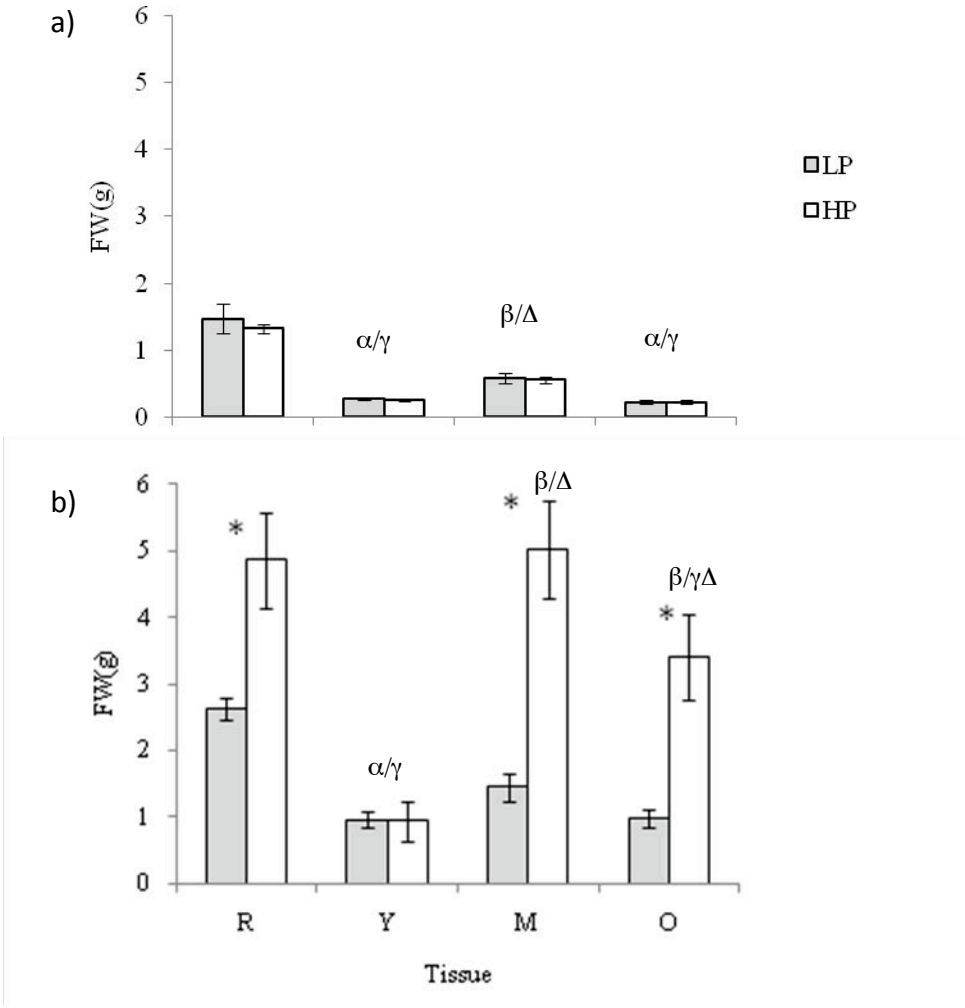


Figure 11. Fresh weight (g) of age specific tissue selected for sampling: root (R) and shoot tissues (Y, M and O) for both *C. jamaicense* (a) and *T. domingensis* (b) when grown at high and low phosphate. (*) Indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, n=6) Values ± SE_{Mean}.

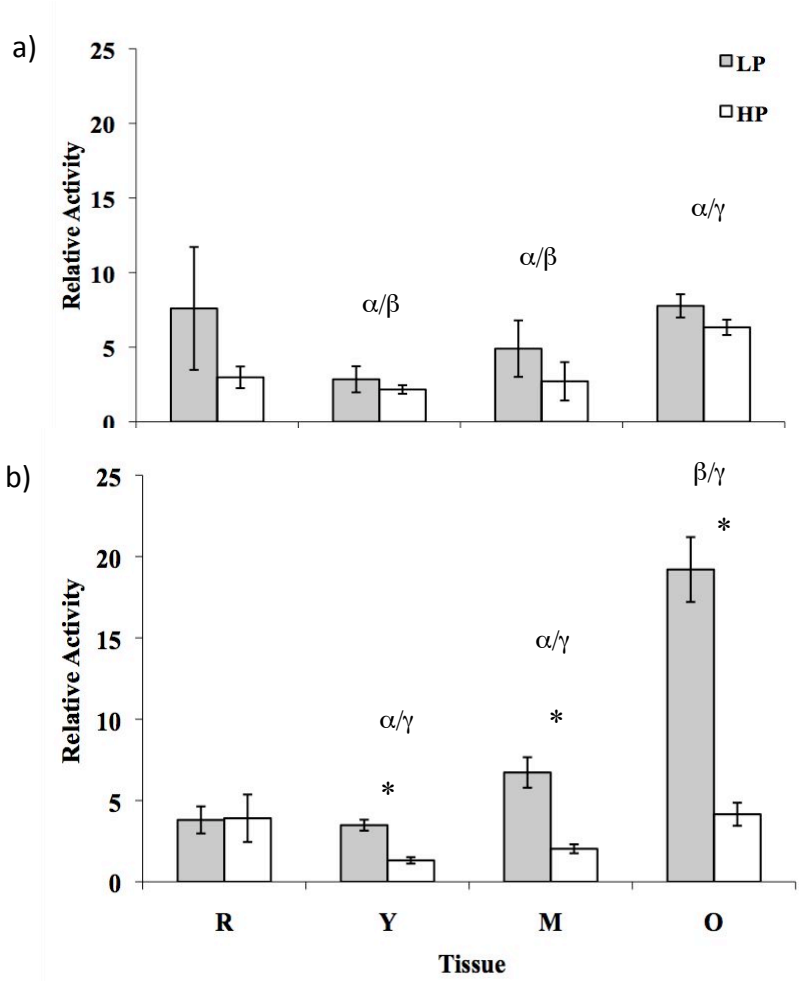


Figure 12. Relative acid phosphatase activity in *C. jamaicense*, a) and *T. domingensis*, b) root (R) and individual shoot tissues (Y, M and O) when grown at high and low phosphate concentrations, LP and HP respectively. (*) indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, $n = 3$) Values ± SE_{Mean}.

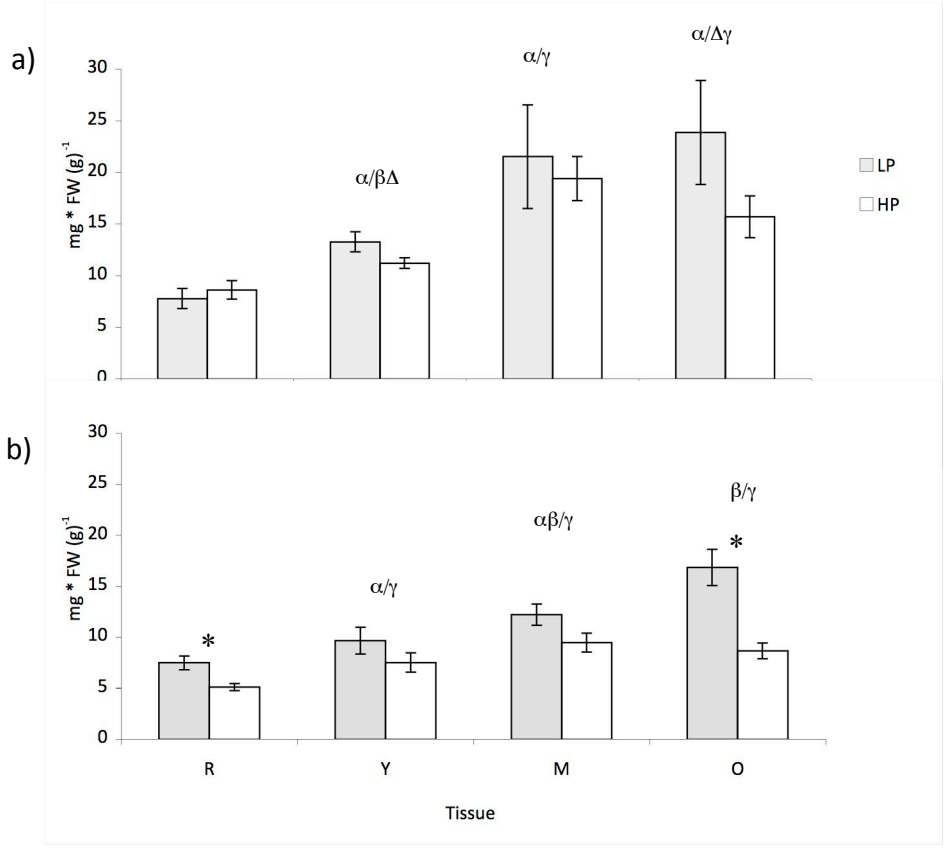


Figure 13. Total soluble carbohydrate in *C. jamaicense* (a) and *T. domingensis* (b) in both root (R) and shoot tissues (Y, M and O) when grown at LP and HP. (*) Indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, $n = 3$) Values ± SE_{Mean}.

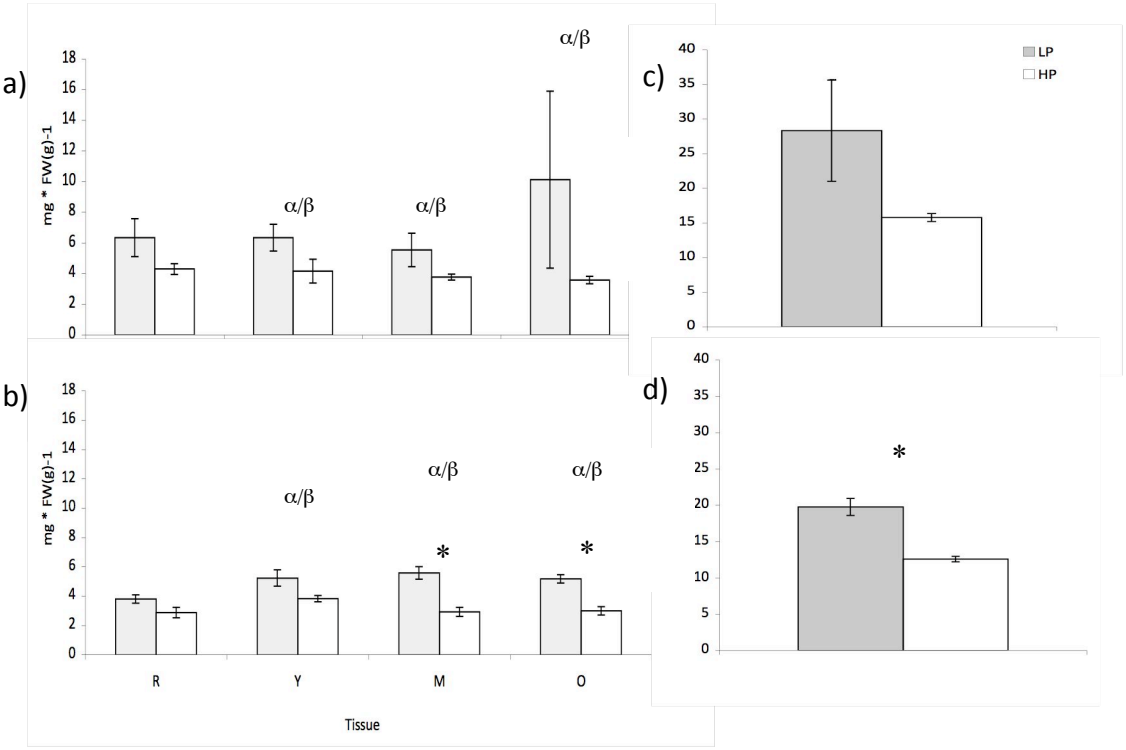


Figure 14. Total insoluble carbohydrate, TIC, in *C. jamaicense* (a) and *T. domingensis* (b) in both root (R) and shoot tissue (Y, M and O). c) and d) represent sum total TIC of R, Y, M and O tissues for *C. jamaicense*, c) and *T. domingensis*, d). (*) Indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, $n = 3$) Values ± SE_{Mean}.

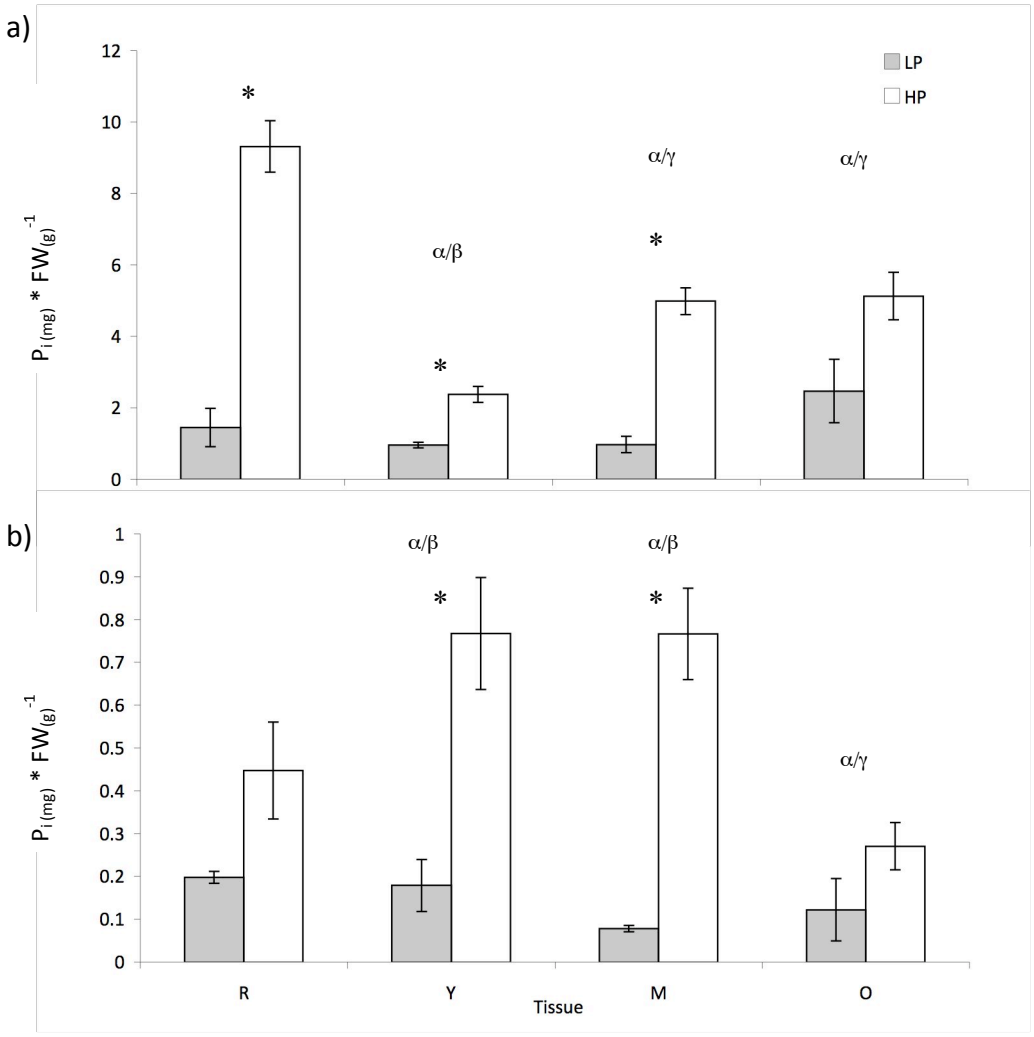


Figure 15. P_i accumulation in both *C. jamaicense* a) and *T. domingensis* b) within specified tissues root (R), Y, M and O when grown at LP and HP treatment levels. (*) Indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, n=3) Values ± SE_{Mean}.

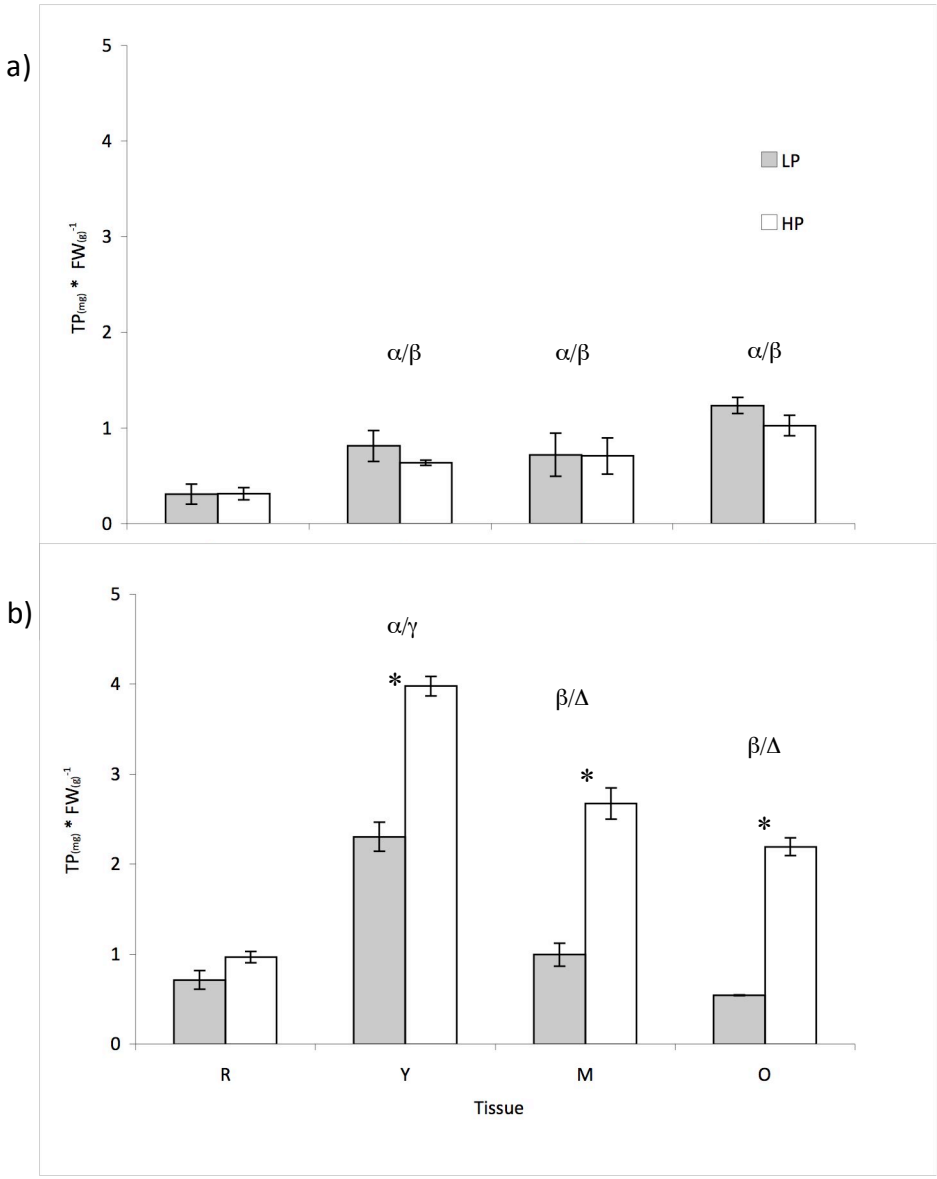


Figure 16. Total protein content of both *C. jamaicense* a) and *T. domingensis* b) root (R) and Y, M and O shoot tissues, when grown at LP and HP. (*) Indicates significance between treatment groups. Greek letters represent significance among the shoots for LP or HP treatments, LP/HP, respectively. ($p < 0.05$, n=3) Mean values ± SE_{Mean}.

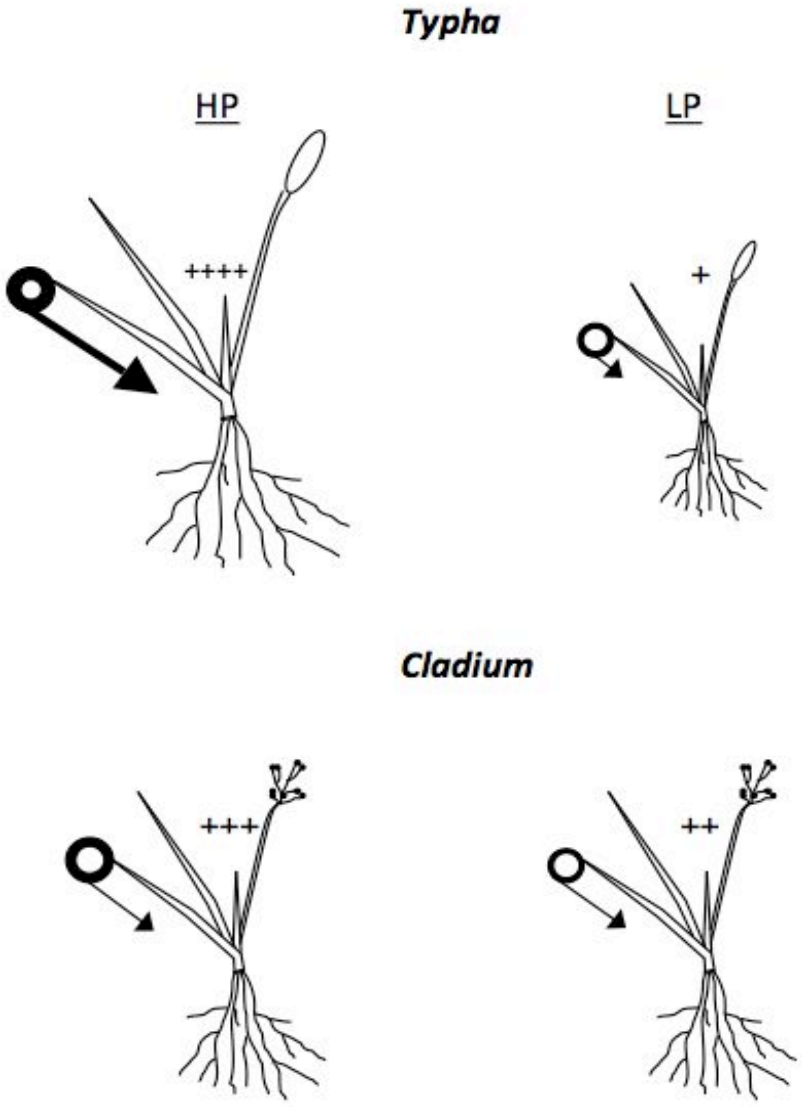


Figure 17. Hypothetical model of TSC trafficking in relation to the sink strength as determined by the P_i status of both *T. domingensis* (top) and *C. jamaicense* (bottom) under deficient and sufficient conditions. Darkened rings represent proportion of TSC being exported from older non-senescing shoots. Empty, interior circles represent proportion of TSC retained in older shoots. (+) signifies relative sink strength of the youngest apical shoot. Relative sink strength is a product of the sink size and activity level.

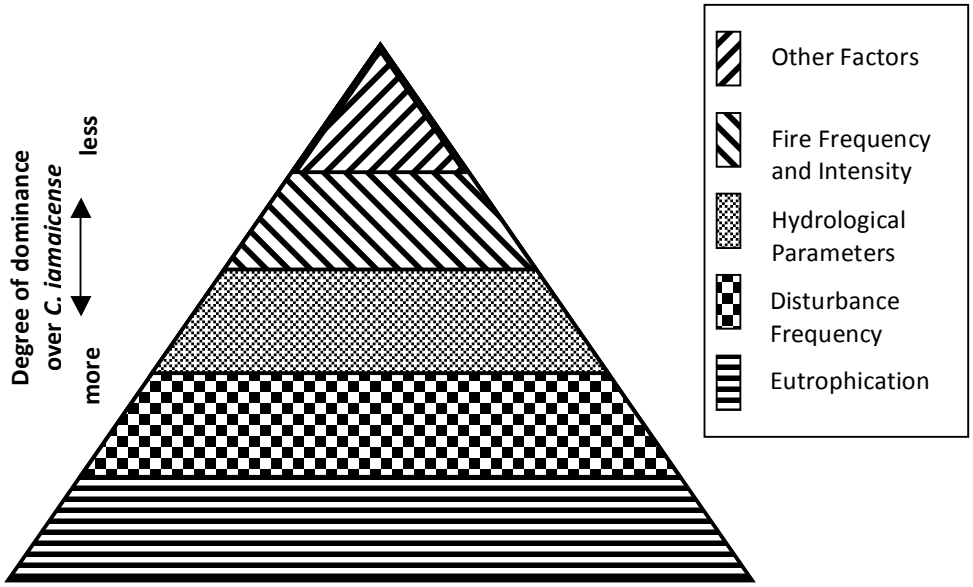


Figure 18. Conceptual model of factors influencing *T. domingensis* dominance over *C. jamaicense* in the modern Florida Everglades system.

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