Cooperative Extension and Propagation for Caño Negro, Costa Rica

by

Rachel Allen Meriwether

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Approved by

Amy Noelle Wright, Chair, Associate Professor of Horticulture
Kenneth Tilt, Professor of Horticulture
Robert Boyd, Professor of Biological Sciences

Abstract

A research relationship was established with colleagues in Costa Rica. These included not only members of academia but also non-profit organization workers, government officials, community members, and private research partners. After extensive consultation with an advisory committee formed by members from each of these entities, a research project was formed. Caño Negro National Wildlife Refuge was identified as an optimal location to begin research by all parties, and two plant species native to this location were selected on which to begin propagation experiments. *Hylocereus stenopterus* cuttings were subjected to several different treatments to determine the most effective propagation technique. Half of the cuttings were stuck immediately, while the other half were allowed to remain uncovered on a bench in the greenhouse for 72 h (cicatrization). Prior to sticking, the basal ends of half of the cuttings were treated with a commercial rooting hormone while the other half received no hormone. Once stuck, half of the cuttings were covered with a plastic tent. Thus, treatments were arranged in a 2 hormone x 2 cicatrization x 2 tent factorial design or 8 total treatments with 20 replications per treatment for a total of 160 cuttings. Additional cuttings of *Hylocereus undatus* and *Hylocereus costariensis* received one of three hormone treatments: untreated (no hormone applied, control), 0.1% indole butyric acid (IBA) or 0.8% IBA. *H. stenopterus* cuttings responded well to both rooting hormone and mist tent but showed very little response to cicatrization time. However, cuttings from all three species

showed excellent survival, vigorous root growth, as well as subsequent shoot growth regardless of treatment. The same was true of *H. undatus* and *H. costariensis*. *H. costariensis* had a 100% survival rate regardless of treatment. Experiments were also conducted on the palm *Acoelorraphe wrightii*. Seeds of this plant were subjected to several preplanting scarification techniques including manual scarification, hot water soaking, and sulfuric acid scarification. In all experiments, seeds scarified by either acid or hot water had higher germination percentages and rates than untreated (control) or manually scarified seeds. Highest germination percentages were observed in the 1:1 peat:perlite mix.

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Literature Review

Costa Rica

Ecotourism has become one of the most important contributors to the Costa Rican economy, surpassing in 1993 the earnings for export products such as coffee and bananas (ICT, 1993). The rise of ecotourism to this point began in the 1950s when, around the world, international tourism began to increase (Latham, 1994). In 1955 the Costa Rican Tourism Institute was established, and in 1960 tourism was officially designated an 'industry' within the country (Campbell, 2002). Over the next several decades, ecotourism expanded rapidly and Costa Rica established itself as the most popular Latin American ecotourism destination (Schluter, 1993). Unfortunately, wherever it is found, ecotourism can be responsible for the loss of local employment when companies bring in outside employees as well as destruction of the environment upon which it relies (Wild, 1994). Some of the negative effects of ecotourism have been attributed to the lack of local ownership of not only hotels and hostels but also tour companies, restaurants, shops, and assorted associated businesses (Burton, 1998; Honey, 2008). If owners were also local inhabitants, there might be a stronger local investment in the long term conservation goals of a sustainable ecotourism program. This theoretical assumption goes hand-in-hand with a general shift in conservation strategies away from exclusionary tactics toward consumptive use conservation, where local inhabitants are allowed access to and use of resources in a sustainable manner, and community based conservation

(CBC) projects. CBC programs are those that focus efforts not only on conserving an area and its environment but doing so in a way that actively involves the local population in activities, planning sessions, and any eventual profits.

Perhaps one of the most well developed consumptive use programs in Costa Rica to date is the Payments for Environmental Services (PES) program (Pagiola, 2006). Begun in 1997, this program offers a method for administrators and land owners to translate external, non-market services provided by the environment into actual financial incentives. Currently, the PES program recognizes four services provided by Costa Rican forests: 1) mitigation of greenhouse gases; 2) hydrological services; 3) biodiversity conservation; and 4) provision of scenic beauty for recreation and tourism (Pagiola, 2008). Interested land owners can apply and, if accepted, will receive monetary compensation for allowing lands that provide at least one of the above services to remain in natural forest cover. As of 2000, roughly 300,000 hectares of land were receiving funding through the PES program with a waiting list of similar magnitude (Pagiola, 2008).

One example of a CBC and consumptive use program is the legal harvest of green sea turtle eggs in the Ostional Wildlife Refuge (Campbell, 1999). The village of Ostional lies within the boundaries of the Refuge, both of which are located along the Pacific coast of the country on the Nicoya Peninsula. This area is home to large numbers of nesting sea turtles, particularly the olive ridley sea turtle (*Lepidochelys olivacea* Eschscholtz), and these turtles have become a successful tourist attraction (Campbell et al., 2007). In conjunction with the tourism revenue, members of the community take part in a legal egg harvest. Eggs are then packaged onsite and distributed nationwide as a food product.

Ostional community members and researchers from the University of Costa Rica worked together to generate a legal harvest limit based on natural egg losses due to unique olive ridley nesting behavior. Olive ridley turtles at Ostional, unlike not only other species of sea turtle but also other groups of olive ridleys in different locations, congregate for mass nesting events (Hughes & Richard, 1974). These last from three to ten days and usually occur once a month. Eggs laid on the first days of these events would normally be lost as other turtles arrive and dig new nests on top of the ones already present. Thus it was determined that harvesting the first wave of laid eggs did not affect the overall population of olive ridley sea turtles (Cornelius et al., 1991). Researchers from the University of Costa Rica and Ostional community members lobbied the government for permission to harvest eggs, which they were granted upon meeting the condition of creating a governing body. This body was called the Ostional Development Association and became active in 1985. Since that time the project has yielded recognizable socioeconomic benefits, including, but not limited to, financial assistance for the community from the Costa Rican Tourism Institute, involvement of the community with the University of Costa Rica (which has provided a research lab and nature guide training to interested parties), and 70% of all households surveyed in the Ostional community in 2007 reported egg harvesting as their primary source of income (Campbell et al., 2007).

In northern Costa Rica the Caño Negro National Wildlife Refuge (NWR) is an area with the capacity for both conservation collaboration as well as the opportunity for economic development. Though the majority of the country is relatively stable economically, this remote region is facing other challenges. The Costa Rican economy currently relies heavily on agriculture, electronics exports, and tourism (CIA, 2010).

Neither of the first two options is feasible in the Caño Negro area: agriculturally because of the sensitive nature of the various ecosystems that dominate the region and electronics manufacture is unreasonable due to the remote nature of the village as well as the lack of necessary infrastructure. Furthermore, the few available jobs in the region are subject to decreased wages due to extensive illegal immigration from neighboring Nicaragua. It has been suggested, however, that there is potential for program development and capitalization of the ecotourism industry which, if handled properly, could be a boon to both the economy as well as the ecosystem (Orozco-Obando, 2009). Internationally targeted cooperative extension programming can bring together not only the local and national resources of an area but also a global perspective that can aid the development of ecosystem assets and enhance both economic opportunities and conservation goals (Harder et al., 2010; Ludwig, 2002; Place et al., 2008).

The Caño Negro NWR is also highly significant as an ecosystem. The majority of the area is a tropical dry forest, the most endangered large ecosystem on Earth (Janzen, 1988). It also contains portions of a vast seasonal wetland that feeds Lake Nicaragua to the north and has extensive populations of birds, caimans, and unique plant life. Caño Negro has been recognized by the Ramsar Convention as a wetland of international significance (RAMSAR, 2010) and is also part of the Mesoamerican biological hotspot established by the organization Conservation International (CI, 2010). It is also one of the most remote parks in Costa Rica's national park system and has, thus far, escaped the degradation of native plant and animal communities often associated with increased in visitor traffic. Economic opportunities in the area are associated with the wetland and, as tourism continues to expand, improper planning might introduce these problems to Caño

Negro as well. The area is also in danger from large scale agricultural interests. As international pineapple growers are shifting out of Hawaii and into cheaper production areas, most notably Brazil and Costa Rica, the wetland areas of Caño Negro have come under particular attack (Niesse, 2006). Portions of the wetland have already been drained to make way for pineapple fields, and Caño Negro community members are being actively approached with regard to selling their lands for more of the same (Ruiz, 2010).

Program Development

The overall and ongoing goals of this program will be to facilitate rural community economic development and bring in supplemental income for local citizens by providing new crops with commercial value as well as to establish an ecotourism destination in the village of Caño Negro which is located within the Caño Negro NWR. The effect of ecotourism on both rural development and conservation goals has been studied in several countries with mixed results that are attributed to several variables (Bookbinder et al, 1998; Campbell, 1999; Hvenegaard & Dearden, 2002; Potvin et al., 2003; Trejos & Chiang, 2009). The proximity of a protected area to Caño Negro represents an opportunity for collaboration with larger government entities and promises a higher chance of success in the development of the local economy (Plummer & Fennell, 2009). Utilizing recommendations from previous research, our educational extension program will provide farmers and local residents an economic alternative to selling their lands to the large pineapple producers that are moving into the area (Ruiz, 2009). This will help not only preserve a unique and beautiful community but further protect a globally significant area of species diversity.

To develop an extension program in Costa Rica, extensive research was done to identify appropriate collaborating entities. These included the Universidad Nacional de Costa Rica (UNA), the Instituto Tecnologico de Costa Rica (ITCR), the Instituto Nacional de Biodiversidad (INBio), the Organization for Tropical Studies (OTS), ProNativas, the Ministerio de Ambiente y Energia (MINAE), and the Ministerio de Agricultura y Ganaderia (MAG). Preliminary research to identify regional native plant species appropriate for this project was corroborated by consultation with Costa Rican academic colleagues, regional experiment stations, and community farmers. Individuals from each of these groups have formed an informal working group and advisory committee that provided local and national educational and political contacts to facilitate both conservation and development activities.

Administration and faculty from UNA were among the first contacts made when efforts began to evaluate the biological, political, and economic factors associated with the feasibility of this project. A relationship had already been established in February 2008 between Auburn University (AU) and UNA personnel. Later that same year a memorandum of agreement between AU and UNA was drafted and signed in order to facilitate future cooperative efforts between these two institutions. This project represents the next step in the development of that relationship.

UNA is a public institution and therefore, within Costa Rica receives funding directly from the government (UNA, 2010). It is a four year university with both undergraduate and graduate degrees in the social sciences and multiple fields within the biological sciences, as well as philosophy and liberal arts. It includes six separate

campuses; the main campus is in San Jose with satellite campuses in Sarapaqui, Nicoya, Benjamin Nunez, Coto, Perez Zeledon, and Liberia.

Another university contacted in Costa Rica that focuses on agriculture and the biological sciences in general is ITCR. ITCR also concentrates on community and applied technologies. Interdisciplinary studies are recognized as being of great benefit and every effort is made to foster collaboration with other universities. There is a fully developed distance learning PhD program which is organized in conjunction with several universities in several countries.

INBio is a nonprofit organization that has been expanding its influence across the globe. Started in 1989, INBio has become an international authority on programs and projects that conserve and promote tropical biodiversity (INBio, 2010). During the late 1980's Costa Rica officially recognized the need to protect its wealth of biodiversity by passing Executive Decree No. 19153. Among other things, this decree established the Planning Commission for the National Institute of Biodiversity, a board composed of members from various governmental institutions and also from higher education centers and conservation organizations. This board recommended to the Costa Rican government that a state biodiversity institute be created which functioned with a high degree of autonomy. Due to issues within the government itself, this recommendation was ignored. At this point members of the board decided to take the situation into their own hands and create a private non-profit organization. This institution was granted official recognition by the standing government and has been supported by successive governments as well. The members then went about the laborious process of securing funding, eventually finding it in the form of a loan for 80 million colones as well as

backing from the Swedish Cooperation Agency (SIDA) and the MacArthur Foundation. On 26 October 1989 a location was selected in Santo Domingo de Heredia, and this is where INBio is still housed today (INBio, 2010). Several services are now offered through INBio including bioprospecting, consulting, education and training, as well as general information services. In 2000, a theme park called INBioparque was added as well. Visitors can tour an organic farm, view a green built house, enjoy a butterfly garden, as well as many other nature based attractions. Atta, the database where all information generated and collected by INBio is stored, is an extensive resource and almost indispensible for someone working with tropical biodiversity.

OTS is another major research association in Costa Rica. AU has a long standing relationship with this organization and has been an OTS member institution since 1987. Classes are offered on a number of subjects pertaining to tropical ecology at both the graduate and undergraduate levels and in multiple countries. There are three research stations in Costa Rica and each specializes in a particular area of tropical ecology. La Selva research station near Sarapaqui was established in 1954 by Dr. Leslie Holdridge as an experimental farm and passed into the hands of OTS in 1968. Since then it has become one of the most important sites in the world for research on tropical primary forests (OTS, 2010). Many research projects are actively being pursued at this location, and a significant portion of them relate to conservation of native plants.

ProNativas, a non-profit organization, is a consortium of individuals and organizations that are concerned with the conservation and use of native plants in Costa Rica. Having only started within the last decade through the efforts of Willow Zuckowski, ProNativas is still in the process of establishing their presence in the

landscaping network of Costa Rica. Collaborative research by ProNativas on the use of certain native plants for both the private home owner as well as for ecosystem restoration is proceeding at the La Selva OTS station as well as several other locations including private residences, commercial nurseries, and universities.

In Costa Rica, privately owned businesses and individuals are often deeply involved with environmental conservation. Selva Verde Lodge in Chilimate is a Costa Rican ecolodge that is invested on several levels with the conservation of its surrounding environment. Considered a pioneer of sorts, the lodge was built in the 1970's and still remains committed to the tenents of sustainable tourism. Currently it holds a rating of three leaves from the Certification for Sustainable Tourism (CST) Rating Program. As a 500 acre private reserve, Selva Verde provides protection to both primary and secondary rainforest ecosystems and the biodiversity contained within.

The CST program was formed to help categorize tourism operations based on a single set of guidelines (Honey, 2008). Certification is based on four criteria. First, physiological-biological parameters are used to assess the interaction of the company with its surrounding habitat. Second, the infrastructure and services are evaluated based on management policies and operational systems for lodging companies (this relates to energy conservation practices, waste disposal, and water pollution). Third, the level to which the company interacts with its patrons to encourage participation in the sustainability practices of the establishment is assessed. Finally, the socio-economic environment is evaluated. In this lat category, the interaction of the company with the local community is evaluated based on the degree to which the tourist company assists with growth and development of the region and the generation of new employment. Leaf

levels 1-5 are assigned yearly, and participation is entirely voluntary and open to any company that is interested. Only seven lodges in Costa Rica currently hold a rating of 5 leaves, and only four tour companies hold 5 leaf ratings. The official entity in charge of this program is the Instituto Costarricense de Turismo (ICT). The University of Costa Rica (UCR) represents the educational sector, while INBio and the Ministerio de Ambiente y Energia (MINAE) represent the public sector (CST, 2010). All members are part of the evaluation process.

Several government agencies are involved in the process of environmental conservation as well. The majority of responsibility and influence lies with two particular entities: MINAE and MAG. MINAE is responsible for implementation, management and the administration of programs associated with biodiversity in Costa Rica (MINAE, 2010). This government agency coordinates all issues regarding conservation and natural resources in the country. Units within MINAE include (1) the Costa Rican Technical Unit, (2) Geology and Mining, (3) Meteorology, and (4) the Sistema Nacional de Areas de Conservacion (SINAC). SINAC specializes in policies related to conservation, environmental management, and the network of conservation areas in Costa Rica. During the past few years MINAE has been focused on ensuring that the information accumulated from various studies is actively utilized for the benefit of protected areas. Ecotourism, biosphere projects, environmental service payments, and medicinal plantations are just some of the programs that have been implemented. MAG, on the other hand, is specifically responsible for managing issues related with agricultural crops and livestock (MAG, 2010). This includes issuing phytosanitary certificates for the export of any live plant or animal material out of the country. Scientific research is also

conducted through this organization on topics relating to the fields of agriculture and livestock production; including pest and disease control, economic development, and marketing (to name a few).

A number of other organizations in Costa Rica are actively participating in environmental conservation and/or ecotourism. After careful research those described above presented the most promising opportunities for collaboration based on mutual interests and a common geographical focus. This in no way permanently excludes any omitted organizations from future consideration nor guarantees a working relationship with those identified so far. Two research trips to Costa Rica have been undertaken thus far to further determine the appropriateness of each organization and ascertain whether they would be amenable to working collaboratively with researchers from AU.

Plant Propagation

In the interests of facilitating both the expansion of conservation techniques as well as the overall conservation effectiveness of Costa Rica, propagation protocols for the following two species native to the Caño Negro area of northern Costa Rica were investigated: *Hylocereus stenopterus* (F.A.C. Weber) and *Acoelorraphe wrightii* Griseb. & H. Wendel.

Hylocereus stenopterus

H. stenopterus is a member of the Cactaceae, and was first described by Britton & Rose (1963). It is a tropical hemiepiphyte (an epiphyte that can secondarily develop roots in the ground) that displays a liana-like growth habit, or growth where plants use trees and occasionally other objects for vertical support. Development of adventitious roots allows this plant to acquire nutrients at microsites along the bodies of living plants

it climbs (Nobel and Barrera, 2002). *H. stenopterus* is found only in Costa Rica. Other members of the genus *Hylocereus* can be found throughout Latin America, where they are thought to have originated, as well as around the world in tropical and subtropical regions (Britton & Rose, 1963; LeBellec et al., 2006). There are currently 16 recognized species in the genus *Hylocereus* (Britton & Rose, 1963). These are all perennial plants that can grow quite rapidly and have high flower and fruit yield (Ben-Asher et al., 2006). As with many other members of the Cactaceae, these species utilize Crassulacean Acid Metabolism (CAM) (Britton & Rose, 1963). The majority of species grow primarily in tropical dry forest habitats in the mid to lower canopy of forest trees (Britton & Rose, 1963). In Latin America several species in this genus, and their fruit, are collectively known as pithaya or pitahaya (other spellings can be found as well: pitahaja, pitajuia, or pitalla). In Vietnam it is called thang loy or dragon fruit (Nobel, 2002). Though little known a decade ago, the pitahaya fruit is now an established item in the exotic fruit market (Mizrahi et al., 1997) as well as in domestic markets within production areas. It continues to be recognized as a promising new crop (Janick, 1999), and the results of this research are intended to facilitate the advancement of its production not only as a cash crop for Caño Negro, Costa Rica but for other agriculturalists and horticulturists around the world.

H. stenopterus consists of three-ribbed stems with a medium to large epigynous berry for a fruit (Nerd et al., 2002). Britton and Rose (1963) describe it as a homoblastic species, meaning it displays few apparent changes in appearance between its juvenile and adult stages. Stems are triangular, succulent, and feature spines 2-4 mm wide and 5-10 mm long. Flowers are hermaphroditic and 20-30 cm wide. Most flowers of the various

species in the genus *Hylocereus* are creamy white, the two exceptions being *H. stenopterus* and *H. extensus*, which have red or pink blooms. All bloom nocturnally. Though the cultivation of pitahaya is spreading, the majority of its consumption and use remains confined to the areas where it is grown. As almost all commercial propagation within this genus has revolved around the one species *Hylocereus undatus*, the remainder of this review will focus on research done on this species.

H. undatus is grown commercially in several countries in nurseries for retail sale as an ornamental plant as well as agriculturally for fruit production (Choi et al., 2007; Esquival et al., 2007; Merten, 2003; Yussof et al., 2008). Large scale production in South and Central America occurs primarily in Columbia and Nicaragua with only a small amount occurring in Costa Rica (Barbeau, 1990), though several Asian countries now produce *Hylocereus* as well. These include Thailand, Laos, Indonesia, Cambodia, and Japan with the majority of Asian production occurring in Vietnam (Nobel, 2002). Large operations have also begun to appear in Australia, Reunion Island and Israel (Jacobs, 1998; Le Bellec & Judith, 2002; Nerd & Mizrahi, 1997). Other countries that produce *H. undatus* include the United States, Ecuador, Mexico, Guatemala, New Zealand, Peru, Spain, and the Philippines (Nobel & Barrera, 2002). Overall, this species is considered to be fairly robust. It can grow in temperatures from 12 to 40^{o}C and at altitudes up to 2,750 m above sea level (De Dios, 2004). All species can grow in various soils so long as they are well drained (Barcenas, 1994) and all species can survive and recover from drought with ease (Ben-Asher et al., 2006; Nobel & Barrera, 2002). Optimal light levels for specific species in this genus are unclear as studies show different levels for different species in the genus *Hylocereus* (Nerd et al., 2002; Raveh et al.,

1998), but if grown in an extremely hot, dry climate some shade will be necessary (Raveh et al., 1998).

In a commercial setting *H. undatus* is grown on some form of support, either vertical or inclined, and pruning is required (LeBellec et al., 2006). Though these plants are capable of surviving drought, when regular flower and fruit set is desired regular watering is required (LeBellec, 2003; Nobel & Barrera, 2002; Nerd et al., 2002). Le Bellec et al. (2006) recommend local micro-irrigation. Suggested irrigation levels range from daily watering (Cavalcante & Martins, 2008) to watering once every two weeks (Thomson, 2002) and is affected by season and weather conditions in general. Several papers explore methods for commercial propagation of this species though the practice only began around 15 years ago (Andrade et al., 2006; Cavalcante & Martins, 2008; De Andrade et al., 2007; De Andrade et al., 2008; Michel-Rosales & Farias-Larios, 2007; Mohamed-Yasseen, 2002). Both asexual and sexual propagation have been attempted. Though propagation by seeds is possible, germination is often inconsistent (LeBellec, 2003). Pollination can be problematic in agricultural settings (Weiss et al., 1994) as the majority of pollination in the wild occurs via pollination by bats (Nerd & Mizrahi, 1997). Hand pollination is fairly easy to accomplish however and is only restricted by the labor associated with it for large scale production (Weiss et al., 1994). Micropropagation has also met with success, but the cost of production is currently not met by the profit of distribution (Mohamed-Yasseen, 2002). LeBellec (2003) concluded that asexual propagation by cuttings represents the most effective and promising form of propagation, at least for agricultural purposes. He concluded that propagation by cuttings allows for reliable production of specific varieties, and the fruiting stage is reached three times as

quickly than with plants grown from seed. This is due to ontogenetic aging, wherein the removed cuttings perpetuate the ontogenetic age of the parent plant and are already at a mature enough stage to begin flower and fruit production. Cuttings can also be taken with ease directly in the field as opposed to other plants that might require special care, or the laborious process associated with producing and collecting seeds from this particular plant.

Using cuttings, LeBellec (2003) achieved up to 90% rooting success. Cavalcante & Martins (2008) suggested that cuttings be taken from juvenile growth with the basal end cut being made just above a joint in the fleshy green stem and the terminal end being an actual joint. Suggested cutting lengths vary between 25 and 50 cm (Cavalcante & Martins, 2008; LeBellec, 2003). Bastos et al. (2006) concluded that application of a rooting hormone aids in rooting success, but Thomson (2002) reported no significant effect. Thomson (2002) also suggest allowing cuttings to cure or cicatrize for up to 5 days before planting while Cavalcante & Martins (2008) planted cuttings immediately.

All these studies indicate that, for *H. undatus*, cicatrization time and application of rooting hormone may influence propagation success. Further research is necessary to make more specific recommendations for asexual propagation of not only *H. undatus* but *H. stenopterus* and *H. costariensis* as well. The current research will investigate stem cutting propagation for all three species.

Accoelorraphe wrightii

Aracaceae is an isolated and distinct group within the plant kingdom and is composed entirely of woody monocotyledonous plants collectively and commonly known as palms (Jones, 1995). Palms are found around the world in tropical ecosystems.

There is no agreement on exactly how many species belong to this family: Meerow (2002) estimated 2600 species spread among about 200 genera. All are perennial, and the family contains both monoecious and dioecious species. Reproduction by palms in nature is almost exclusively by seed, and most palms have a fairly recognizable canopy with a prominent trunk and a characteristic crown of leaves. A few species are acaulescent (trunkless) and others are even climbers (Jones, 1995; Riffle & Craft, 2003). When trunks are present, they are found as a single stalk or as multiple clumping or caespitose trunks.

Within this family, *A. wrightii*, also commonly known as Paurotis palm or Everglades palm, is a member of the Corypheae tribe and the Coryphoideae subfamily (Meerow, 2002). It is found in Florida, the West Indies, and coastal or wetland areas of Central America (Farruggia, 2008; Jones, 1995; Meerow, 2002; Riffle & Craft, 2003). The genus *Acoelorraphe* is monotypic with *A. wrightii* the only species (Jones, 1995). This is a swamp-growing species with a clumping style growth habit. It will often form dense thickets in brackish waters. It has palmate fans that are divided more than half the way up the leaf into several slender sections (Meerow, 2002). The trunk is covered with a think fibrous sheath that can be quite prickly (Jones, 1995; Meerow, 2002). Moisture is extremely important to this species, and individuals in dry locations grow very slowly (Meerow, 2002).

A. wrightii produces bisexual white flowers, and there are no published reports on its pollination method in the wild. It is a relatively tolerant species when it comes to habitat, being one of the few palms that can grow in saturated soils (Meerow, 2002). It

can have problems with manganese deficiencies but responds well to fertilization and has no known pest problems (Meerow, 2002).

Though reproductive maturity is reached in four to five years by some species of palms, others can take up to forty or fifty years (Meerow, 2004). Once flowering begins most palms are pleonanthic (flower regularly throughout their lives) though a few are monocarpic. Pollination was historically considered to be by wind, but it is now thought that most pollination is done by beetles, flies, bees, and bats (Jones, 1995). Seeds come in a range of sizes, with one species in this family (*Lodoicea maldivica* J. Gmelin) producing the largest seed of any plant in the world that can grow up to 40 pounds, yet seeds of some species are only a few millimeters long (Meerow, 2004). This family also contains the species (*Raphia regalis* Becc) with the longest leaf of any plant at 24 m and the plant (*Corypha* Becc) with largest inflorescence, which can reach up to 7.5 m tall and contain thousands of tiny flowers (Jones, 1995).

Propagation of palms is almost entirely done by seed, though vegetative propagation is possible by air layering, division, or even more recently, tissue culture (Jones, 1995). The information on seed germination for palms tends to be inconsistent as much of it comes from hobbyists not researchers, and no clear conclusion can be drawn for more than only a few commercially important species. Vegetative propagation is almost exclusively used by enthusiasts and collectors while tissue culture is used by researchers, collectors, and large scale commercial operations. Neither tissue culture nor vegetative propagation is feasible for the average nursery or farm.

Unlike *A. wrightii*, seed germination in most palms is notoriously difficult with some seeds taking years to germinate (Odetola, 1987). It has been estimated that up to

25% of all palms take over 100 days to germinate and often achieve less than a 20% germination rate (Tomlinson, 1990). Germination of *A. wrightii* usually occurs within 2 to 4 months (Carpenter, 1988; Jones, 1995; Meerow, 2002). Efforts to produce palms commercially persist, however, as certain species are significant in the ornamental and edible markets (Jones, 1995). Some species are also important in indigenous lifestyles as fiber, food, and building material (Potvin et al., 2003).

There is no clear evidence why this difficulty in germination occurs, as very few seed germination studies have been conducted for palms (Meerow, 2004). Most palms do have small embryos compared to the size of the seed and the endosperm (Corner, 1966). Often, the embryo of palms is not fully developed when the seed is disseminated. Several species have an embryo that initially consists of a small disk of cells located near the operculum (Ginieis, 1957; Corner, 1966). Only after a series of complicated chemical changes does the embryo break dormancy, develop fully and fill the entire seed (DeMason, 1985).

Germination itself begins when certain enzymes reach the endosperm of the seed and mobilize the food resources there. The endosperm itself can be easily recognized in the species *Cocos nucifera* L. as the white 'meat' and milk in a coconut. In *A. wrightii* the endosperm is extremely hard and has the appearance and consistency of hard plastic (personal observation). In general, the endosperm of palm seeds is made up primarily of cellulose, a non-nitrogenous storage material, (Orozco-Segovia et al., 2003) and lipids which are also an important source of energy in embryo development (DeMason, 1986). Germination in several species begins with the differentiation of the cotyledon into three structures; the tubular base, the petiole, and the haustorium (Tomlinson, 1990). The

haustorium is the structure that absorbs nutrients from the endosperm while the embryo and cotyledon swell and develop. Seed germination among palms occurs in several forms but can generally be divided into two initial categories; remote or cryptocotylar germination and adjacent or hypogeal germination (Meerow, 2004; Orozco-Segovia et al., 2003). Seed germination in *A. wrightii* occurs by the remote germination pathway (Meerow, 2002). With remote germination the first structure to emerge from the seed is called the cotyledonary petiole; this is commonly mistaken for the first seedling root. However, the first seedling root will actually emerge from this structure after it has grown down into the soil. At the other end, the seedling shoot or plumule will emerge. The actual cotyledon remains inside as the absorptive structure and is known as the haustorium. Adjacent germination involves only a small portion of the cotyledon emerging from the seed; this is known as the button (Meerow, 2004). Then, the radicle and plumule emerge from the button. The radicle in this type of germination is very short lived and is quickly replaced by adventitious roots that form around the stem base. Like remote germination, the part of the cotyledon known as the haustorium remains inside the seed and absorbs nutrients from the endosperm. These different germination strategies have been associated with establishment in different ecosystems (Orozco-Sergovia et al., 2003).

Seed dormancy has been identified as an important aspect of palm seed germination and may be a significant part of understanding the long and sporadic germination of many palm species (Orozco-Segovia et al., 2003). Other possibilities that have been proposed to explain sporadic palm seed germination include embryo immaturity at time of dissemination, hormonal controls, or inadequate environmental

factors to induce germination (Baskin & Baskin, 1998; Corner, 1966; Hussey, 1958; Nagao et al., 1980). Moisture is one of the primary factors to consider when overcoming dormancy in palm seeds. Indeed the most common form of scarification for palms is extended soaking in water for anywhere from 12 hours to up to 7 days (Broschat & Donselman, 1986; Kheong, 1992; Moussa et al., 1998). This practice is thought to help soften the often hard seed coats of many palm seeds, although well documented studies on the effectiveness of this treatment are in short supply (Orozco-Segovia et al., 2003, Ehara et al., 2001). In cases of extremely hard or thick seed coats, soaking in concentrated sulfuric acid can be effective for shortening germination time (Merlo et al., 1993). Physical scarification accomplished by nicking the seed coat has also been used to reduce germination time and increase germination rate (Holmquist & Popenoe, 1967; Nagao et al., 1980; Odetola, 1987).

Temperature can also play an important part in palm seed germination. Most palms are thermophilous with optimum germination temperatures between 30-40^{o} C (Carpenter, 1987; Ehara et al., 1998; Odetola 1987; Rees, 1962). Cold stratification has been used in a few instances, but heating is far more common (Hussey, 1958; Nagao et al., 1980; Rees, 1962). In general, the interaction of several factors affecting seed dormancy and germination is very poorly understood for palm seeds.

In nurseries, appropriate germination temperatures are often achieved by the use of heated greenhouses and/or bottom heat cables. Other methods reported as effective include covering containers with plastic or placing containers on heat-retaining surfaces (Meerow, 2004). Appropriate light levels are important as well. The majority of palm seeds can germinate and will grow in full sun but a few do require shade as they grow

naturally in the understory of thick rainforests (Meerow, 2004). Planting depth can be adjusted to help compensate for overly sunny conditions. Generally palm seeds can be planted at a depth equal to the diameter of the seed, but this is not always the case. For many of the very large palm seeds, it is best to simply push the seed down into the substrate while part of the seed remains exposed (Jones, 1995; Meerow, 2004). If a palm species usually grows in a shady location it can be planted at a deeper depth in a sunny location and still germinate effectively (Meerow, 2004). Substrate for palms needs to be well-drained yet have good moisture holding capacity. Germinating palms do not respond well to alternating periods of dry and wet. A common mix in the nursery industry is a 1:1 ratio of peat moss:perlite (Meerow, 2004). Palms have also been germinated in perlite or peat moss, but a 1:1 mix of the two appears to be most effective (Carpenter, 1988; Jones, 1995; Koebernik, 1971).

Containers for palm propagation come in many shapes and sizes with the primary consideration being that they allow adequate growing space for the seedling to develop and that they be well drained (Meerow, 2004). Flats are not generally recommended because of shallow soil depth, therefore specially made individual cells or containers are used. In the tropics, many palms are sown directly in large raised beds, and some palms can only be grown in the ground because as they grow a sinker root that can be up to a meter long before a leaf shows above ground. Once this long root has developed, the plant will often not tolerate transplanting (Jones, 1995).

Most palms require uniform moisture during germination and subsequent seedling development (Meerow, 2004). It is important that the substrate stay damp but not be saturated since palm seeds can be susceptible to rot (Koebernik, 1971). Fertilization of

palms is not important until late in seedling development. For the first few months after germination the endosperm acts as the only source of nutrition for the developing seedling and added nutrients can damage the new seedling (Meerow, 2004).

Only one reported research project directly studied propagation of *A. wrightii*. It was concluded that *A. wrightii* will germinate best at temperatures above 35^{o} C and within a range of 33^{o} to 39^{o} C (Carpenter, 1988). The current research will investigate the effect of scarification method and sowing substrate on the germination rate and final germination percentage of *Acoelorraphe wrightii* seeds.

Research Objective

Plant propagation and subsequent outplanting can be used as an effective conservation technique (Potvin et al., 2003), and ecotourism can benefit both local economies and the local environment (Campbell, 2002). Based on these two observations, the objective of this research will be to determine effective propagation techniques for two plant species native to the Caño Negro NWR so that they may be produced, harvested, and transplanted by community members to stimulate the economy of Caño Negro village and help conserve the unique ecological community of the Caño Negro NWR.

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Chapter II

Development of a Cooperative Extension Program in Costa Rica

Key Words: Costa Rica, International extension, Caño Negro, conservation

Abstract: A joint extension program between Auburn University (AU) and the Universidad Nacional de Costa Rica (UNA) was developed in an effort to expand educational opportunities at participating universities, conserve global biodiversity, and support community level economic development. AU researchers made two research trips to Costa Rica in order to develop this relationship in Sept. 2009 and May 2010. In Sept. contact was made with several professional entities as well as community members of Caño Negro village to assess the feasibility and validity of the proposed research program. Professional relationships were established with not only UNA Sarapaqui but also with the Instituto Nacional de Biodiversidad (INBio) and the Ministerio de Ambiente y Energia (MINAE). Upon returning in May, a research bed was built at the UNA Sarapaqui campus, and relationships with all appropriate parties were reinforced.

Introduction

In northern Costa Rica the Caño Negro National Wildlife Refuge (NWR) is an area with the capacity for both conservation collaboration as well as the opportunity for economic development. Though the majority of the country is relatively stable economically, this remote region is facing other challenges. The Costa Rican economy currently relies heavily on agriculture, exports by the electronics industry, and tourism (CIA, 2010).

Neither of the first two options is feasible in the Caño Negro area: agriculturally because of the sensitive nature of the various ecosystems that dominate the region and industrially because electronics manufacture is unreasonable due to the remote nature of the village as well as the lack of necessary infrastructure. Furthermore, the few available jobs in the region are subject to decreased wages due to extensive illegal immigration from Nicaragua. It has been suggested, however, that there is potential for program development and capitalization of the ecotourism industry which, if handled properly, could be a boon to both the economy as well as the ecosystem (Orozco-Obando, 2009). Internationally targeted cooperative extension programming can bring together not only the local and national resources of an area but also add a global perspective that can aid the development of ecosystem assets and enhance both economic opportunities and conservation goals (Harder et al., 2010; Ludwig, 2002; Place et al., 2008).

The Caño Negro NWR is also highly significant as an ecosystem. The majority of the area is a tropical dry forest, the most endangered ecosystem on Earth (Janzen, 1988). It also contains portions of a vast seasonal wetland that feeds Lake Nicaragua to the north and has extensive populations of birds, caimans, and unique plant life. Caño Negro has been recognized by the Ramsar Convention (RAMSAR, 2010) as a wetland of international significance and is also part of the Mesoamerican biological hotspot established by the organization Conservation International (CI, 2010). Additionally it is one of the most remote parks in Costa Rica's national park system and has, thus far, escaped the degradation of native plant and animal communities often associated with an increase in visitor traffic. Economic opportunities in the area are associated with the wetland. As tourism continues to expand, improper planning might introduce these

problems to Caño Negro as well. The area is also in danger from large scale agricultural interests. As international pineapple growers are moving out of Hawaii and into cheaper production areas, most notably Brazil and Costa Rica, the wetland areas of Caño Negro have come under particular attack (Niesse, 2006). Portions of the wetland have already been drained to make way for pineapple fields and Caño Negro community members are being asked to sell their lands for more of the same (Ruiz, 2010).

Los Chiles is the only municipal area in close proximity to the refuge that could be considered to be larger than a village. This is the northernmost town in the Huetar Norte Region of Costa Rica, lying only 10 km south of the Nicaraguan border. From this location, tourists can take boat tours along the San Juan River, the official border between Nicaragua and Costa Rica. However, this is a border that is still at times violently contested, with many Nicaraguans claiming that the entire river belongs to their country, and Costa Ricans claiming that the border is along the middle of the river. One of the guides encountered during lunch in Los Chiles said that there is even occasional gunfire exchanged along the river. This issue has been ostensibly resolved by the governments of these neighboring countries, but reality is not so cut and dry.

The overall and ongoing goals of this project will be to facilitate rural community economic development and bring in supplemental income for local citizens by providing a new crop with commercial value as well as establish an ecotourism destination in the village of Caño Negro which is located within the Caño Negro NWR. The effect of ecotourism on both rural development and conservation goals has been studied in several countries with mixed results that are attributed to several variables (Bookbinder et al, 1998; Campbell, 1999; Hvenegaard & Dearden, 2002; Potvin et al., 2003; Trejos &

Chiang, 2009). Propagation of native plants and subsequent out planting has been an effective conservation tool (Potvin et al., 2003). In Nepal, it was observed that ecotourism did not contribute significantly to the local economy (Bookbinder et al., 1998) but other studies are more optimistic about the benefits of ecotourism (Campbell, 1999; Hvenegaard & Dearden, 2002). Challenges to development include finding ways to keep monetary benefits within a community and targeting the focus of that community on how their natural environmental resources can be utilized to enhance their economic opportunities. The proximity of a protected area to Caño Negro represents an opportunity for collaboration with larger government entities and promises a higher chance of success in the development of the local economy (Plummer & Fennell, 2009). Utilizing recommendations from research based models, our educational extension program will provide farmers and local residents an economic alternative to selling their lands to the large pineapple producers that are moving into the area (Ruiz, 2009). This will help not only preserve a unique and beautiful community but further protect a globally significant area of species diversity.

To develop a program in Costa Rica, extensive research was done to identify appropriate collaborative entities. These included the Universidad Nacional de Costa Rica (UNA), the Instituto Tecnologico de Costa Rica (ITCR), the Instituto Nacional de Biodiversidad (INBio), the Organization for Tropical Studies (OTS), ProNativas, the Ministerio de Ambiente y Energia (MINAE) and the Ministerio de Agricultura y Ganaderia (MAG) among others. Preliminary research to identify regional native plant species appropriate for this project was verified by consultation with Costa Rican academic colleagues, regional experiment stations and community farmers. Individuals

from each of these groups have formed an informal working group and advisory committee that provided local and national educational and political contacts to facilitate both conservation and development activities.

Administration and faculty from UNA were among the first contacts made when efforts began to evaluate the biological, political, and economic factors associated with the feasibility of this project. A relationship had already been established in February 2008 between Auburn University (AU) and UNA personnel. Later that year, a memorandum of agreement between AU and UNA was drafted and signed to facilitate future cooperative efforts between these institutions. This project represents the next step in the development of that relationship.

UNA is a public institution and receives funding directly from the Costa Rican government (UNA, 2010). It is a four year university with both undergraduate and graduate degrees including the social sciences and multiple fields in the biological sciences, as well as philosophy and liberal arts. It includes six separate campuses; the main campus is in San Jose with satellite campuses in Sarapaqui, Nicoya, Benjamin Nunez, Coto, Perez Zeledon, and Liberia.

Another university contacted in Costa Rica that focuses on agriculture and the biological sciences in general is ITCR. ITCR also concentrates on community and applied technologies. Interdisciplinary studies are recognized as being of great benefit and every effort is made to foster collaboration with other universities. There is a fully developed distance learning PhD program which is organized in conjunction with several universities in several countries.

INBio is a nonprofit organization that has been expanding its influence across the globe. Started in 1989, INBio has become an international authority on programs and projects that conserve and promote tropical biodiversity (INBio, 2010). During the late 1980's Costa Rica officially recognized the need to protect its wealth of biodiversity by passing Executive Decree No. 19153. Among other things, this decree established the Planning Commission for the National Institute of Biodiversity, a board composed of members from various governmental institutions and also from higher education centers and conservation organizations. This board recommended to the Costa Rican government that a state biodiversity institute be created which functioned with a high degree of autonomy. Due to issues within the government itself, this recommendation was ignored. At this point members of the board decided to take the situation into their own hands and create a private non-profit organization. This institution was granted official recognition by the standing government, and has been supported by successive governments as well. The members then went about the laborious process of securing funding, eventually finding it in the form of a loan for 80 million colones as well as backing from the Swedish Cooperation Agency (SIDA) and the MacArthur Foundation. On October 26th of 1989 a location was selected in Santo Domingo de Heredia and this is still where INBio is housed today (INBio, 2010). Several services are now offered through INBio including bioprospecting, consulting, education and training, as well as general information services. In 2000, a theme park called INBioparque was added as well. Visitors can tour an organic farm, view a green built house, enjoy a butterfly garden, as well as many other nature based attractions. ATTA, the database where all

information generated and collected by INBio is stored, is an extensive resource and almost indispensible for someone working with tropical biodiversity.

OTS is another major research association in Costa Rica. AU has a long standing relationship with this organization and has been an OTS member institution since 1987. Classes are offered on a number of subjects pertaining to tropical ecology at both the graduate and undergraduate levels and in multiple countries. There are three research stations in Costa Rica and each specializes in a particular area of tropical ecology. La Selva research station near Sarapaqui was established in 1954 by Dr. Leslie Holdridge as an experimental farm and passed into the hands of OTS in 1968. Since then it has become one of the most important sites in the world for research on tropical primary forests (OTS, 2010). Many research projects are actively being pursued at this location and a good portion of them relate to conservation of native plants.

ProNativas, a non-profit organization, is a consortium of individuals and organizations that are all concerned with the state of native plants in Costa Rica. Having only started within the last decade through the efforts of Willow Zuckowski, ProNativas is still in the process of establishing their presence in the landscaping network of Costa Rica. Joint research on the use of certain native plants for both the private home owner as well as for ecosystem restoration is proceeding at the La Selva OTS station as well as several other locations including private residences, commercial nurseries, and universities.

In Costa Rica, privately owned businesses and individuals are often deeply involved with environmental conservation. Selva Verde Lodge in Chilimate is a Costa Rican ecolodge that is invested on several levels with the conservation of its surrounding

environment. Considered a pioneer of sorts, the lodge was built in the 1970's and still remains committed to the tenents of sustainable tourism. Currently it holds a rating of three leaves from the Certification for Sustainable Tourism (CST) Rating Program. As a 500 acre private reserve, Selva Verde provides protection to both primary and secondary rainforest ecosystems and the biodiversity contained within.

The CST program was formed to help categorize tourism operations based on a single set of guidelines (Honey, 2008). Certification is based on four criteria. First, physiological-biological parameters are used to assess the interaction of the company with its surrounding habitat. Second, the infrastructure and services are evaluated based on management policies and operational systems for lodging companies (this relates to energy conservation practices, waste disposal, and water pollution). Third, the level to which the company interacts with its patrons to encourage participation in the sustainability practices of the establishment is assessed. Finally, the socio-economic environment is evaluated. In this lat category, the interaction of the company with the local community is evaluated based on the degree to which the tourist company assists with growth and development of the region and the generation of new employment. Leaf levels 1-5 are assigned yearly and participation is entirely voluntary and open to any company that is interested. Only seven lodges in Costa Rica currently hold a rating of 5 leaves and only four tour companies hold 5 leaf ratings. The official entity in charge of this program is the Costa Rican Board of Tourism (ICT). The University of Costa Rica (UCR) represents the educational sector while INBio and the Ministerio de Ambiente y Energia (MINAE) represent the public sector (CST, 2010). All members are part of the evaluation process.

Several government agencies are involved in the process of environmental conservation as well. The majority of responsibility and influence lies with two particular entities: MINAE and MAG. MINAE is responsible for implementation, management and the administration of programs associated with biodiversity in Costa Rica (MINAE, 2010). This government agency coordinates all issues regarding conservation and natural resources in the country. Units within MINAE include (1) the Costa Rican Technical Unit, (2) Geology and Mining, (3) Meteorology, and (4) the National System of Conservation Areas (SINAC). SINAC specializes in policies related to conservation, environmental management, and the network of conservation areas in Costa Rica. During the past few years MINAE has been focused on ensuring that the information accumulated from various studies is actively utilized for the benefit of protected areas. Ecotourism, biosphere projects, environmental service payments, and medicinal plantations are just some of the programs that have been implemented. MAG, on the other hand, is specifically responsible for managing issues related with agricultural crops and livestock (MAG, 2010). This includes issuing phytosanitary certificates for the export of any live plant or animal material out of the country. Scientific research is also conducted through this organization on topics relating to the fields of agriculture and livestock production; including pest and disease control, economic development, and marketing (to name a few).

A number of other organizations in Costa Rica are actively participating in environmental conservation and/or ecotourism. After careful research those described above presented the most promising opportunities for collaboration based on mutual interests and a common geographical focus. This in no way permanently excludes any

omitted organizations from future consideration nor guarantees a working relationship with those identified so far.

Materials and Methods

The first exploratory mission to Costa Rica took place from 27 September 2009 through 4 October 2009 (Figure 1). A combination of teaching, research, and extension personnel were present including Dr. Amy Noelle Wright, horticulture associate professor and primary investigator, Rachel Allen Meriwether, horticulture graduate research assistant at Auburn University, Warner Orozco-Obando, horticulture graduate research assistant at Auburn University as well as liaison and translator for the trip, and Monica Jenkins DeTure, director of distance learning at Auburn University.

Monday, 28 Sept., was spent in meetings with various officials from both non-profit and academic institutions. On the first morning after arrival contact was made with the non-profit organization INBio. A meeting was held with Nelson Zamora, Director of Botany, Alvaro Herrera, Director of Research, Jesus Ugalde-Gomez, Associate Director of Biodiversity, and Elena Galante Marcos, Director of the Rio Frio Project. Discussion began with introductions by both groups (INBio and AU) highlighting their various programs and interests and proceeded into details on how a cooperative relationship might develop between INBio and Auburn. Once the formal meeting was complete, everyone toured INBioparque.

AU personnel then traveled to the campus of UNA in Heredia to discuss collaborative opportunities with this institution. While visiting UNA, a presentation was given by the author outlining the project and the objectives for research in Costa Rica. Several faculty and staff members from UNA attended and were encouraged to make

suggestions and consider becoming involved with the project. After the meeting, Victor Ruiz Obando, Regional Coordinator for the Huetar Norte Region, expressed interest in the project, even offering assistance during further travels through the country.

Tuesday 29 Sept., the party left Heredia. Driving north from the Central Valley, the travel ended in Chilimate at Selva Verde Lodge, one of the very first ecolodges built in Costa Rica. While in Chilimate AU personnel were joined by Victor Ruiz and the opportunity became available to also visit the UNA Sarapaqui campus in Rio Frio for a tour of their facilities. After the tour of UNA Sarapaqui on Tuesday, Ruiz and AU personnel traveled to the La Selva OTS field station for a basic nature tour with one of the OTS volunteers/guides. The following day was spent traveling to Batan where a tour was given of the new UNA aquaculture research center.

On 1 Oct., the group traveled north to Los Chiles and Caño Negro. The first stop up north was made in the town of Los Chiles followed by a brief stop was made at the Nicaraguan border. From the border, the next stop was the actual village of Caño Negro for two nights. Upon arrival a meeting was scheduled with several community leaders from Caño Negro as well as the few resident biologists in the area. It is extremely important when making decisions about development to be assured of community participation (Honey, 1999). Those present included Don Lolo, one of the oldest inhabitants of Caño Negro village, Oscar Gutierez, Oscar's young son, and Oscar's wife Christina who is a biologist with the Ministerio de Medio Ambiente y Recursos Naturales (MARN). MARN is an environmental organization based in El Salvador and is concerned with environmental assessments and international cooperation. Also in attendance were Bernadita Aguilar Lopez, a woman who owns a small restaurant and

guest house called Tablitas located not far from Caño Negro and Victor Ruiz. Ruiz is the equivalent of an extension agent for the area and is based out of the UNA Sarapaqui campus. Mick Wigal, a member of the United States Peace Corp, was also present. Warner Orozco lead the meeting since it was almost exclusively in Spanish. He began by describing the design and intention of the AU project to those present. It was also made clear that the primary reason for calling this meeting was to receive suggestions from those present on how to make the project applicable and appropriate for Caño Negro. Once the introduction was complete, the discussion was opened up to questions and suggestions. At this point Juan Diego Alfaro Hernandez, director of research for the Huertar Norte conservation area, and Carlos Alvarez arrived and joined the discussion.

On the following day, 2 Oct., a visit was paid to the local MINAE office. Juan Diego Alfaro Hernandez took the time to meet us in Caño Negro to discuss our plans and theirs for the area. A presentation was given by Hernandez outlining plans for a large visitor and research center to be built in Caño Negro. After the meeting at MINAE, a boat tour into the wildlife refuge was taken with a local tour company. Kingfisher Lodge and Tours is owned by Antonio Sequeira and his wife, both of whom have lived in the Caño Negro area most of their lives. The opportunity to see the lodge was not presented.

Once the boat tour was completed a further tour was given by Don Hernandez of the building site where the new MINAE facility will be located. After the tour of the proposed new facilities was complete, AU personnel left Caño Negro traveling west and stopped for lunch and further discussion and planning at Rancho las Tablitas, the restaurant and bar run by Bernadita Aguilar Lopez. Following lunch, the group traveled to Cuidad Quesada (San Carlos), the home of the Instituto de Tecnologico de Costa Rica

(ITCR). Overnight accommodations were provided by the school which has a hotel facility that is also a training area for Tourism students at ITCR.

On 3 Oct., a meeting was held with Dr. Tomas de Jesus Guzman Hernandez, the director of the Doctoral program in Natural Sciences at ITCR. A short tour was given of the campus by Ing. Oscar Lopez Villegas, a professor in computer sciences. He works with small businesses primarily in the tourism industry and helps owners learn how to use the internet to advertise and develop their businesses. This concluded activities at ITCR, and the remainder of the afternoon was spent traveling back to San Jose. The final day spent in Costa Rica was 4 Oct. with all party members flying home that afternoon.

A second research trip was conducted 5-16 May 2010 (Figure 1). Along with following up on relationships established on the first trip, the intention of this trip was to establish research plots in Caño Negro. Plans also included collecting cuttings of *H. stenopterus* to bring back to Auburn to continue propagation research there. Participants were limited to Dr. Amy Wright and Rachel Meriwether. Warner Orozco-Obando was once again of assistance in organizing the trip and acquiring supplies, though he was unable to participate in the actual trip. Departure and arrival locations were the same as on the first trip.

Immediately upon landing in San Jose a visit was made to INBio for a short visit with Nelson Zamora. There was not time to conduct further business on this day, and the remainder of the evening was spent at Hotel Bougainvilla making sure all preparations had been made for the following two weeks.

Early on the morning of 6 May a meeting was scheduled at the Ministerio de Agricultura y Ganaderia offices. It had been discovered that this was the location where

the phytosanitary certificates necessary to bring plants from Costa Rica into the USA are issued. The office is located on one of the UNA campuses in San Jose. Ing. Magda Gonzalez Arroyo is a director for the office of Servicio Fitosanitario del Estado (State Phytosanitary Services) and was kind enough to give a presentation on a research project her office had conducted several years before. At the conclusion of this meeting, Warner Orozco was delivered to the airport and returned to Auburn. Wright and Meriwether continued on to Sarapaqui and Selva Verde Lodge with a driver and van provided by the travel company Costa Rica Ambiance.

A visit was paid to UNA Sarapaqui on the morning of 7 May. After touring the campus, a more formal meeting was held with the current director of the UNA Sarapaqui campus Alvaro Villalobos and the future director Esteban Araya. During this visit permission was given to use the greenhouse for our cooperative research project. Any supplies that had not already been purchased were acquired from a nearby hardware store and all materials were left at the UNA campus near the greenhouse area until work could commence on the propagation bed. Due to the decision to use the pre-existing greenhouse, work was ahead of schedule and the rest of the day was spent at the hotel working on AU related tasks.

On 8 May time was spent catching up on work and making sure everything was in readiness to travel north to Caño Negro the following day. Unfortunately, due to complications with the van, it was not possible to take the trip to Caño Negro and an additional four nights were spent at Selva Verde Lodge. On 9 May Wright and Meriwether participated in a guided hike that took travelers through the forests located on Selva Verde Lodge property. On 10 May Wright and Meriwether met with Victor Ruiz

at Selva Verde to discuss plans for continued collaboration. This was a short meeting, so the majority of discussion occurred late that night over dinner in Chilimate. On 11 May an investigatory boat tour was taken down the Rio Frio to determine what plant life was visible in the area as well as assess the quality and style of services for tourists.

Work began the following day, 12 May, putting in propagation experiments in the greenhouse at UNA Sarapaqui. On the way to the campus from Selva Verde a visit was paid to the La Selva OTS station. It had been learned during the course of the trip that a gentleman worked there who was also interested in propagation of native plants and was connected with Pro Nativas. Orlando Vargas is the head of Scientific Operations at the La Selva Biological Station and has several projects with native plants in progress. Once conversation was concluded, Mr. Vargas gave a brief tour of his onsite propagation facility.

Once the visit to OTS was concluded, Wright and Meriwether proceeded to the UNA campus. On this first day of work lumber for the raised bed was treated with Penta-Cell, the mist system was constructed, and all necessary landscape fabric was cut. Penta-Cell is a liquid product that is mixed with either water or diesel fuel and seals lumber with active the active ingredients borate and chromate. This also served to announce the beginning of construction in the greenhouse so that the following day, when work that required assistance would occur, the necessary people would be available. Upon returning the following day, 13 May, it was communicated that UNA staff had learned of our presence and were available to assist with completing the experimental set up. Carmen Daly assisted with the project and conversation revealed that she is the manager for day to day operations in and around the greenhouse. She also helps direct student

workers from the university that have duties in the greenhouse area. Work on constructing the propagation beds in the greenhouse resumed almost immediately this day. This included nailing together the frame, lining it with ground fabric, filling it with substrate, and putting the mist system in place. Once this was complete, work began on preparing the seeds for planting. *A. wrightii* seeds were planted with the assistance of Evelyn Jimenez, a student worker from UNA. She was responsible for managing the propagation experiment once AU personnel departed. *A. wrightii* seeds received one of two scarification treatments, or no scarification (control) before planting. Scarification treatments were soaking in water and scratching with a rat tail file. All were planted in 100% perlite in traditional black square 4 inch pots. Seeds of *Inga sensiblis* purchased a few days before from a roadside vendor were also planted. These were planted in a mixed substrate consisting of equal parts sand and perlite and smaller but equal portions of organic compost, saw dust, and coconut fibers. *I. sensibilis* seeds in the pod are covered by a thick white pulp that is the edible portion of the fruit. One hundred and sixty seeds were planted, half with white pulp removed and half with white pulp left intact. Seeds were planted in the black plastic bags that are typical for tree propagation in Costa Rica. These bags appear to be made of number 4 low-density polyethylene plastic similar to typical trash can liners in the USA. They were roughly 20 cm tall and 13 cm wide (when lying flat), with three rows of evenly spaced drainage holes running from top to bottom. A portion of the *H. stenopterus* cutting that was gifted from Orlando Vargas was also planted. This concluded work at UNA Sarapaqui. All left over supplies were donated to the campus, and instructions for maintenance were provided. Contact

information was exchanged with Ms. Daly, and arrangements were made for updates on the propagation experiments to be sent to Auburn.

The morning of 14 May was spent traveling from Chilimate to San Jose. Upon arrival in San Jose, a quick visit was made to INBio to visit with Nelson Zamora. Seeds from *A. wrightii* were left with Zamora with the understanding that propagation experiments would begin on this plant in Costa Rica.

On the last full day in Costa Rica, 15 May, the University of Costa Rica (UCR) botanic garden, Lankester Jardin Botanico, and Orosi, one of the more prominent agricultural regions of San Jose, were visited. Travel back to the United States took place on 16 May.

Results

INBio

During the first visit to INBio, it was discovered that this organization has an existing aquaculture research center in Caño Negro. This project is jointly funded and administered by INBio and a Spanish company (the actual name of this company was not made available). Plans for the aquaculture research center and the Caño Negro area in general focus on sustainable development, ecotourism, education and outreach, watershed management, and community development. Unfortunately, there are limited funds available for this program so it is proceeding slowly. Within the relationship, INBio has only an advisory role on this project and is primarily available to make scientific recommendations when necessary. The Spanish company involved is particularly interested in production of organic food crops but is currently focused on commercialization and marketing of these crops rather than actual production. Members

of INBio felt that this may become problematic in Caño Negro as many communities in the area only collect and sell crops but do not produce them. There is apparently also a new interest in developing an urban landscapes program that seeks to educate the general public on the use of native plants in home gardens. Propagation of native plants and expansion of nurseries that sell them would be needed in order for this project to develop. It was recognized that the AU project could facilitate this plan.

All the members of INBio present at the meeting felt that the AU project was extremely interesting and worth pursuing. They felt that this project had the potential to begin filling in some of the knowledge gaps in their research on the Caño Negro area. The idea of being involved with *in situ* conservation also appealed to Nelson Zamora in particular. They also suggested incorporating a global warming component into the research project somehow, perhaps as a way to elicit further funding.

During travel in May 2010 and the second visit to INBio current development of the project was discussed as well as progress on both sides. Consideration was given to the possibility of establishing another propagation facility at INBio where research being conducted at AU and UNA can be replicated as well as work with native plants and their growth. It was also agreed that on the next research trip of AU personnel to Costa Rica, Zamora would plan to travel north with us to Caño Negro to visit research sites and that further effort would be made to solidify the relationship between Auburn and INBio by beginning formal propagation experiments at the INBio offices. Construction of a greenhouse at INBio was discussed.

UNA

The meeting and presentation at UNA in Heredia in May received mixed response. Some individuals did seem interested in the project, but communication with them occurred primarily after the main meeting. A few helpful suggestions were made, including the need for an economic analysis of the plants selected for research. Establishment of a relationship with Victor Ruiz Obando was the most significant benefit of this meeting. Some discussion did occur about the possibility of having a student from UNA being directly involved in the project, however, to date no relationship has been established with the UNA campus in San Jose.

Instead, an affiliation has been developed with UNA Sarapaqui. The campus itself is only a few years old with the clear focus being one of extension and environmental programs of various kinds. There are specific areas dedicated to various aspects of farming, agriculture, and ecotourism. Construction and development of facilities is increasing rapidly. Don Ruiz gave an exceptional tour that clearly demonstrated his own pride in the work there as well as his wish to develop collaborative research with AU. Accommodations for future trips were offered on campus as well as research space in any and all facilities onsite. This was reiterated the next day during a brief final visit in the morning with the director of the campus, Alvaro Villalobos.

On the second visit to UNA Sarapaqui it was clear that the campus had developed quite a bit since the previous visit in October. This happened to include a recently completed greenhouse with full overhead irrigation. It had been the intention of Wright and Meriwether to build a small hoop house in which to house propagation experiments but, upon finding the new structure, the aim became securing access to and use of this facility. During the meeting with the campus directors it was established that everyone

was agreeable to research proceeding on the campus in general but that special permission would be needed to use the greenhouse. Meryll Arias and a colleague were in charge of managing use of the greenhouse if not the actual maintenance of the structure and joined the discussion. After fully explaining the project all UNA representatives were satisfied with allowing use of the greenhouse for the plant propagation project. Some interest was expressed in incorporating the forestry and agriculture programs into the project, possibly even using the tree propagation experiments for teaching purposes.

Over the evening meal with Don Ruiz on the trip in May, he expressed a particular interest in growing *H. stenopterus* on the campus of UNA Sarapaqui. He explained that the majority of this fruit consumed in Costa Rica is actually imported at high cost from Columbia. He felt that if dragon fruit were to be produced locally, it would be an excellent source of income for participating communities. There is already an agriculture program as well as a focus on community development at UNA, and Victor Ruiz seemed to think that it would be possible to incorporate research on this plant into the pre-existing departments. Though there was not much time to discuss future plans in detail, mutual agreement was reached that the project was proceeding as it should and both parties remained pleased with the relationship.

During the trip in Oct. 2009, the OTS tour was not very successful. Though the tour itself was somewhat informative and some wildlife was seen, the group left feeling slightly disappointed. The opportunity to speak with actual researchers was never presented and so no contacts were made. There was more success upon returning to OTS in May. After a brief explanation of the AU project, Orlando Vargas expressed definite interest in being involved. He was particularly interested in the palm and the cacti. *A.*

wrightii was not a species with which he was familiar and he was quite excited to learn more. *H. stenopterus* did turn out to be one of the plants he is working with and he was even kind enough to make a gift of a cutting from one of the cacti growing on a tree just outside his office. This was the first time we were able to see this particular species of cactus growing in Costa Rica. It was agreed that every attempt would be made for Orlando to travel north to Caño Negro on the next research trip to discuss collaborative work further. Until then, communication would be maintained via the internet, and results of interest would be shared.

On the trip in Oct. 2009 the tour of the aquaculture facility proved to be fascinating and informative. Though aquaculture was neither directly related to the authors' research nor an area of expertise for anyone in the party, we were able to see its relevance to future projects and the potential for further collaboration with the AU Fisheries Department. It also continued developing the relationships that will be crucial for success of the overall project. UNA has thus far created an exceptional aquaculture facility that is maintaining a focus on community development and sustainable harvesting methods. There are some long term goals that pertain to this area of research and the possibility of incorporating research on lotus (*Nelumbo lutea*), which is being conducted at Auburn University by Dr. Kenneth Tilt and Warner Orozco-Obando. Caño Negro also is an area that could benefit from the research being done at the UNA aquaculture center. With an existing gar fish (or gaspar as it is locally known) production program in place, improved methods of gar farming are in high demand.

Caño Negro

Another unexpectedly interesting location was the international border between Costa Rica and Nicaragua; where the Costa Rican road literally ended in a field and the border itself was marked by a single strand of yellow caution tape stretched between four rusty barrels. There was a guard station on site manned by four individuals. A small camouflage hut was located just across the border on the Nicaraguan side. It was unclear how many individuals were actually working at this guard house but at least one man was seen with a large automatic weapon. There was no clear sign of an entrance road into the Nicaraguan side. While there, several people walked across from Nicaragua into Costa Rica with no questions from the Costa Rican guards. The people making this crossing were of normal Costa Rican appearance, being dressed in cotton clothing and primarily shod in flip-flops; one woman had on a flowered dress and seemed to be carrying a shopping bag of some sort. There was no sign of any habitation or settlement on the Nicaraguan side. The majority of the vegetation on the Nicaraguan side appeared to be orange plantations. Upon speaking to the Costa Rican guards we were informed that these were indeed massive orange plantations on the other side of the border. They further explained that land and labor in Nicaragua had recently become very cheap. The fruit juice companies Dole and Ticofruit have for many years had large plantations in Costa Rica, but when prices began to fall in Nicaragua, they sold off their lands in Costa Rica and moved just across the border. Apparently the original factories in Costa Rica are still in use, but the majority of the fruit and labor comes from Nicaragua now. They said that many of the people in the area had lost jobs due to this migration, and some of them had little choice but to travel into Nicaragua to continue what they had been doing before, despite the fact that they were paid less and obviously had to travel further.

Though this was a short and unexpected stop it provided further insight into the agricultural economy of Costa Rica.

Driving north through Agua Zarcas on the way to Los Chiles and the border it was clear that agriculture has become the primary economic activity of the area. Aside from the occasional fields of ornamental plants, the land is dominated by sugar cane and pineapple production. It also became clear upon arriving in Los Chiles that the town actively advertises itself as being the gateway to Caño Negro NWR, while in fact the town is not located in Caño Negro, and the boat tours never even enter the protected area. Many of the boats have the name Caño Negro stenciled on the side, and many other signs and stores indicate that this is Caño Negro.

During meeting with community members and officials of Caño Negro, it was explained that sometime in the past the Ministerio de Recursos Naturales (MRN) had attempted a development project in the area, but when the administration changed within MRN no more support was received and the project was dropped. There was quite a bit of concern over the same thing happening again with our project. A clearer picture was also given of the gar project in the area as well as a river turtle project that is ongoing. The river turtle project in particular was used as an example of a successful project. It was not clear what group started the project, but apparently they were very successful at involving and educating the local people about the project and its benefits. It had been several years since the project had been handed over in full to the community members, and it was still operational and lucrative. Each year, participating families collect turtle eggs from within the protected area. These eggs are then hatched and the hatchlings raised in the research facility near the new MINAE offices. Once the turtles have reached

the appropriate maturity level, one portion of the population is released back into the wildlife refuge and the other portion is sold for profit. The entire operation is also used as a tourist attraction. This is apparently not a community wide project, only about 3 or 4 families are responsible for the majority of the work, but it appeared that everyone in the community is supportive and recognizes the benefits of this effort.

The gar project is still in its infant stages, but those involved seemed excited about its prospects. Apparently the plan will be to establish an aquaculture facility within the Caño Negro community that will fulfill multiple purposes. First, it will act as a source of food and income for the residents. Second, it will be used as an environmental restoration tool, as a portion of the fish that are raised will be released into the Caño Negro NWR and the San Juan River to build up the natural population. This also has the potential to act as a source of revenue for the community if they are able to capitalize on the increased fish population through the attraction of sport fishermen, a sport which is quite popular in Costa Rica. There are clearly similarities between these two projects and interestingly enough, both have the same similarities with the AU plant propagation and production project. Ultimately the propagation of plants in Caño Negro would attempt to fulfill the same goals of generating food and revenue for the community while also providing material to begin environmental restoration efforts.

Caño Negro community members are interested in developing the ecotourism industry in the area. Concern is mounting over the incursion of transnational pineapple corporations that are buying land on the edges of the wetland and draining it without concern or consideration being given to the environment or the community. There is already some level of tourism though much business is lost to Los Chiles due to the

confusion about it being Caño Negro. There is a large sign in the main square of Caño Negro village proclaiming it as the "real Caño Negro". Most tourists do make the mistake of thinking that Caño Negro is just a wildlife refuge and not an actual village. The hope is that as more businesses related to tourism open up in Caño Negro, it will be easier for travelers to find the village.

People present at the meeting were also adamant about the project remaining in the hands of local residents. The one hotel in Caño Negro is owned by a foreigner, who does not contribute or participate in the community, so that tourism revenue does not remain in the area. There are also other difficulties for Caño Negro as it is extremely remote and can be fairly hard to reach for the average traveler. Thus it would be important to have businesses and attractions that will make it a sought after destination. Caño Negro is a young village having only been officially recognized in 1945 so many of the entities that tourists expect may not be present. Perhaps the most common and lucrative tourism opportunity in the area is the boat tours that operate up and down the Rio Frio and San Juan River and take interested parties into the wildlife refuge. Most of the tour boats are privately owned and operated by residents of Caño Negro village. It would be hard to call any of these operations actual tour companies as no one person owns more than one or two boats, but the tours are run professionally and are quite enjoyable. A few of the boat operators also offer a fishing tour option. Boats that are used are quite typical for this sort of operation in Costa Rica. They are 8 to 10 meter long flat bottom boats with rows of seats on each side, a roof, and are motor operated. The majority of guides are at least partially bilingual. There is also some hope that the native plant restoration efforts along the rivers will increase success of the boat tours. As

previously mentioned, the river banks along the areas where the tour boats operate have become severely degraded and invasive plants continue to proliferate. If native plants were to be outplanted along the banks, the natural beauty of the environment would be enhanced, particularly if the plants selected provided bold, ornamental features. Plants that are good for attracting wildlife would also be beneficial as the majority of tourists are interested in viewing birds or monkeys rather than only the plants. As with any ecosystem related project, there is no real end point or finite limit to the opportunities for positive impact.

Suggestions were solicited and received during the meeting on which plant species would be most beneficial to the community. There was agreement that the species already being considered as candidates for research were an acceptable beginning. One additional species of tree in particular was mentioned, *Lecythis ampla* or the monkey pot tree. A member of the Brazil nut tree family, this large tropical tree is quite rare. Ultimately this dictated why this particular species was not selected for research due to the difficulty in finding an adequate number of stock plants for propagation. A general suggestion was made to focus on tree species as these would be the most likely to have large environmental and ornamental impact and be lucrative for the community. Additionally, it was noted that there are several rare tree species in the area. These suggestions will be prioritized and considered not only during this project but in future projects as well.

A small MINAE office/field station is currently in use in Caño Negro with a few permanent employees and volunteers on site at any one time. Community members are also often involved with the activities of the office. There are dorms where guest

researchers and volunteer firefighters can stay if necessary. There was a decent lab building already present. A large two story building with lab facilities on the bottom floor and office spaces on the second floor stands not far from the edge of the wetland itself and is much closer to the main square of the village than the current office space. This structure appears to be well built and will act as a separate research facility in the upcoming visitor complex. This research center will be jointly administered by MINAE and UNA. Behind this building were two large shade cloth tents that covered several raised benches and large aquatic tanks. The benches are roughly a meter high and 4 meters long. They are filled with a peat based substrate in which the river turtle eggs are hatched before they are transferred to the tanks. Everything was empty and not in daily use at this time. It was explained that this is the area where much of the work for the river turtle project goes on, but that it only occurs during part of the year, and during the rest of the time it remains unused. There was discussion on the possibility of using the same facility for plant propagation, as the benches used to hatch the turtle eggs could easily be converted into mist beds for propagation during the part of the year they are not being used for hatching.

Plans for the new visitor center include areas for education, tourism, and research throughout the facility. There was also a plan in place to landscape the facility using native plants and incorporate several green building features.

Additional helpful suggestions were received while visiting the MINAE office, particularly with regard to *Nymphaea ampla*. It was discussed that this plant would be excellent for mitigation of agricultural pollution in the wetland as well as helping to reduce total suspended solids (TSS), both of which are problems in the Caño Negro

wetland as well as the Rio Frio (Fernandez, 2009). This represents additional opportunities for collaborative research and extension efforts. Don Hernandez suggested that it would be of benefit for AU to work jointly with UNA to complete an initial water quality survey of the Caño Negro area before beginning research, both for project planning purposes as well as for comparisons with future data to see what effect outplanting may have on the ecosystem.

Touring the Caño Negro NWR was informative and provided a critical visual impression of where and what research might be needed and appropriate. Don Antonio, the boat tour guide, was obviously familiar not only with the larger fauna of the area but also the flora and all their many interactions. When the project was described to him he agreed it was a good idea and even mentioned that he knew of only one location where a stand of the *Acoelorraphe* palm remained in the wetland. Unfortunately it was not possible to view these as the water was too low to reach that area at that particular time of year. Another tree was mentioned, a species of *Inga*, that Don Antonio said may be especially good for fisherman because this tree grows along river banks and drops its fruit in the water there. This in turn attracts various fish that eat these fruit at the surface, making the fish easier to spot and catch. During the boat tour it was clear that the banks along the Rio Frio had indeed become extremely eroded and were continuing to erode. Abundant wildlife was seen. Caimans (*Caiman crocodiles* L.) were perhaps the most common large reptile seen. There were a few basilisk or Jesus Christ lizards (*Basiliscus basiliscus* L.) but these were far outnumbered by the iguanas which could be seen at almost every turn, even swimming in the water. The guide said he had seen caimans eat iguanas swimming across the open water. Of course the bird population was extensive as

well. Anhingas, or cormorants, (*Anhinga anhinga* L.) were by far the most frequently sighted bird on the tour: these are also known as snake birds because of the way they swim with their bodies under the water and their long necks protruding above the waterline. Several green kingfishers (*Chloroceryle americana* Gmelin) were also seen. A few mantled howler monkeys (*Alouatta palliata* Gray) were seen, but they were very high up in the trees and napping during the heat of the day. Some farm animals, cows and horses, were either in fields adjacent to the river or on drinking swales. These swales or erosion ditches were almost completely devoid of plant life. There are stream side management laws in Costa Rica to prevent this, but they are difficult to enforce and thus rarely followed (Ruiz, 2009). Making the elimination of these swales economically advantageous would benefit the views and the environmental ecology of the area. Plant life along the river banks was typical of the area with huge numbers of bromeliads growing in almost every tree. Creeping vines occurred just as numerously, and large specimens of many tree species. Several species of palm were seen along the banks as well, along with more than one species of *Inga*. Once the wetland area was reached multiple species of water plants were seen. No orchids or large flowering plants were seen along the bank, though this is not uncommon. Perhaps most fascinating of all was the large number of epiphytic cacti seen in the trees. One island in particular had several large *Inga* trees on which it was difficult to discern the trunks due to the masses of cacti hanging down from the branches. It was not possible to stop and closely examine the cacti so there is no certainty as to what species were present but *Hylocereus costariensis* and *Epiphyllum hookeri* are the most likely candidates. Identifying and mapping native plants along the river would be an excellent collaborative project for AU and UNA

graduate students. Throughout the tour cacti were seen in multiple species of trees and at various heights in the canopy. All species of these cacti are night blooming which could be used to promote night tours, a practice that is gaining popularity in Costa Rica. If cacti were planted intentionally, stands could be monitored so as to know what nights blooms would be available for viewing during the flowering season.

At Rancho las Tablitas, fresh tilapia from a neighbor's aquaculture ponds is served in the restaurant. The proprietor, Ms. Lopez, has a small botanic garden and even a labyrinth started behind the restaurant area. Cabins are available for rent and spaces for camping are also available. Most of the food served is raised on site and Bernadita Lopez is very involved with the community and its projects. She currently works with a women's group in the area that has been started in collaboration with the local Peace Corps volunteer. Her reaction to the project and its goals was positive, and she did encourage contact with the women's group. This stop was a pleasant look into the everyday resources and activities of the area.

Other Organizations

During the visit to ITCR, specifics of the educational and degree process were presented. The possibility of AU becoming a part of this venture though distance education was discussed and all agreed that the idea deserved further consideration and development. It was interesting to learn Oscar Villegas' focus on small business development with a focus on technology application. This would be an exceptionally good aspect of training for those developing the tourism industry in Caño Negro to receive.

While visiting in May 2010, the meeting at MAG was highly informative and provided many ideas for subsequent cooperation with this agency. The results of the study recently concluded by MAG and presented to AU personnel was an extensive investigation on what pests are most likely to be found on the most common export crops of Costa Rica, with a particular focus on ornamental plants. Apparently there is a major problem because import standards imposed by other countries are too onerous for small farmers. Consequently export profits have fallen. The MAG study was intended to act as a recommendation reference for small farmers on how to effectively manage pests based on export standards. At the conclusion of this presentation further discussion ensued on the possibilities for collaboration between Auburn and MAG. These included internships, joint research, and study abroad programs, among other things. Assistance from MAG on future research trips by AU was also offered.

During the visit with Orlando Vargas, Wright and Meriwether learned that for several years he has been actively trying to remove invasive plant species from the OTS station property while replacing these with various native species. In the future he hopes to turn this project into an educational program to help the public learn about problems and concerns pertaining to invasive plants. He has also been instrumental in developing the La Selva online database of native plants, La Flora Digital de La Selva (La Selva, 2010), which was begun with the help of an NSF grant. Recently he has indeed become involved with Pro Nativas and has begun preliminary germination and propagation experiment on several native Costa Rican plants. This work will be combined with the work on removal of invasive plants to promote the use of native plant species in the landscape. His propagation area was composed of four raised beds and several plants

planted directly in the ground. The area displayed a Pro Nativas sign. A cutting of *H. stenopterus* was growing amongst these plants. Vargas gave AU personnel a cutting from an *H. stenopterus* plant growing on the OTS campus

Chilimate

While on the hiking tour at Selva Verde, it was discovered that Selva Verde participates in the PES program, and the forest is well protected. It was also well worth taking the boat tour in Chilimate in order to observe the contrast between it and the one taken in October in Caño Negro. Transportation from the hotel to the site of the boat tour launch was organized through Selva Verde Lodge, as was the boat tour itself. Boats left from a pier located in Chilimate and traveled up the river for about an hour. The style of craft was the same as that seen in Caño Negro. The guide for the trip was knowledgeable and spoke English. Almost immediately it was obvious that this area had not experienced the same environmental degradation as that of the Caño Negro area. Obvious erosion was not prevalent, and only one cow wallow was seen. Not nearly as many caimans or iguanas were seen, but basilisk lizards were more plentiful. A blooming bromeliad was seen, and the guide said that it was native only to the Rio Frio area. Several epiphytic cacti were seen, probably *Epiphyllum hookeri*, but once again it was not possible to examine any of them closely enough to make a positive identification. Kingfishers were once again present as were anhingas, though not in the same high numbers as in Caño Negro. In general, though the animal life was not as obvious, the vegetation was thicker and more over grown in this southern location than further north in Caño Negro. Differences in the presence of various animal species can be attributed to differences in ecosystem type: the one in the north being a wetland and home to more crocodilians and

water fowl and the one in the south being a river. Environmental status could be a reflection of the differences in history of the two areas. Chilimate has been a center for tourism since the industry began to flourish in the early seventies. Thus, for years the income of many of the local residents has been based on travelers coming to enjoy the beauty of the local area. Therefore, it was in their best interests to protect that environment, whereas further north, in Caño Negro, agriculture was the primary industry and only very recently has tourism begun to expand there. Protecting the environment was secondary to raising crops and cattle.

The UCR botanic garden was well maintained, diverse, and well organized with several distinct styles and areas laid out. Perhaps the most spectacular feature of the garden was the orchid house, where hundreds of varieties of orchids at every conceivable life stage could be seen. No particular effort had been made to inform visitors about or encourage use of native plants, though plants were organized according to ecoregions. None of the plants currently being investigated were present.

Orosi was also well worth seeing. For many years this area has focused on agriculture and in particular shade grown coffee. During the time of European colonization, immigrants settled primarily in this area as it is one of the first hospitable regions reached when traveling to the central valley from the Caribbean coast. It is a good example of how agriculture is an integral part of Costa Rican society and not relegated to rural areas.

Plans are being made for a return research trip. It will be important to visit UNA Sarapaqui and check on the progress of the experiments in the greenhouse there. There will also be a plan to begin similar propagation experiments at INBio in San Jose as well

as to pay a visit to Caño Negro, hopefully in the company of Orlando Vargas, Victor Ruiz, and Nelson Zamora. An attempt will also be made to return with cactus cuttings from the Caño Negro area to continue experiments at AU.

Discussion

In retrospect it would have been impossible to make appropriate decisions about program development without having taken the two described research trips. Though large amounts of useful information can be gleaned from reading books and articles on ecotourism and the dynamics of research in Costa Rica, it would be extremely difficult to fully understand the intricacies of sociological interactions without experiencing them firsthand.

Working relationships have been established at this point with both UNA Sarapaqui and INBio. Neither of these organizations were primary candidates for cooperation at the beginning of the first trip. Though there was communication with UNA San Jose before the trips, it wasn't even known that UNA Sarapaqui existed, and now it has evolved into the most promising candidate for a long term research partner. The only experiments implemented are located on the UNA Sarapaqui campus. Victor Ruiz, its regional director, is now a personal friend and very involved with the project. Without the personal visit to the actual area, it is likely that this connection would never have been made. The process progressed to jointly signing a Memorandum of Agreement with INBio to fully formalize and stimulate the relationship. It is possible that this relationship as well would never have occurred if research trips had not been taken. During preliminary research, this non-profit was simply considered a valuable information source as opposed to a research partner. At this point, no formal experiments

have begun at INBio but Nelson Zamora is in possession of *A. wrightii* seeds. The intent is to begin experiments at INBio later this year.

Several positive meetings with potential partners were conducted during both research trips that have not yet translated into working relationships. ITCR and OTS are two examples. MAG has the potential of forming an excellent working relationship. There was talk of developing reciprocal internship programs between AU and MAG sometime in the future. Work with OTS and Orlando Vargas offers a promising relationship for the future and is another example of the value of international travel to meet people and assess research goals and potential commitment to a collaborative program. This connection may also establish a relationship with ProNativas. Though no formal meeting has occurred to date, there has been communication via email with several members of Pro Nativas as well as its founder, Willow Zuckowski. It is clear that the goals of the Auburn project and Pro Nativas are similar and there is a strong possibility for this relationship to develop into a long-term commitment.

Though the overall goals of the project remain the same (to facilitate community development and conservation by encouraging and teaching propagation and use of native plants) the methods by which this will be accomplished have shifted to some degree. There is less intention to focus solely on the community level. Community participation and development is still of paramount importance, but more general program development may need to occur if there is to be a chance of success. If anything the goals for the project have become more long-term and expansive, creating a program with the potential to grow beyond the Caño Negro area and become an integral part of two or more universities. So the original goals have expanded to include the creation of a

multi-level program with an interdisciplinary influence that draws on resources from multinational sources; these sources being two educational institutions, multiple governmental agencies, and at least one non-profit organization. Ultimately this program will include aspects of both education and research involving student and faculty study abroad and possible internships programs with concurrent and coordinated research in multiple locations. Information and international models developed from this program will then be applied in rural communities such as Caño Negro and other international regions to further the goals of worldwide conservation and community development.

Figure 1: Map of research trips taken in September 2009 and May 2010.

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Chapter III

Propagation of *Hylocereus stenopterus* and *Acoelorraphe wrightii*

Key Words: *Hylocereus*, *Acoelorraphe wrightii*, propagation, scarification, palm, cacti

Abstract: Effects of rooting hormone, cicatrization time and use of a mist tent on rooting, root and stem growth, and fresh weight relative growth rate (RGR) of *Hylocereus stenopterus* cuttings was studied. Effects of rooting hormone on rooting, root and stem growth, and fresh weight relative growth rate (RGR) of both *Hylocereus undatus* and *H. costariensis* cuttings was also studied. Effects of scarification and substrate on germination rate of *A. wrightii* seeds was also studied. All experiments took place in a glass house in Auburn, AL. All three *Hylocereus* species showed increased root growth and RGR when rooting hormone was applied. *H. stenopterus* cuttings also showed higher RGR when enclosed in a mist tent. *A. wrightii* seeds showed highest germination percentages when propagated in a 1:1 peat:perlite mix. Increased germination of *A. wrightii* seeds occurred in all experiments when seeds were scarified with water or sulfuric acid. Manual scarification had lower germination percentages than other forms of scarification or unscarified (control) seeds.

Introduction

Conservation of biodiversity has become an issue of international concern (CBD, 2010). Ecosystems do not adhere to political boundaries and the solution to slowing the loss of biodiversity must in some part involve international cooperation. Research suggests that plant propagation and subsequent outplanting can be used as

an effective conservation technique (Potvin et al., 2003). It has also been observed that ecotourism can benefit local economies as well as the local environment (Campbell, 2002). Opportunities exist for researchers at Auburn University (AU) to collaborate with colleagues in Costa Rica to develop propagation protocols for plant species native to the Caño Negro National Wildlife Refuge. The research objective of this program is to develop reliable and clear propagation techniques for two multi-use (edible, economically viable, aesthetic, in need of conservation) plants native to the Caño Negro NWR so that they can be produced, harvested, and transplanted by community members to stimulate the economy of Caño Negro village and help conserve the unique ecological community of the Caño Negro NWR.

As part of ongoing cooperation between AU and Universidad Nacional de Costa Rica (UNA), and in the interest of facilitating both the expansion of conservation techniques as well as the overall conservation of Costa Rican biodiversity, propagation protocols for the following two species native to the Caño Negro area of northern Costa Rica were investigated: pitaya or *Hylocereus stenopterus* (F.A.C. Weber) Britton & Rose and Paurotis palm or *Acoelorraphe wrightii* Griseb. & H. Wendel.

Hylocereus sp.

H. stenopterus is a member of the Cactaceae and was first described by Britton & Rose (1963). It is a tropical hemiepiphyte (an epiphyte that can secondarily develop roots in the ground). *H. stenopterus* consists of three-ribbed stems that are triangular, succulent, and feature spines 2-4 mm wide and 5-10 mm long (Nerd et al., 2002). Flowers are perfect and 20-30 cm wide. Most flowers in the genus *Hylocereus* are

creamy white: two exceptions are *H. stenopterus* and *H. extensus*, which have red or pink flowers. All bloom nocturnally.

H. stenopterus is found only in Costa Rica. Other members of the genus *Hylocereus* can be found throughout Latin America, where they are thought to have originated, as well as around the world in tropical and subtropical regions (Britton & Rose, 1963; LeBellec et al., 2006). There are currently 16 recognized species in the genus *Hylocereus* (Britton & Rose, 1963). In Latin America several plants in this genus, and their fruit, are collectively known as pithaya or pitahaya (other spellings can be found as well: pitahaja, pitajuia, or pitalla). In Vietnam it is called thang loy or dragon fruit (Nobel, 2002). It continues to be recognized as a promising new crop (Janick, 1999). Results of this research are intended to facilitate the advancement of its production, not only as a cash crop for Caño Negro, Costa Rica, but for agriculturalists and horticulturists world-wide. As almost all reports of commercial propagation within this genus have revolved around one species, *Hylocereus undatus*. The remainder of this review will focus on research done on this species.

H. undatus is grown commercially in several countries in nurseries for ornamental retail sale as well as horticulturally for fruit production (Choi et al., 2007; Esquival et al., 2007; Merten, 2003; Yussof et al., 2008). Several papers explore methods for commercial propagation of this species, though the practice only began around 15 years ago (Andrade et al., 2006; Cavalcante & Martins, 2008; De Andrade et al., 2007; De Andrade et al., 2008; Michel-Rosales & Farias-Larios, 2007; Mohamed-Yasseen, 2002). Both asexual and sexual propagation have been attempted. Though propagation by seeds is possible, germination is often inconsistent (LeBellec, 2003). Micropropagation has

also met with success, but the cost of production is currently not profitable (Garcia et al., 2009; Mohamed-Yasseen, 2002). LeBellec (2003) concluded that asexual propagation by cuttings represents the most effective and promising form of propagation, at least for horticulture purposes.

Up to 90% rooting success can be achieved using cuttings (LeBellec, 2003). Cavalcante & Martins (2008) suggested that 25 to 50 cm cuttings be taken from juvenile growth with the basal end cut being made just above a joint in the fleshy green stem extending to the terminal end joint. Bastos et al. (2006) concluded that application of a rooting hormone aided in rooting success, but Thomson (2002) reported no effect. Thomson (2002) also suggested allowing cuttings to cure or cicatrize for up to 5 days before planting while Cavalcante & Martins (2008) planted cuttings immediately.

These studies indicate that, for *H. undatus*, cicatrization time and application of rooting hormone may influence propagation success. Further research is necessary to make more specific recommendations for asexual propagation of not only *H. undatus* but *H. stenopterus* and *H. costariensis* as well. Current research investigates stem cutting propagation for all three species.

Accoelorraphe wrightii

Aracaceae is an isolated and distinct group within the plant kingdom and is composed entirely of woody monocotyledonous plants collectively and commonly known as palms (Jones, 1995). Palms are found around the world in tropical ecosystems and most have a fairly recognizable canopy with a prominent trunk and a characteristic crown of leaves. All are perennial, and the family contains both monoecious and

dioecious species. Reproduction by palms in nature is almost exclusively by seed (Meerow, 2002).

Within this family, *A. wrightii*, also known as Paurotis palm or Everglades palm, is a member of the Corypheae tribe and the Coryphoideae subfamily (Meerow, 2002). It is found in Florida, the West Indies, and coastal or wetland areas of Central America (Farruggia, 2008; Jones, 1995; Meerow, 2002; Riffle & Craft, 2003). The genus *Acoelorraphe* is monotypic with *A. wrightii* the only species (Jones, 1995). It is a relatively tolerant species to habitat conditions, being one of the few palms that can grow in saturated soils (Meerow, 2002). It can have problems with manganese deficiencies but responds well to fertilization and has no known pest problems (Meerow, 2002).

Though reproductive maturity is reached in four to five years by some species of palms, others can take up to forty or fifty years (Meerow, 2004). Unlike *A. wrightii*, seed germination in most palms is notoriously difficult, with some seeds taking years to germinate (Odetola, 1987). It has been estimated that up to 25% of all palms take over 100 days to germinate and often achieve less than a 20% germination rate (Tomlinson, 1990). Germination of *A. wrightii* usually occurs within 2 to 4 months (Carpenter, 1988; Jones, 1995; Meerow, 2002). There is no clear evidence why this difficulty in germination occurs, as very few seed germination studies have been conducted for palms (Meerow, 2004). Efforts to produce palms commercially persist, however, as certain species are significant in the ornamental and edible markets (Jones, 1995).

Propagation of palms is almost entirely done by seed, though vegetative propagation is possible by air layering, division, or even more recently, tissue culture (Jones, 1995). Vegetative propagation is almost exclusively used by enthusiasts and

collectors while tissue culture is used by researchers, collectors, and large scale commercial operations. Neither tissue culture nor vegetative propagation is feasible for the average nursery or farm due to cost and equipment needs.

Seed germination among palms occurs in several forms but can generally be divided into two initial categories; remote or cryptocotylar germination and adjacent or hypogeal germination (Meerow, 2004; Orozco-Segovia et al., 2003). Seed germination in *A. wrightii* occurs by way of the remote germination pathway (Meerow, 2002). With remote germination the first structure to emerge from the seed is called the cotyledonary petiole; this is commonly mistaken for the first seedling root (radicle). However, the first seedling root will actually emerge from this structure after it has grown down into the soil. At the other end, the seedling shoot or plumule will emerge. The cotyledon remains inside as the absorptive structure and is known as the haustorium. Adjacent germination involves only a small portion of the cotyledon emerging from the seed; this is known as the button (Meerow, 2004). Then, the radicle and plumule emerge from the button. The radicle in this type of germination is very short lived and is quickly replaced by adventitious roots that form around the stem base. Like remote germination, the part of the cotyledon known as the haustorium remains inside the seed and absorbs nutrients from the endosperm. These different germination strategies have been associated with establishment in different ecosystems (Orozco-Sergovia et al., 2003). Information on seed germination for palms tends to be inconsistent as much of it comes from hobbyists observations and not controlled research. No clear propagation protocols can be described for more than a few commercially important species.

Seed dormancy has been identified as an important aspect of palm seed germination and may be a significant part of understanding the long and sporadic germination of many palm species (Orozco-Segovia et al., 2003). Several methods are used in plant propagation to break seed dormancies, the most common among palms being seed scarification. Scarification for palms commonly utilizes extended soaks in water for 12 h up to several days (Broschat & Donselman, 1986; Kheong, 1992; Moussa et al., 1998; Odetola, 1987). In cases of extremely hard or thick seed coats, soaking in concentrated sulfuric acid can be effective for shortening germination time (Merlo et al., 1993). Physical scarification accomplished by nicking the seed coat has also been used to reduce germination time and increase germination rate (Holmquist & Popenoe, 1967; Nagao et al., 1980; Odetola, 1987).

Substrate for palms requires well-drained yet moist conditions. Most palms require uniform moisture during germination and subsequent seedling development and do not respond well to alternating periods of dry and wet. It is important that the substrate stay damp but not be saturated since palm seeds can be susceptible to rot (Koebernik, 1971). A common substrate in the nursery industry is a 1:1 ratio of peat moss:perlite (Meerow, 2004). Palms have also been germinated in only perlite or peat moss, but a 1:1 combination appears to be the most effective (Carpenter, 1988; Jones, 1995; Koebernik, 1971). Fertilization of palms is not important until late in seedling development. For the first few months after germination the endosperm acts as the only source of nutrition for the developing seedling and further nutrients can damage the seedling (Meerow, 2004).

Only one study (Carpenter, 1988) directly investigated propagation of *A. wrightii*, and concluded that *A. wrightii* germinated best at temperatures above 35^{o} C with an optimum range of 33^{o} to 39^{o} C. Current research will investigate the effect of scarification method and sowing substrate on the germination rate and final germination percentage of *Acoelorraphe wrightii* seeds.

Materials and Methods

Hylocereus sp.

All experiments with *Hylocereus* sp. were conducted in a glass house at the Paterson Greenhouse Complex on the Auburn University campus in Auburn, AL (32.6^{o}N latitude). Ground beds 1 m wide, 4.6 m long and 15.2 cm tall were used for propagation. Beds were lined with black fabric groundcloth and filled with 100% sand.

Hylocereus stenopterus

Cuttings of *H. stenopterus* were obtained from California in February 2010 (Matt's Landscape, San Juan Capistrano, CA). Thirty-three cuttings, each measuring an average of 18 cm long, were harvested on 6 February, bundled in newspaper, mailed via USPS, and arrived in Auburn, AL on 16 February. Upon receipt in Auburn, cuttings were sorted into groups based on stem diameter, recut into smaller pieces, each measuring approximately 3.8 cm in length, and fresh weight (FW) was recorded for each cutting. Cuttings from each stem diameter were distributed evenly among treatments. Care was taken to maintain proper polarity of all cuttings. Half of the cuttings were then stuck immediately, while the other half were allowed to remain uncovered on a bench in the greenhouse for 72 h (cicatrization). Prior to sticking, the basal end of half of the cuttings were treated with 0.1% IBA (Hormodin®, OHP, Inc., Mainland, PA) while the

other half received no hormone. Once stuck, half of the cuttings were covered with a plastic tent. Mist tents were 91 cm tall, 127 cm wide, 178 cm long, and constructed of 1" Schedule 40 PVC™ pipe and Husky® 3.5 mm clear plastic sheeting. Mist tents were placed directly on top of the propagation bed. Thus, treatments were in a 2 hormone x 2 cicatrization x 2 tent factorial design for a total of 8 treatments with 20 replications per treatment for a total of 160 cuttings. Cuttings were stuck such that the basal 1 cm was below the surface of the sand. Experimental design was a split plot design with the presence or absence of plastic tent forming the main plot (total four main plots). Within each main plot, treatments (hormone x cicatrization) were arranged randomly.

Mist was applied initially for 20 s once an hour then adjusted as needed throughout the experiment. For the first month cuttings were misted for 20 s every hour. This appeared to be too much moisture as rot began to occur on the cut surface of each cutting. On 11 March mist was discontinued, and the substrate was allowed to dry for 4 days. Beginning on 15 March, cuttings then received mist once a day for 10 min a day between 8:00 a.m. and 10:00 a.m. Beginning 14 June cuttings were fertilized at a rate of 100 ppm N 20-20-20 Jack's® Professional (J.R. Peters, Inc., Allentown, PA) water soluble fertilizer, applied to the bed once a week by hand. This was increased to twice a week beginning 12 July.

On 3 May, mortality, new shoot length, length of longest root, and total plant fresh weight were recorded. To measure fresh weight, cuttings were carefully dug out of the sand by hand and the roots rinsed with water to remove sand before weighing. To account for initial cutting size, relative growth rate (RGR) was calculated using the

formula (Final FW – Initial FW)/Initial FW. On 17 June shoot lengths were measured again.

On 30 June, cuttings with new shoots longer than 5 cm were either removed from the bed and potted or the new shoot was removed from the original cutting, treated with hormone and stuck in the same sand bed adjacent to the original cutting. Cuttings that were potted were planted either with the original cutting entirely below the substrate surface or with only the basal 1 cm of the cuttings below the surface (similar to position in the propagation bed). Equal numbers of cuttings (37) were used for each "replanting" technique. On 22 July the original cuttings still in the bed were evaluated, and cuttings with new shoots over 5 cm were again either potted using the two potting methods described above or the new shoot was removed and stuck using the same procedures as on 30 June. When potted, cuttings were planted in 3.8 L black plastic pots in 1:1:2 perlite:peat moss:sand and placed on a raised bench in the same greenhouse as the sand ground bed. Potted cuttings were fertigated by hand twice a week at the same rate and analysis of fertilizer previously given. For all repotted and restuck cuttings, shoot lengths were measured on 24 Aug. and 29 Sept. Relative growth rate of the shoots for cacti that were potted was calculated using the following formula ((Initial length-Final length)/Initial length)/Number of days between measurements. Cuttings that were cut and stuck in the same bed were only observed for survival.

H. undatus* and *H. costariensis

Sixty cuttings of *H. undatus* and 15 cuttings of *H. costariensis* were harvested 22 May 2010 in CA and shipped to Auburn, AL. Experiment location, cutting source, and shipping method were the same as with *H. stenopterus*. *H. undatus* cuttings were re-cut

so that each new cutting would have at least 3 nodes on at least 2 ribs. Once recut, cuttings were sorted into three size classes based on fresh weight, and each size class was distributed evenly among each treatment. *H. costariensis* cuttings were all of a similar size and there was no need to sort them based upon size. Care was again taken to maintain proper polarity with all cuttings.

For both species three hormone treatments were used: untreated (no hormone applied, control), 0.1% IBA or 0.8% IBA. *H. undatus* had 30 replications in each treatment for a total of 90 cuttings. *H. costariensis* had 9 cuttings per treatment for a total of 27 cuttings. Experiments for both species were completely randomized. All cuttings received overhead mist for 10 min a day between 8:00 a.m. and 10:00 a.m. Beginning 14 June all cuttings were fertigated once a week at the same rate and with the same fertilizer solution as that used previously. Beginning 12 July cuttings were fertigated twice a week at the same rate.

Initial fresh weight and length of all cuttings were measured for both species. Mortality, length of new shoots, longest root length, and total plant fresh weight (as described above) were recorded for both species on 13 July. RGR was calculated as described above. Lengths of new shoots were measured again for *H. undatus* on 16 Sept. Lengths of new shoots, longest root length, and total fresh weight (as described above) of *H. costariensis* cuttings were measured again on 24 Aug.

All growth and rooting data for all cacti were analyzed using PROC GLM in SAS 9.1 (SAS Inc., Cary, NC). Means were separated using PDIFF at $\alpha = 0.05$ (5%). Results are not reported for any parameter showing no significant differences.

Acoelorraphe wrightii

Experiments with this species began in February 2010. Seeds were obtained from the Banana Tree (Easton, PA). All experiments with *A. wrightii* were conducted in a glass house in Auburn, AL on raised metal benches at the Paterson Greenhouse Complex on the Auburn University campus (32.6^{o}N latitude). All containers were hand watered on a daily basis. Seeds were stored in the cooler at the Paterson Greenhouse Complex at 8^{o}C. For experiments 1 and 2 seeds were planted in 20 cm x 13 cm squat mum pots (Nursery Supplies, Inc. Kissimmee, FL). In experiment 3 seeds were planted in 4" individual pots and placed in plastic mesh flats. All seeds were planted at a depth of 1 cm (1.5 times mean seed diameter). For all experiments, experimental design was a completely randomized design.

Experiment One

To investigate the effect of scarification on germination of *A. wrightii*, seeds received one of three scarification treatments: non-scarified (control, unsoaked), abrasion scarified, and hot water scarified. There were 10 pots per treatment with 5 seeds per pot. Abrasion scarification was accomplished by hand using a rat tail file to rasp out a 1-2 mm deep groove in a single location on each seed coat. Seeds that were hot water scarified were placed in water initially heated to 65.5^{o}C and left to soak for 24 h. All seeds were planted on 23 February 2010 in a 1:1 peat:perlite mix. Data collection included whether seeds germinated and when seedlings reached a height of 7 cm.

Experiment Two

To investigate the effect of scarification on germination of *A. wrightii*, seeds received one of eight scarification treatments: unscarified (control in which seeds were soaked in room temperature water for 30 min), immersion in 100% H_2SO_4 for 10, 20, or

30 min, or immersion in 80%, 60%, 40%, or 20% H_2SO_4 for 30 min. There were 10 pots per treatment with 3 seeds per pot. All seeds were planted on 5 April 2010 in a 1:1 peat:perlite mix. Data collected included date of germination (defined as when new growth was visible above the soil surface) and date seedlings reached a height of 5 cm.

Experiment Three

To investigate the effect of scarification as well as substrate on germination of *A. wrightii*, seeds received one of twelve treatments. There were four substrate treatments and three scarification treatments in a factorial design of 12 total treatments. Scarification treatments included unscarified (control), hot water scarification (methodology being the same as that for experiment one), and acid scarification of 30 min immersion in 100% H_2SO_4. Substrate treatments were a 1:1 peat:perlite mix, 100% perlite, 100% aged whole pine tree substrate (AP) (aged 9 months outdoors in 63 ft^3 bulk bags), and 100% fresh whole pine tree substrate (FP) (harvested and processed the same week seeds planted). There were 10 pots per treatment with 5 seeds per pot. All seeds were planted on 11 June 2010. Data collected were the same as that for experiment two.

All data were analyzed using PROC GLM in SAS 9.1 (SAS Inc., Cary, NC). Means were separated using PDIFF at $\alpha = 0.05$ (5%). Results are not reported for any parameter showing no significant differences.

Results

Hylocereus stenopterus

For data collected 3 May, there was a significant interaction between rooting hormone and mist tent for root length. Cacti that did not receive rooting hormone and were also not under a tent had less root length than other cuttings (2.5 cm (SE=0.69,

p=0.03) vs. 6 cm (SE=0.69, p<0.0001) average in all other treatments). On 17 June a significant difference in RGR of shoots occurred between cacti cuttings that were covered by a tent (5.5 cm (SE=1.06, p<0.0001)) as compared to those that were not (2.8 cm (SE=1.06, p=0.007)).

Cacti repotted on 30 June showed no significant differences among replanting techniques in average shoot length on 24 Aug. or 29 Sept. Cacti repotted on 22 July showed no differences in RGR of shoots when data were taken on 24 Aug. However, on 29 Sept. RGR for shoots of cacti with the original cutting planted above the substrate surface were significantly higher than that for those planted below the substrate surface (45 cm and 31 cm, respectively (SE=3.1, p<0.0001)).

H. undatus* and *H. costariensis

On 13 July, for *H. undatus*, root length and date of new shoot development were different among treatments. Root length was longer for untreated cuttings (16 cm (SE=1.36, p<0.0001)) than for cuttings receiving either treatment with 0.1% IBA or 0.8% IBA (12 cm (SE=1.08, p<0.0001) and 11.8 cm (SE=0.98, p<0.0001), respectively). Untreated cuttings also developed shoots in a significantly shorter period of time (25 days (SE=2.09, p<0.0001)) as compared to either treatment with 0.1% IBA or 0.8% IBA (31 (SE=1.66, p<0.001) and 35 (SE=1.51, p<0.0001) days respectively). There were no significant differences among treatments for any of the measurements taken on 13 July for cuttings of *H. costariensis*.

On 16 Sept., RGR for new shoots of *H. undatus* cuttings treated with 0.8% IBA was higher (14.7 cm (SE=0.53, p<0.0001)) than for cuttings treated with 0.1% IBA (8.8 cm (SE=0.70, p<0.0001)) and untreated cuttings (10.2 cm (SE=0.87, p<0.0001)). There

was also a difference in the number of days until shoot development for cuttings treated with 0.1% IBA (46 days (SE=5.6, $p<0.0001$)) and 0.8% IBA (57 days (SE=4.2, $p<0.0001$)), but there was no difference between untreated cuttings (52 days (SE=6.9, $p<0.0001$)) and either of the two other treatments.

On 24 Aug., there was a difference in RGR of new shoots between untreated *H. costariensis* cuttings and those treated with 0.8% IBA (19 g (SE=2.3, $p<0.0001$) and 26 g (SE=2.3, $p<0.0001$) respectively).

Acoelorraphe wrightii

Experiment 1

Germination percentages for the control and water scarified seeds (40% (SE=0.059, $p<0.0001$) and 50% (SE=0.059, $p<0.0001$), respectively) were not significantly different from each other, while that for the abrasion scarified seeds (2% (SE=0.059, $p=0.7$)) was significantly lower than both treatments. Of the seeds that germinated there were no significant differences among treatments as to the speed at which seeds developed to a height of 7 cm.

Experiment 2

Seeds scarified in 100% sulfuric acid for 30 min had a higher germination percentage (80% (SE=0.05, $p<0.0001$)) than seeds in any other treatment. Seeds scarified in 100% acid for 10 and 20 min had higher germination percentage (56% (SE=0.05, $p<0.0001$) and 50% (SE=0.05, $p<0.0001$), respectively) than seeds in all other treatments. In all other treatments (seeds scarified for 30 m in 80%, 60%, 40%, and 20% acid and control seeds) germination percentages were not significantly different from each other (0%, 0%, 0%, 3%, and 0% respectively).

Of the seeds that germinated, those scarified in 100% acid for 30 min germinated more quickly (65 days (SE=1.42, $p<0.0001$)) than seeds scarified in 100% acid for 20 min (67 days (SE=1.95, $p<0.0001$)) and seeds scarified in 100% acid for 10 min (78 days (SE=1.75, $p<0.0001$)). These treatment differences were the same for number of days after sowing until seedlings reached the height of 5 cm.

Experiment 3

In experiment 3, there was an interaction between scarification and substrate treatments for germination percentage. Germination percentage for seeds scarified in acid and planted in 1:1 peat:perlite was significantly higher (64% (SE=0.04, $p<0.0001$)) than all other substrate/scarification combinations (Table 1).

Of the seeds that germinated, those soaked in 100% acid and planted in 1:1 peat:perlite had fewer number of days between sowing and germination (43 (SE=2.3, $p<0.0001$)) than all other treatments (Table 2). There were no differences between treatments pertaining to number of days following germination until seedlings reached 5 cm.

Discussion

***Hylocereus* sps.**

Results for all three species of *Hylocereus* suggest that this genus has the potential to be an easily propagated and maintained crop for ornamental or edible field crop purposes. High survival was observed for all three species regardless of treatment, cuttings rooted quickly and readily, growth was vigorous once cuttings were established, and plants were easily transplanted with only minimal mortality. Like LeBellec (2003), high overall survival rates with cuttings were documented for all species regardless of

treatment (*H. stenopterus*: 85%, *H. undatus*: 77%, *H. costariensis*: 100%). Within treatment, *H. undatus* had 97% survival rate for cuttings treated with 0.8% IBA. One hundred percent survival was also seen for *H. stenopterus* cuttings that were covered by a mist tent but neither received rooting hormone nor were allowed to cicatrize. In practice however, no treatment produced dramatic enough results to indicate a particular advantage when propagating this plant. Both LeBellec (2003) and Cavalcante and Martins (2008) suggest using cuttings that are longer than 25 cm. However, cuttings of *H. stenopterus* used for this study only average 3.8 cm long and were still successful.

Rooting hormone did have an effect on the rooting and subsequent growth of cuttings from all the *Hylocereus* species investigated. On 3 May, *H. stenopterus* cuttings that received rooting hormone had a significantly higher RGR and longer roots than other cuttings. On 13 July *H. undatus* cuttings that were treated with 0.8% IBA had longer shoots than other treatments and cuttings treated with 0.1% IBA developed shoots more quickly than other treatments. On 24 Aug. *H. costariensis* cuttings treated with 0.8% IBA had a higher RGR and longer shoots than all other treatments. This is consistent with research by Bastos (2006), which suggested that the application of rooting hormone would help with survival of *H. undatus* cuttings.

Cicatrization did not affect survival subsequent growth of *H. stenopterus* cuttings. However, both Thomson (2002) and Cavalcante and Martins (2008) found it beneficial to allow cicatrization time. During this investigation, on 3 May, cuttings that had cicatrized had a higher RGR than other treatments, but this was no longer the case when cuttings were measured again on 17 June.

Acoelorraphe wrightii

Research on this palm suggests that it would be a good candidate for commercial production within the ornamental market and for conservation oriented outplanting due to its relatively short germination time, relatively high germination percentages, robustness of seedlings, and ease of transplant. Similar to results reported by Carpenter (1988), this research indicates that germination time for *A. wrightii* seeds, particularly when subjected to scarification, can be relatively short compared to other palm species (Meerow, 2004). Seeds scarified in 100% acid for 30 min in both Experiments 2 and 3 resulted in shorter germination times than other treatments. Seedlings were robust after germination; no seedling mortality was observed in any experimental trials. Speed of growth for seedlings was remarkably uniform as well. Once seeds germinated, seedlings consistently took 9 to 12 days to reach a uniform height.

Scarification of seeds affected not only germination time but also germination success. In Experiment 1, seeds scarified in water had a higher germination percentage (50%) than either control seeds (40%) or seeds that were manually scarified (2%). It is possible that the method used to abrasion scarify seeds damaged embryos. The exact location of the embryo inside *A. wrightii* seeds is unclear so it is possible that scarification actually damaged the embryo. Nagao et al. (1980) suggested a similar explanation for low germination percentages in palm seeds that were scarified manually with a file. In all experiments some level of scarification, either by water or acid, increased percent germination. This is consistent with findings from Odetola (1987) who reported that scarification improved germination in several other palm species. In this, study the highest germination percentages were recorded when seeds were soaked in 100% H_2SO_4 for 30 min.

Choice of substrate affected rate of germination in Experiment 2. Consistent with recommendations by Meerow (2004), a 1:1 peat:perlite substrate produced highest germination rates. Koebernik (1971) suggests it is possible to grow palm seeds more successfully in 100% perlite. This was not the final conclusion of this research, where seeds grown in 100% perlite had some of the lowest germination percentages over all.

Conclusions

All species of *Hylocereus* investigated in this research grow well in 100% sand. To speed up rooting, rooting hormone can be applied but is not necessary to assure final rooting success. On latitudes even with or north of AU, it is necessary to grow cacti inside a structure that provides shelter from freezing temperatures. However, the use of a mist tent is not crucial.

A. wrightii seeds are easily stored at cool temperatures and respond with increased germination rates and percentages to scarification with water or acid. The lack of seedling mortality and ease of transplant make this palm a good candidate for nursery production. It is important to make sure these palms receive adequate water and are fertilized once seeds have finished germinating.

Both these species appear to be good candidates for both commercial production as well as conservation oriented propagation and outplanting in Costa Rica. All experiments indicate that both species are easy to work with, vigorous, attractive, and easy to propagate. Collaborative entities in Costa Rica are also motivated to work with these species and remain cooperative toward ongoing research goals.

Table 1: Effect of scarification treatment and germination substrate on germination percentage of *A. wrightii* seeds grown in a greenhouse in Auburn, AL.

Mean Germination Percentages (SE=0.04)				
Scarification Treatments[z]	Substrates[y]			
	PP	AP	FP	P
Acid Scarification (100% H_2SO_4 for 30 min)	64Aa	16Ba	22Ba	2C
Water Scarification (24 hr soak in water initially heated to 65.5°C)	22Ab	0Bb	13Bb	2B
Control (Unscarified)	16Ab	6Bb	2Cb	0C

[z]Treatments included acid scarification (A) (soak in 100% sulfuric acid for 30 min), water scarification (W) (24 hr soak in water heated to 65.5°C), and unscarified (C) (control).
[y]Substrates used included 1:1 peat:perlite (PP), aged pine tree substrate (AP), fresh pine tree substrate (FP), and 100% perlite (P).
Means separation using PDIFF at $\alpha = 0.05$ indicated by uppercase letters in rows and by lowercase letters in columns.

Table 2: Effect of scarification treatment and germination substrate on number of days until germination of *A. wrightii seeds* grown in a greenhouse in Auburn, AL.

Mean Days Until Germination			
Scarification Treatments[z]	Substrates[y]		
	PP	AP	FP
Acid Scarification (100% H_2SO_4 for 30 min)	43Ab[x] (SE=2.3)	73B (SE=5.3)	67B (SE=4.5)
Water Scarification (24 hr soak in water initially heated to 65.5°C)	94Aa (SE=4.5)	--[w]	66B (SE=5.4)
Control (Unscarified)	85a (SE=5.2)	--	--

[z]Treatments included acid scarification (A) (soak in 100% sulfuric acid for 30 min), water scarification (W) (24 hr soak in water heated to 65.5°C), and unscarified (C) (control).
[y]Substrates used included 1:1 peat:perlite (PP), aged pine tree substrate (AP), fresh pine tree substrate (FP), and 100% perlite (P).
[w]Data not available due to insufficient germination.
[x]Means separation using PDIFF at $\alpha = 0.05$ indicated by uppercase letters in rows and by lowercase letters in column

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Appendix

Nymphaea ampla

Introduction

Preliminary research was begun on the propagation of the water lily *Nymphaea ampla*. Trials were undertaken to investigate not only germination of *N. ampla* seeds but also subsequent growth and fertilization of seedlings. Due to insufficient results no statistical analysis was performed and only a literature review and cursory results will be presented here.

Literature Review

N. ampla is a member of the Nymphaeaceae in the subgenus Brachyceras (Wunderlin, 1980). This family contains seven genera and about 65 species, 12 of which are members of the subgenus Brachyceras (Muhlberg, 1981). All are aquatic plants with rhizomes or tubers and leaves that are arranged in basal rosettes (Muhlberg, 1981). Most grow in sheltered areas of standing or slowly flowing water and can even withstand short periods of drought (Speichert & Speichert, 2004). Within the genus *Nymphaea*, an important distinction is made between tropical water lilies and hardy water lilies which can grow in cooler climates. Hardy water lilies flower only during the daytime while tropical water lilies have both night and day blooming varieties (Slocum & Robinson, 1996).

N. ampla is a tropical water lily that flowers only during the day (Landon, 2009). Some tropical species also display viviparous germination (when seeds germinate while

still attached to the parent plant), but Slocum & Robinson (1996) assert that *N. ampla* is not one of them. For this species all flowers are white and have a pronounced fragrance. New leaves grow out as a bronzy red but quickly develop a deep green color. Leaves usually have dark splotches and prominent veins. Leaf margins are dentate with a wavy perimeter. This species is native to several countries in Central and South America as well as areas of the southern United States (Conard, 1905; Wunderlin, 1980). This plant has been used for centuries as a pharmaceutical and some of this use continues today (Bertol, 2004). Aporphine is an active compound which can be found in the bulbs and roots of at least three species of *Nymphaea* including *N. ampla* (Bertol, 2004). In the human body, aporphine is hydroxylated into the active compound apomorphine. Traditionally this has been used as a hallucinogen in rituals and religious ceremonies, not only in ancient times but regularly during the 1960s and 1970s. Today, research is ongoing as to its effectiveness to treat erectile dysfunction (Bertol, 2004).

While it is possible to propagate *N. ampla* from seed or rhizomes, seed propagation is rarely used in industry (Purcell, 1995). Reasons for this are not necessarily related to difficulties with seed propagation, but rather because named cultivars of water lilies are almost all hybrids and must be propagated from tubers or rhizomes in order to produce genetically identical individuals (Landon, 2009). Some species have seeds which are extremely sensitive to desiccation and can only be germinated if planting occurs within a few weeks of harvesting (Conard, 1905). Certain species in the subfamily Brachycerus are not among these and can withstand extended periods of drought (Conard, 1905). Seed propagation on a commercial scale is restricted to the species *Nymphaea pygmaea* Salisb. and *Nymphaea odorata* Aiton (Case, 1991).

In general, fully mature seeds of tropical water lilies are hard and black and surrounded by a jelly-like substance which supposedly helps with flotation and dispersion in water (Speichert & Speichert, 2004). Seeds of *N. ampla* measure only a few millimeters across, not including the jelly like substance. Before planting, the jelly is removed by wiping it off to keep the seed from floating at the surface. Then, growers generally use one of two methods. First, seeds can be allowed to float in the water column and germinate before they are planted into a solid substrate. Second, some growers will actually plant seeds by spreading them over soil or substrate, covering them with a fine layer of sand (no more than 2-3 mm deep), and finally covering again with several centimeters of water (Speichert & Speichert, 2004; Case, 1991). For germination, most water lily seeds need to be submerged in 5 to 30 centimeters of water (Conard, 1905). Germination time is poorly documented, and reports vary widely for Nymphaeaceae in general. In one experiment, one set of seeds from a single species germinated in two weeks while a second set of the same species germinated after more than a year (Conard, 1905). Factors that appear to affect germination time are temperature, light level, crowding of seeds, storing time, sowing depth and water depth (Case, 1991; Conard, 1905; Slocum & Robinson, 1996; Speichert & Speichert, 2004).

N. ampla has a small presence in the literature, but the closely related species *Nymphaea odorata* has been the subject of more research. Else & Riemer (1984) investigated several aspects of germination for *N. odorata*: seed crowding, light level, stratification, freezing, desiccation, ethylene, and seed coat puncture. Investigating seed crowding was meant to determine whether or not a germination inducer is released into the water by the seeds and, if present, what that substance might be. Else and Reimer

(1984) determined that the level of seed crowding affects germination for this species, reporting that after replicating treatments over three growing seasons there was zero germination in experimental vials containing 20 seeds or less in 100 mL of water (1 seed/5 mL). The highest germination percentages in this research occurred in vials with 100 seeds in 20 mL of water or 5 seeds/mL of water. They also observed that when water from crowded vials was transferred to uncrowded vials, germination increased, indicating there was a substance released from the seed into the water that promoted germination. After further experimentation it was hypothesized that the substance was a water soluble gas. Other studies have shown that ethylene can affect germination in witchweed, cocklebur, clover, peanut, and a number of other species leading researchers to decide to investigate the effect of this gas on *N. odorata* (Egley & Dale, 1970; Egley, 1980; Esashi & Leopold, 1964; Ketring & Morgan, 1969; Letham et al., 1978). Application of ethylene to tightly capped seed vials did produce higher germination percentages than in controls in all trials. Restricted light level reduced germination percentages, and freezing severely reduced germination. Periods of drying also significantly inhibited germination. Seed coat puncturing did not assist germination. There did appear to be an interaction effect between cold stratification and seed crowding. Stratified seeds showed increased germination in crowded situations after 5 months of chilling but no effect in uncrowded situations. Seeds stratified for 7 to 9 months showed nearly 100% germination in crowded conditions and improved germination in uncrowded conditions. Conard (1905) reported that seeds from the subgenus Castalia, of which *N. odorata* is a member, are particularly sensitive to drought and temperature fluctuations, but no such information is available for the subgenus

Brachyceras. The current research will investigate the effect of crowding, light level, and sowing method on germination of *Nymphaea ampla* seeds.

Results

Seeds were both ordered from an online supply company (The Banana Tree, Easton, PA) and provided by a private grower. Seeds procured online were dry and had been stored for an unknown period of time. Seeds provided by the grower were fresh from the plant and were never allowed to dry completely. All seeds were germinated in petri dishes. The only definitive result that can be reported is that dry seeds, barring any unknown dormancy breaking requirements, will not germinate. Regardless of treatments applied, fresh seeds had almost 100% germination while not a single dry seed germinated. Contrary to Slocum and Robinson (1996), viviparous germination was observed. Fatal algal blooms were encountered when attempts were made to subsequently grow seedlings in pots. Three different fertilization techniques were attempted with no success in any pots that actually received fertilizer. Only unfertilized (control) pots did not produce algal blooms but seedlings also did not continue developing.

Rhizomes were not available at the time of this research but if some could be found this would perhaps be the best way to continue research on the growth of *N. ampla*. Future research on seedling growth may be more successful in containers that provide a larger body of water. Communication with Ken Landon after initial attempts to grow seedlings failed indicated that due to evaporation, chemical levels in small amounts of water can reach toxic levels for water lily seedlings regardless of any other parameter.

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