

# **PREDICTING SEEDLING ESTABLISHMENT IN TROPICAL DRY FORESTS USING PLANT TRAITS AND MICROSITES**

By

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## Abstract

Tropical dry forests are considered the most threatened terrestrial ecosystem due to their conversion into agricultural lands. Reforestation of these sites is critical to restore forest structure, function, and diversity. Understanding the environmental factors that affect sapling survival and establishment could help improve success in reforestation projects. This study investigated the ability of different species to adjust physiological leaf traits in response to harsh environmental conditions in tropical dry forest, and the influence of different microsite characteristics on plant growth and survival. Leaf chlorophyll content, leaf water content, photosynthesis, stomatal conductance, transpiration, water use efficiency (WUE), specific leaf area (SLA), growth, and survival rate were determined in an experimental planting of 9 tree species growing under three different vegetation covers (full sun, full shade, partial shade) in an experimental planting in Puerto Rico. Experimental results were compared to physiological measurements on 21 other tree species observed in dry forest remnants in Southwestern Puerto Rico. The relationships between microsite characteristics and growth and survival were also evaluated for these species in each location. High variability in water use efficiency (WUE) resulted in greater growth and survival while high specific leaf area (SLA) was associated with lower survival rates. This suggested that plant species might develop the ability to adjust water use efficiency (WUE) to respond to variable water supply and thereby increase survival. Microhabitats with moderate slope ( $\leq 20\%$ ), deep soil ( $> 20$  cm) and concave microtopography increased plant survival and establishment. In water-limited areas the

surviving individuals were mostly found in microhabitats that maximized water availability in the soil. To accelerate restoration of tropical dry forests both physiological and microsite traits should be considered in selecting species for restoration projects.

## Resumen

Los bosques secos tropicales son considerados los ecosistemas terrestres más amenazados debido a su conversión en tierras agrícolas. La reforestación de estos sitios es fundamental para restaurar la estructura, función y diversidad del bosque. La comprensión de los factores ambientales que afectan la supervivencia de árboles jóvenes y el establecimiento podría ayudar a mejorar el éxito en los proyectos de reforestación. Este estudio se investigó la capacidad de las diferentes especies para ajustar las características fisiológicas de la hoja en respuesta a las condiciones ambientales rigurosas en el bosque seco tropical, y la influencia de diferentes características de microhábitat sobre el crecimiento y la supervivencia. El contenido de clorofila y de agua de la hoja, la fotosíntesis, conductancia estomática, transpiración, la eficiencia del uso del agua (WUE), el área foliar específica (SLA), el crecimiento y la tasa de supervivencia fue determinados en una plantación experimental de 9 especies de árboles que crecen bajo tres diferentes tipos de vegetación cubre (a pleno sol, sombra total, sombra parcial) en una plantación experimental en la finca Mataró, Lajas, Puerto Rico y 21 otras especies de árboles observados en bosque seco remanentes del área suroeste de Puerto Rico. Se evaluaron también las relaciones entre las características de microhábitat y el crecimiento y la supervivencia para estas especies en cada localidad. En ambos estudios, la alta variabilidad en el uso eficiente del agua dio lugar a un mayor crecimiento y la supervivencia, mientras que alta SLA se asoció con tasas de supervivencia más bajas. Esto sugiere que las especies de plantas podría desarrollar la capacidad de ajustar WUE para responder a la variabilidad de alimentación de agua y por lo tanto aumentar la supervivencia. Microhábitats con pendiente moderada ( $\leq 20\%$ ), suelos profundos ( $> 20$

cm) y la microtopografía cóncava aumento de la supervivencia de plantas y establecimiento. En zonas limitadas de agua las especies que sobreviven se encuentran principalmente en microhábitats que maximiza la disponibilidad de agua en el suelo. Para acelerar la restauración de los bosques secos tropicales ambas características fisiológicas y microhábitat se deben considerar en la selección de especies para los proyectos de restauración.

## **Dedication**

This thesis is dedicated to my first source of inspiration Jehovah my God The Great I AM “Who gives wisdom and from Whose mouth come knowledge and understanding” (Proverbs 2:6, Holy Bible), to my dearest family Josee Vedrine-Pauléus and Mishael J. Pauléus my second source of inspiration, and to all scientists, my third source of inspiration, especially those who know that “there is a spirit in man and the breath of the Almighty gives them understanding”(Job 32:8).

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### List of variables used in the text

Variable	Definition	Units
SLA	Specific leaf area (leaf area: leaf dry weight)	cm <sup>2</sup> g <sup>-1</sup>
E	Transpiration rate per unit leaf area	Umol/g.s
WUE	Water-use efficiency of photosynthesis (CO <sub>2</sub> fixed per unit water transpired)	mmol m <sup>-2</sup> s <sup>-1</sup>
LAI	Leaf area index (total one-sided area of leaf tissue per unit ground surface area )	m <sup>2</sup> /m <sup>2</sup>
SM	Soil moisture (percentage of water in the soil)	%
CC	Chlorophyll content (percentage of Chlorophyll content)	%
LWC	Leaf water content (percentage of water in the leaves)	%
g <sub>s</sub>	Stomatal Conductance (rate of passage of CO <sub>2</sub> and water vapor through the stomata)	mmol/ m <sup>-2</sup> s <sup>-1</sup>
A <sub>max</sub>	Maximum photosynthesis (maximum increase CO <sub>2</sub> assimilation)	Umol/g.s
G_R_H	Growth rate in height (percentage of height growth )	%
G_R_D	Growth rate in diameter (percentage of diameter growth)	%

## CHAPTER 1: GENERAL INTRODUCTION

### 1.1 Introduction

The Earth's tropical and subtropical landmass is comprised of 40% opened or closed forests of which 42% is dry forest, 33% moist forest and 25% wet and rain forest (Murphy and Lugo, 1986a). Tropical dry forest [TDF, i.e., forests that exist in tropical regions characterized by a highly variable rainfall distribution (400-1700 mm/year), and a pronounced period of drought (Miles *et al.*, 2006; Gerhardt and Hytteborn, 1992)] represents the highest percentage of all tropical forest types (Murphy and Lugo, 1986). It is one of the most threatened ecosystems among all major tropical forest types due to conversion into agricultural land (Janzen, 1988).

Today in the tropics, most of the remaining dry forests are secondary (Gerhardt, 1996). Different vegetative recovery processes have been used in restoration of degraded forest. The most common approach is to use natural succession after abandonment of degraded land. This tends to be slow and low in biodiversity because of species' dispersal limitations, grazing, fire, and invasive species (Griscom *et al.*, 2009). Abandoned lands are usually dominated by early successional, fast-growing species (mostly invasive and a few native species) for a short period of time (up to 15 years) followed by the establishment of a few long-lived species for many years (e.g., Denslow, 1985; Uhl *et al.*, 1988; Guariguata *et al.*, 1997). More manipulative restoration uses seeding methods that often have low species survival without the use of herbicide (Brooks *et al.*, 2009) or transplanting seedlings or saplings of mostly native early successional fast-growing species that can accelerate succession and establish plant

communities over a shorter period of time (Lugo, 1992,1997; Aide *et al.*, 2000; Cabin *et al.*, 2002) than using native late successional species that may be limited from abandoned fields by dispersal, light, or soil conditions. According to Gómez-Popma and Vázquez Yanes (1981), native fast-growing species represent only 20% of the diversity in the tropical forest. That means, transplanting fast growing species only to speed up forest restoration will not necessarily restore forest biodiversity. However, late successional species with high intraspecific variation in leaf traits, transplanted in early successional environments, were shown to have higher survival rates than pioneers (Martínez-Garza *et al.*, 2005). Thus, physiological traits could predict reforestation success. However, in harsh early successional environments, pioneers could serve as nurse trees providing shade and nutrients (in case of N-fixing trees) and reducing competition by grasses for later successional species (Ray and Brown, 1995; Santiago-García *et al.*, 2008).

Several other factors may influence plant survival and establishment in TDF. For example, TDF are subject to human and natural disturbances (fire, clearing, hurricane, flooding, road construction, etc.), microsite heterogeneity, climatic fluctuation, and life history attributes of plant species, each of which tends to lengthen forest regeneration. Regeneration is a long-term and dynamic process, and tends to be slow and stochastic in TDF because of water shortages that limit plant growth and establishment. As a result, vegetation cover might alter microsite environments and could create areas of well-developed vegetation and some rigid zones for plant growth and survival. For example, Ray and Brown (1995) showed that shade is essential for the establishment of native species in dry forest. However, other limiting factors may influence the development and persistence of those communities and other dry land ecosystems. Considering that much

habitat in the dry zone of Puerto Rico and elsewhere in the tropics are degraded, and that development is happening in those areas, restoration projects have been increasingly important, but remain challenging because of environmental limitations to successful tree establishment.

## **1.2 Ecological Restoration**

Ecological restoration deals with the recovery process after disturbance (caused primarily by humans) to the diversity and the dynamics of indigenous ecosystems (Jackson *et al.*, 1995), and provides experimental designs to test ecological theory. The main goal of restoration is to speed up successional processes, which tend to be slow, by incorporating principles of forest ecology to return the forest to its initial structure and function. Different models try to re-establish forest structure and function by using a reference condition or historical evidence to determine restoration goals. To do so, both active and passive restoration approaches may be needed. A passive restoration approach involves the removal or suppression of anthropogenic or novel disturbances, and allows natural recovery. This approach is based on succession as the primary mechanism. After the removal of the perturbation, active restoration approaches attempt to re-introduce historically appropriate disturbances and other inputs such as introduction of propagules or alteration of structure. It may also require the re-introduction of different native plant species according to historical evidence.

Dryland restoration is limited not only by abiotic factors such as water stress or lack of available nutrients, but also by biotic factors that increase competition and can intensify water and nutrient stress. A number of studies have shown that as environmental

conditions (e.g. soil moisture, soil compaction, light availability, microbial activities, invasion rate, predation, adaptation to environmental conditions) change, selective pressures differentiate microsites and stress plant species differently in accordance to historical habitats. Plant species have evolved different physiological traits to respond to stress (Cowles, 1899; Pywell *et al.*, 2003; Shipley *et al.*, 2006; Westoby and Wright, 2006). In contrast, using an experiment of different plant species at the earliest stage of succession, Walker *et al.* (2006) showed that plant traits cannot predict colonization success depending on severe microsite differences. However, very few studies focus on long-term establishment and plant traits that predict establishment success. These are important factors that influence the development and persistence of plant communities.

### **1.3 Conceptual Support**

Different conceptual foundations provide patterns to understand the processes by which the number of species can survive in a particular area. Assembly theory emphasizes controversial ideas on whether ecological communities are deterministic (converge toward a common structure) or historically contingent (divergent in structure depending on localities) (Fukami *et al.*, 2005) while succession theory accentuates different sets of stages (with certain common characteristics) of ecological communities after disturbance. Ecological communities from different starting points may tend to return to a state similar to the initial one (climax) (Young *et al.*, 2001) but that depends on the strength, duration, and nature of the disturbance. Given the theories above, if communities move toward a predictable end-point even with different starting points, or if historical land use leads communities to multiple stable points under identical environmental conditions,

restorationists should question under what conditions and how often do communities move toward climax (Young *et al.*, 2001) and when is the opposite observed. An experimental design combining both assembly versus succession theories will allow ecologists to explain the difference between both of these conceptual foundations explicitly and to suggest good tools for reforestation success.

These two theories, though often treated separately, should be seen as complementary. Practitioners cannot transplant saplings that may be suitable to the sites and think that the process of succession by itself will drive a forest community toward a desired state identical to its initial state. This state may be one possible solution over many other alternatives that will suggest several management decisions among which practitioners will have the opportunity to decide on the most suitable option either ecologically or historically (Young *et al.*, 2001). Thus, restoration should consider both the single stable state and the multiple ecological states theories in order to succeed. The convergence versus the divergence of communities may depend on the level of community organization. On one hand, at the species composition level, communities tend to diverge due mostly to initial biotic variation of community assembly; on the other hand, trait-group composition tends to converge (Fukami *et al.*, 2005). Research is needed on different functional groups, especially trees.

#### **1.4 Leaf Traits and Microsite Characteristics**

Daehler (2003) predicted outcomes of interactions between native species and invaders on the basis of resource availability and disturbance regime. More forest disturbance will create more forest degradation and will generate more resource availability which will

favor invasive species. In dry forests, one of the most important limiting factors is water availability (Murphy and Lugo, 1986a). Thus, as invasion increases, competition for water will be higher (Daehler, 2003). In TDF, with water shortages, native plants have developed physiological traits such as low leaf water potential ( $\psi_{\text{leaf}}$ ) and high leaf thickness that allow them to minimize water loss and increase survival. To facilitate the re-establishment of native woody species and increase the security of the threatened native species in the dry zones, research is needed to increase our knowledge on how management practices can enhance natural succession, and the ecological patterns and processes that drive to successful reforestation. For my purpose, “success” is plant establishment.

Many studies have shown linkages between plant traits and good establishment, as indicated by high growth and survival rates (Pywell *et al.*, 2003). Patterns of variation among growth forms (trees, lianas, understory plants) have revealed the importance of leaf traits within sites (Santiago and Wright, 2007). Mediterranean tree species *Acer opalus* ssp. *granatensis* indicated variability of seedling establishment between microsites in space and time (Gomez-Aparicio *et al.* 2005). In contrast, Walker *et al.* (2006b) found that in early primary succession where severe habitats with strong abiotic filters combine with minor microsite differences, species traits were poor predictors of colonization success. Their study also compared functional plant groups (shrubs, forbs, grasses) to different microsite status and found that functional plant groups did not predict colonization success. However, the same study specified predictable microsite characteristics such as concave microtopography to establishment success at the level of individual species. Del Moral and Wood (1989) and Jones and Del Moral (2005) found

similar results in previous studies of temperate forests. Kitajima (1994) compared the different photosynthetic traits of new leaves under shade and sun treatments for 13 tropical tree species (either shade-tolerant or shade-intolerant) and how physiological traits affected seedling survival. Her study showed that species with higher relative growth rate (RGR) from germination to 2 months in both sun and shade had lower survival rates in the first year under the canopy. Species with high RGR in shade also had higher RGR in sun. She also found that CO<sub>2</sub> exchange did affect seedling survival but that maximum net photosynthesis (A<sub>max</sub>) varied among species within light treatments. Despite studies such as these above from other vegetation types, little is known about plant traits and microsite characteristics that predict dry land restoration success. The study of the ecological patterns and processes that encourage successful reforestation (i.e., microsite traits, succession pathways and rates, species traits, and factors that control and limit their spread) will facilitate restoration efforts of degraded indigenous habitat for native plant communities. It will allow us to better predict whether a reforestation project will succeed.

The research objectives are to:

1. Evaluate the success of native late-successional dry forest species in reforestation and natural regeneration.
2. Determine plant characteristics that are good indicators of reforestation success.
3. Determine microsite characteristics that are good indicators of reforestation success.

## **CHAPTER 2: PHYSIOLOGICAL LEAF TRAITS AS INDICATORS OF PLANT ESTABLISHMENT IN REFORESTATION**

### **2.1 Introduction**

Deforestation of tropical forests is increasing at an alarming rate and almost everywhere in the tropics (Turner, 1996; Bawa and Dayanandan, 1997) causing the decline of biodiversity of forest remnants. Many cultivated and pasture lands have been abandoned for natural forest regeneration by succession with some expectation that the forest cover would be similar to the original forest after a certain amount of time (Moran *et al.*, 1994). This process allows the land to be colonized mostly by early successional fast-growing species for the first 15 to 20 years and the establishment of some late successional long-lived species for several decades.

To accelerate succession, restoration practitioners tend to focus much effort toward recovering the ecosystem rapidly. One of the key management methods involves the use of seeds of native species. On one hand, though high germination and seedling establishment could be observed along with some positive effects to the forest community (such as greater biodiversity, less soil runoff, and increased soil nutrient content), restored forest patches were not similar to the original forest (Lamb and Gilmour, 2003). On the other hand, seed reintroduction may also show low germination, requiring a large amount of seed for certain species and low establishment caused by invasive species competition (Cabin *et al.*, 2002; Lamb and Gilmour, 2003). The use of non-native and some fast-growing native tree species plays an important role in the imitation of mature forest patches (Lugo, 1997; Lamb, 1998). Both groups of tree

species can directly restore structure but not biodiversity, although that may be facilitated in the long term. Fast-growing native species with only 20% of the total diversity of tropical forest not only show low survival rates turning from early to late successional stages than the late successional native species, but also decrease forest biodiversity (Gómez-Popma and Vázquez-Yanes, 1981; Martínez-Garza *et al.*, 2005). Another problem is that water is typically the limiting factor in tropical dry forests and the high annual variability in precipitation can make seedling establishment in restoration difficult (Murphy and Lugo 1986a). Thus, a successful restoration project should include both early and late successional species because the former could provide safer sites for the latter by decreasing competition for water and nutrients by shading out resource-demanding grasses and mitigating daily and annual climate variation (Jumpponen *et al.*, 1999). The question is how to choose the different species that can tolerate the environmental pressures in degraded locations and show long-term establishment.

Plant growth and survival have been assessed by several studies in the tropics (Kitajima, 1994; Walters and Reich, 1999; Poorter *et al.*, 2005; Martínez-Garza *et al.*, 2005; Poorter and Bongers, 2006). One of the best methods used is the investigation of the behavior of different plant species under harsh environmental conditions in order to determine plant traits associated with growth and survival. Then, those traits could be helpful later in the selection of species for forest restoration.

Leaves, as the principal photosynthetic organs of the majority of plant species, play an important role in growth and establishment. The flow of CO<sub>2</sub> and water vapor between the vegetation and the atmosphere and the biogeochemical cycles that link soil, climate and atmosphere are significantly related to leaf traits such as leaf size, nutrient

content, and photosynthetic capacity (Reich *et al.*, 2007). Therefore, any changes in leaf traits caused by environmental heterogeneity may lead indirectly to an alteration in plant growth. As environmental pressure can affect the allocation of leaf resources, variability in leaf traits within and across species can predict growth rates of plants (Poorter and Bongers, 2006; Niklas *et al.*, 2011). They illustrated that species with high specific leaf area (SLA) and high nitrogen concentration will also have high photosynthetic, respiration, and conductance rates. These traits produce species with short-lived leaves and high growth rates. Leaf traits such as SLA, leaf nitrogen (LN), conductance ( $g_s$ ) and maximum net photosynthesis ( $A_{max}$ ) can help in plant growth prediction. Under high nitrogen content and low irradiance, plants increased SLA and  $A_{max}$  which resulted in higher growth rates (Meziane and Shipley, 2001; Daehler, 2003).

As a consequence of environmental heterogeneity, plant species tend to adapt differently and show different physiological traits because they have been under different levels of stress. The establishment and survival rates of a species depends on how well that species responds to stress physiologically. In dry areas, to maintain turgor, plants reduce leaf osmotic potential, which helps them to maintain photosynthetic rates (Nunes *et al.*, 1989). Water limitation in the soil decreases leaf water potential ( $\psi_{leaf}$ ) of plants and could cause a limitation in  $CO_2$  uptake (Niu *et al.*, 2005; Portes *et al.*, 2006). Furthermore, high temperature reduces leaf total dry mass and WUE. Thus, the correlation of WUE with SLA or  $A_{max}$  and the decrease of WUE at high temperature might be an adaptation to heat stress (Craufurd *et al.*, 1999). Thus, species with high WUE should perform better in dry habitats (Fischer and Turner, 1978).

The purpose of most restoration programs is to turn degraded ecosystems back to their initial stage. Successful ecosystem restoration should take into account ecological characteristics of different species, assembly, interaction and function of different species in a community. In other words, practitioners should be aware of the processes that drive the development of restored communities. Does the performance of a species relate to its ecological traits? Pywell *et al.* (2003) explored how plant traits affected their performance in a restoration project. They found that functional groups with good colonization ability (ruderality, high germination rate) showed good establishment in the first year; however, as time progressed traits of competition, vegetation growth and persistence were almost absent. Kitajima (1994) demonstrated that plant survival was associated with traits that tended to enhance plant defense (such as leaf thickness and low SLA) and a well-established root system.

In addition, plant growth and survival depends on alteration of the morphological and physiological traits of different plants species due to microhabitat heterogeneity with forest community (King *et al.*, 1997). Different species show different phenotypic plasticity that allows them to grow and survive in a wide range of environmental conditions. Several studies have documented how leaf traits can predict growth and survival in wet tropical forests (e.g., Martínez-Garza *et al.*, 2005; Poorter and Bongers, 2006). For example, short-lived species showed higher growth and lower survival rates, and species with high variation in leaf traits such as specific leaf mass (SLM) increased growth and survival, apparently in response to variable light availability (Martínez-Garza *et al.*, 2005). The main limiting factor in dry environments is water availability. Therefore, to increase survival, different plant species increase WUE variability and

decrease SLA to maintain the CO<sub>2</sub> assimilation, and invest in tough leaves. These could be used as good plant performance indicators in dry forest restoration. Few studies have focused on physiological variability of leaf traits that can predict seedling establishment in dry forest. In this study, I investigated leaf trait variations that can help to predict species long-term establishment in dry forests.

I hypothesized:

- Species with greater plasticity in traits that respond to limiting resources will have greater growth and survival rate.
- Growth and survival and photosynthesis will be more responsive to water limitation than the other resource limitations.

## **2.2 Materials and Methods**

### **2.2.1 Site background**

This study was conducted in the subtropical dry forest life zone of Puerto Rico, which extends east along the south coast approximately 120 km from the southwest corner of the island, approximately between 18°N 66°35'W and 18°N 67°12'W (Ewel and Whitmore, 1973). The mean annual temperature is about 25°C and the mean annual rainfall ranges from a minimum of about 350 to a maximum of 1500 mm with a variable distribution between 4-9 months. The annual ratio of potential evapotranspiration (PET) to precipitation (P) ranges from 0.041°C/mm to 0.014°C/mm (Murphy and Lugo, 1986b). Runoff in this region is only about 61 mm/year and occurs in the fall, in association with tropical storm systems. The forest is semi-deciduous and tends to form a complete crown cover composed of tree species with small and succulent leaves. The maximum tree height does not surpass 15 m. Natural regeneration frequently occurs by coppicing and

basal sprouting instead of by seed (Ewel and Whitmore, 1973; Castilleja, 1991; Ray and Brown, 1994; Dunphy *et al.*, 1996; Van Bloem *et al.*; 2003).

### 2.2.2 Study sites

To compare natural regeneration with my experimental results I divided the study into an experimental part and an observational part. The experiment was located Finca Mátaró off Route 394 in the Palmarejo section of Lajas, near the Lajas Agricultural Experiment Station. The observational part was carried out in southwestern Puerto Rico dry forest stands in the Guánica, Yauco, and Cabo Rojo.

The experimental site was a low-density cattle pasture that was historically used for sugar cane production for 30 years or more. The site was in an open secondary forest state dominated by *Albizzia procera* and *Psidium guajava*, with a grass-dominated understory (Figure 1.). The edaphic and topographic conditions of the site were highly variable. The soils were primarily characterized as descabrado clay loam and fraternidad clay (Soil survey staff, USDA, 2012). Slopes varied across the site from 10-60%. Rainfall averaged 1000 mm annually, but was highly variable (Ewel and Whitmore, 1973).

### 2.2.3 Forest transects in SW Puerto Rico

To carry out the observational part of the study, 20 transect lines of 50 m each were established in six forest stands in various locations in an attempt to capture any spatial variability of traits within species. Each transect was located on calcareous soils with thin layer of organic soil (Pérez-Martínez, 2007) and classified either as calciustolls or

aridisols (Lugo-López and Rivera, 1976; Mount and Lynn, 2004). The climate in the forest patches is tropical semiarid with a vegetation mainly consisted of secondary and mature native forest species and very few introduced species mostly at the edges. The average annual temperature ranged from 78 to 80 degrees F., and the average annual rainfall ranged from 508 to 860 mm. Previous land use was for charcoal production unless noted below. One location where I had 3 transects had been used for research previously (Murphy and Lugo, 1986). Other selected locations were the “Camino La Hoya” trail with 4 transects, the “Camino Las Cobanas” trail with 4 transects, the “Camino Ojo de Agua” (a baseball field until the 1950s) with 1 transect, “La Jungla” near Playa Santa with 3 transects, Cabo Rojo near the lighthouse with 3 transects, and Yauco with 3 transects.

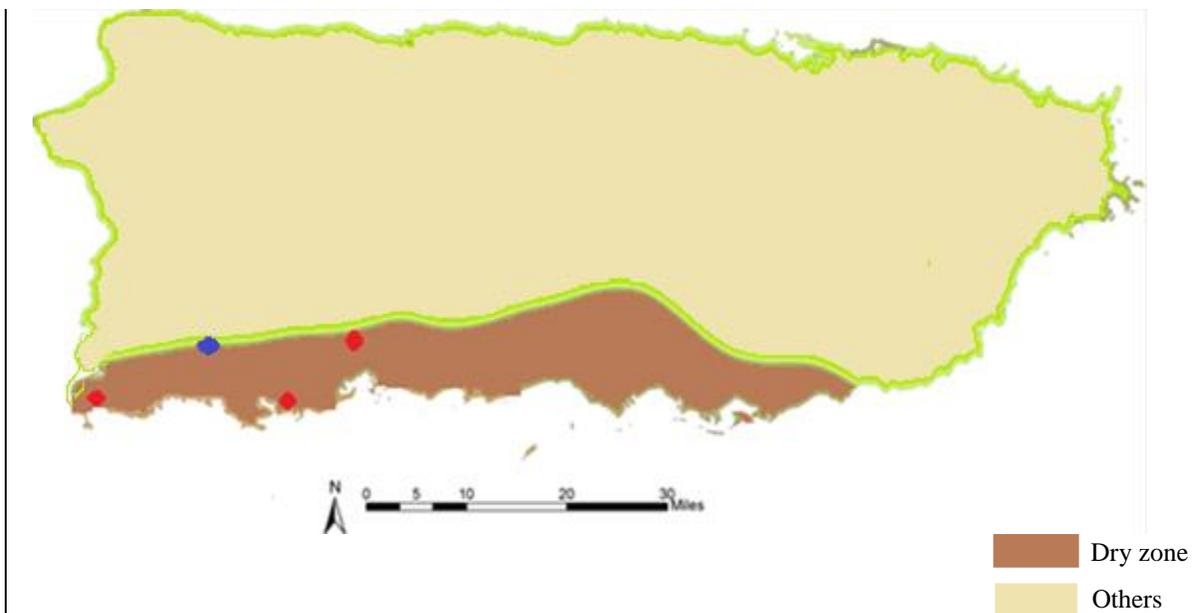


Figure 1. Map of sites showing the different locations of the study at Cabo Rojo, Guánica, Lajas and Yauco in Puerto Rico (approx). Blue dot is designated Lajas and red dots are designated the other sites of the study.

## **2.3 Physiological plant traits in Dry Land restoration in Puerto Rico**

### **2.3.1 Experimental assessment of Growth and Survival**

The experiment was conducted in an old pasture at the Mataro farm. The landscape was suitable for this study because of the variation of micro-topography (flat, concave, and convex) and vegetation cover (shady, partial, and sunny area). A total number of 117 native saplings, consisting of 9 different species from 8 different families were transplanted in October 2009 under three different light treatments (Table 1). The treatments, which were full shade, partial shade, and full sunlight, were determined by visual estimates of percent cover in October 2009. Each species was assigned to all three treatments *a priori*. To calculate growth rates, stem diameters at 10 cm above the ground and sapling height were measured for each transplanted individual in November 2009. Trees were watered by hand weekly and grass was trimmed 50 cm from each sapling at the time of transplanting and for three months after (once every month) to reduce water stress in the first months in order to increase sapling survival. In this study, saplings were preferred rather than seedlings for several reasons. Seedlings are more vulnerable to environment stress. They take more time to grow on the field than in the nursery because of both abiotic and biotic factors of competition and water stress (Daehler 2003). Thus, by planting saplings I attempted to minimize environment stress, especially mortality due to water shortage at seedling stage. Also, most dry forest restoration in Puerto Rico uses saplings. Hence, our methods reflected current management practice.

### 2.3.2 Physiological parameters

Leaf Area Index (LAI) was measured at the top of each surviving individual using the LI-COR 2000 Canopy Analyzer. LAI measurements were taken between 07h00 and 11h00. For each individual, three mature leaves were chosen to measure leaf chlorophyll content (CC) using the Chlorophyll Content Meter CCM-200-Opti-Sciences, Inc. One of the three leaves was taken, weighed fresh and then dried at room temperature for at least 3 days. Leaf Water Content (LWC) was calculated using the formula:

$$\text{LWC (\%H}_2\text{O)} = \left( \frac{\text{FW} - \text{DW}}{\text{FW}} \right) * 100 \quad \text{where FW = leaf fresh weight and DW = leaf dry}$$

weight.

**Table 1.** Successional stage, leaf phenology, and number planted of 9 tropical species used in experimental study at Finca Mataro.

Species	Family	key	Successional stage	Leaf phenology	Number planted
<i>Casearia sylvestris</i>	Flacourtiaceae	Casy	Early	E	6
<i>Cassine xylocarpa</i>	Celastraceae	Caxy	Early	D	6
<i>Colubrina arborescens</i>	Ramnaceae	Colar	Early	D	40
<i>Crescentia cujete</i>	Bignoniaceae	Crescu	Mid	E	19
<i>Guaiacum officinale</i>	Zygophyllaceaea	Guaof	Late	E	3
<i>Pisonia albida</i>	Nyctaginaceaea	Pial	Late	D	18
<i>Pisonia subcordata</i>	Nyctaginaceaea	Pisub	Mid	D	13
<i>Pouteria multiflora</i>	Sapotaceae	Poumul	Mid	E	6
<i>Thespesia grandiflora</i>	Malvaceae	Thesgran	Early	E	6

References: M. Colon and Lugo, 2006; Little and Wadsworth, 1964. Leaf phenology: arrangement of leaves in time. D=deciduous; E=evergreen. Botanical nomenclature: Estudios para la flora de Puerto Rico. Folletos 2-6. Tip. González & Co., Puerto Rico; The Evolution and Classification of Flowering Plants (1988).

To measure physiological parameters, we used the same leaf that was chosen from each surviving individual in each treatment to determine chlorophyll content and LWC. For those species with compound leaves, one leaflet at the same position was taken from three leaves. The measurements were made during the dry season, between February and May 2011 when soil moisture would be at the lowest values. Leaf photosynthesis, stomatal conductance, evaporation, and intercellular CO<sub>2</sub> concentration were measured using a portable infrared gas analyzer (LI-COR, 6400 Portable Photosynthesis system). Measurements were taken between 7:30 AM and 11:30 AM at ambient temperatures (mean T = 76 °F), ambient CO<sub>2</sub> (400 ppm) and humidity (mean H = 69%) and up to 4 min to decrease variation in stomatal conductance. Water Use Efficiency (WUE) and Specific Leaf Area (SLA) were calculated using the formula:

$$WUE = \left( \frac{A_{max}}{E} \right) * 100 \text{ and } SLA = \left( \frac{LA}{LDM} \right),$$
 where  $A_{max}$  = maximum photosynthesis,  $E$  = evaporation,  $LA$  = leaf area,  $LDM$  = leaf dry matter content respectively. Leaf area was determined using ImageJ analysis software (Abramoff *et al.*, 2004).

### 2.3.3 Survival and Analysis

Between February and May 2011, the surviving saplings were counted to determine survival. For the total 2½ year period, survival rate was determined by species, by treatment and for the interaction of species by treatment using the formula:

$$\text{Survival (\%)} = \left( \frac{SI}{TI} \right) * 100,$$
 where  $SI$  = the number of individuals surviving and  $TI$  = number of individuals transplanted.

#### 2.3.4 Observational Study (Natural Regeneration)

The observational study was conducted under natural conditions to investigate different plant physiological characteristics associated with natural plant establishment. The investigation focused on 21 common dry forest species (Table 2). For each transect, 5 individuals from those 21 species sized from 50-200 cm high and  $\geq 0.3$  cm in diameter were selected haphazardly to take measurements. Height, diameter at 10 cm from the stem base, leaf chlorophyll content, leaf fresh weight, and leaf water content (LWC) were measured as described previously. Leaves were dried at room temperature for at least 3 days and weighed for LWC determination. The same sets of measurements of the physiological traits were taken for the experimental study. However, one individual per species was selected randomly between the five sampled to measure the different physiological parameters.

#### 2.3.5 Statistical Analysis

Correlation analysis was conducted to compare the different physiological parameters among themselves. The intraspecific variation for each leaf trait was calculated as its coefficient of variation:  $CV(\%) = \left(\frac{S}{\bar{X}}\right) * 100$ , where  $S$  = standard deviation of given leaf trait and  $\bar{X}$  = Mean of given trait across all microsites.

Linear regression was used to compare growth or survival to the average value or coefficient of variation for each leaf trait ( $A_{\max}$ , E, WUE and SLA). A Chi Square test was used to test the effects of species, the effects of different treatments, and the effects

of the interaction of species by treatment on survival. All analysis were conducted in Infostat version 2011e (Infostat Group, 2011) with  $\alpha= 0.05$ . Among the 9 species, 3 species were removed from the data analysis because of lack of data caused by species mortality.

**Table 2.** Family, successional stage, and leaf phenology of 21 tropical species.

Species	Family	key	Successional stage	Leaf phenology
<i>Amyris elemifera</i>	Rutaceae	1	Late+	E
<i>Bourreria succulenta</i>	Boraginaceae	2	Early ++	E
<i>Bursera simaruba</i>	Burseraceae	3	Mid+	E
<i>Capparis cynophallophora</i>	Caparaceae	4	Early***	E
<i>Coccoloba diversifolia</i>	Polygonaceae	5	Mid+	E
<i>Coccoloba microstchya</i>	Polygonaceae	6	Late ++	Semi D or E
<i>Colubrina elliptica</i>	Ramnaceae	7	Late+	E
<i>Erithalis fruticosa</i>	Rubiaceae	8	Early****	E
<i>Erythroxylon rotundifolium</i>	Erythroxylaceae	9	Late**	D
<i>Eugenia biflora</i>	Myrtaceae	10	Late***	E
<i>Eugenia foetida</i>	Myrtaceae	11	Mid+	E
<i>Eugenia rhombea</i>	Myrtaceae	12	Late***	E
<i>Exostema caribaeum</i>	Rubiaceae	13	Mid+	E
<i>Guaiacum officinale</i>	Zygophyllaceaea	14	Late**	E
<i>Guettarda elliptica</i>	Rubiaceae	15	Early+	D
<i>Gymnanthes lucida</i>	Euphorbiaceae	16	Late**	E
<i>Krugiodendrum ferreum</i>	Ramnaceae	17	Late +	E
<i>Leucaena leucocephala</i> *	Leguminosae- Mimosoideae	18	Early***	D
<i>Pithecellobium unguis-cati</i>	Leguminosae- Mimosoideae	19	Mid+	E
<i>Reynosia uncinata</i>	Ramnaceae	20	Early**	E
<i>Tabebuia heterophylla</i>	Bignoniaceae	21	Early**	D

References: +Ross, *et al.*, 1999; ++Van Bloem *et al.*, 2007; \*\*Santiago-García, *et al* 2008; \*\*\*Little and Wadsworth, 1964; \*\*\*\*Barrett & Stiling, 2006; Leaf phenology: arrangement of leaves in time. D=deciduous; E=evergreen. \*The only invasive species. Botanical nomenclature: Estudios para la flora de Puerto Rico. Folletos 2-6. Tip. González & Co., Puerto Rico; The Evolution and Classification of Flowering Plants (1988)

## 2.4 Results

### 2.4.1 Physiological parameters of experimentally planted trees

For all species, LAI varied from 0.89 to 3.66 m<sup>2</sup>/m<sup>-2</sup> (Table 3) at the end of the experiment. Almost all species showed variable water use efficiency (WUE) values, and low stomatal conductance values varying from 0.32 to 0.58 mol m<sup>-2</sup>s<sup>-1</sup> (Table 4). Species with high SLA had low A<sub>max</sub> and low WUE (Fig. 2, r = -0.83; Fig. 3, r = -0.88,

respectively). Transpiration rate increased with high specific leaf area (Fig. 4,  $r = 0.81$ ). The coefficient of variation of the different physiological parameters did not show significant correlation between one another ( $P$ -value  $> 0.05$ ; Table 5). In dry forest, species with high  $E$  had low  $A_{\max}$  (Fig. 5,  $r = -0.89$ ). No correlation was found between chlorophyll content (CC), leaf water content (LWC) and the other leaf traits.

**Table 3.** Average chlorophyll content (CC), leaf area index (LAI), leaf area (LA), coefficient of variation of CC and LA of the 6 tree species planted at Mataro Farm.

Species	CC (%)	LAI ( $m^2/m^2$ )	LA ( $cm^2$ )	CV-CC (%)	CV-LA (%)
<i>Cassine xylocarpa</i>	30.68	1.74	7.42	64.17	10.63
<i>Colubrina arborescens</i>	30.49	2.09	33.14	20.19	35.28
<i>Crescentia cujete</i>	20.55	2.19	3.93	63.69	68.99
<i>Pisonia albida</i>	21.23	1.4	13	24.76	49.16
<i>Pisonia subcordata</i>	21.63	2.83	22.4	30.71	25.7
<i>Thespesia grandiflora</i>	25.89	2.32	44.42	18.11	39.34

**Table 4.** Average specific leaf area, soil moisture, growth rate in height and diameter, maximum photosynthesis ( $A_{\max}$ ), transpiration rate ( $E$ ), water use efficiency (WUE) conductance ( $g_s$ ) and % survival of the 6 tree species planted at Mataro Farm.

Species	Specific leaf area ( $cm^2/g$ )	Soil moisture (%)	Growth rate in Height (%)	Growth rate in Diam (%)	$A_{\max}$ $\mu mol/g.s$	$E$ $\mu mol/g.s$	WUE $mmol/m^2.s$	$g_s$ $mmol/m^2.s$	Survival (%)
<i>Cassine xylocarpa</i>	25.94±5.18	34.85±8.16	26.28±33.6	37.55±40.9	16.71±7.6	5.14±1.5	2.58±0.8	0.38±2.2	83.33
<i>Colubrina arborescens</i>	18.68±9.47	33.67±9.38	30.92±40.9	73.99±46.8	19.3±8.54	4.17±1.9	4.18±0.7	0.58±2.1	45
<i>Crescentia cujete</i>	21.39±10.3	36.39±9.75	24.53±11.2	25.39±23.14	19.06±8.7	5±1.8	3.77±0.2	0.43±0.5	21.05
<i>Pisonia albida</i>	17.33±5.58	32.87±9.73	97.97±14	165.66±58.84	20.34±5.8	4.6±1.38	3.8±0.7	0.33±0.1	27.78
<i>Pisonia subcordata</i>	13.97±2.5	26.3±4.05	22.24±19.6	68.11±42.2	30.67±9.9	3.3±1.12	7.06±0.7	0.48±0.3	46.15
<i>Thespesia grandiflora</i>	17.02±3.06	34.48±4.52	23.69±52.6	88.37±28.5	22.28±4.3	4.67±1.5	5.23±2.3	0.43±0.1	83.333

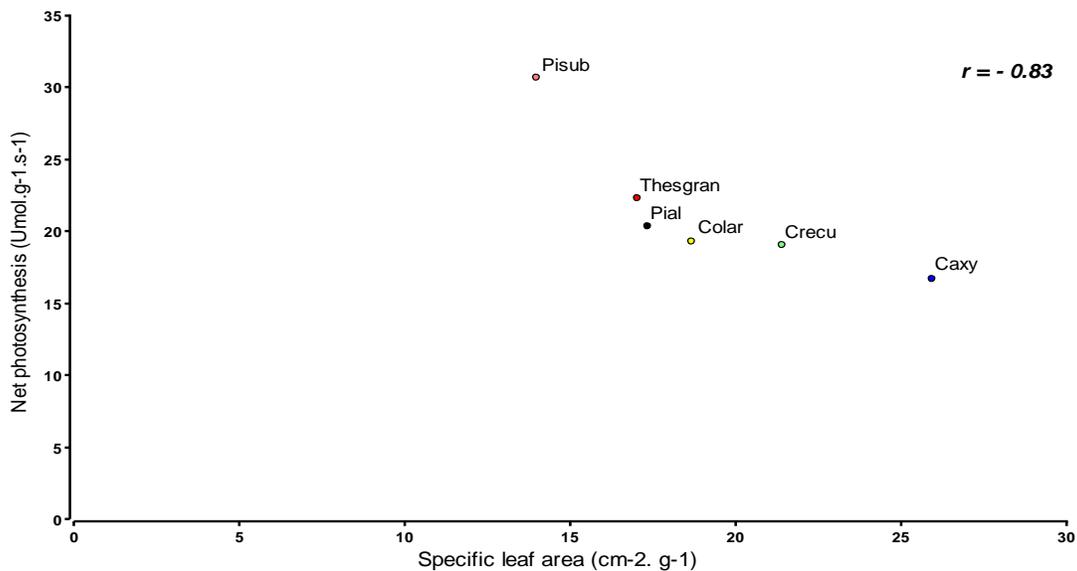


Figure 2. Relationship between maximum net photosynthesis ( $A_{max}$ ) and specific leaf area (SLA) of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.

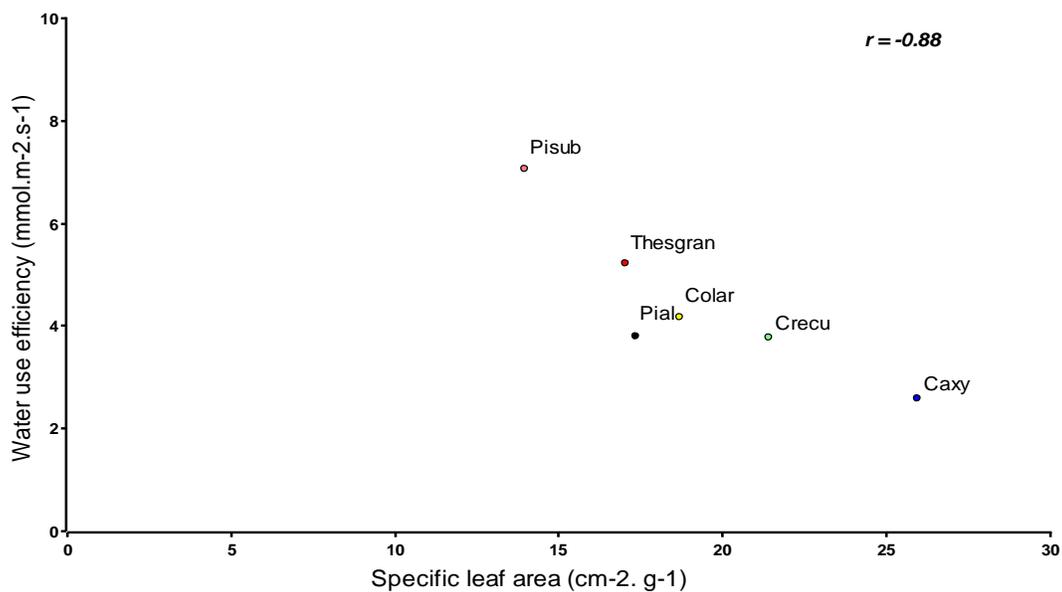


Figure 3. Relationship between water use efficiency (WUE) and specific leaf area (SLA) of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.

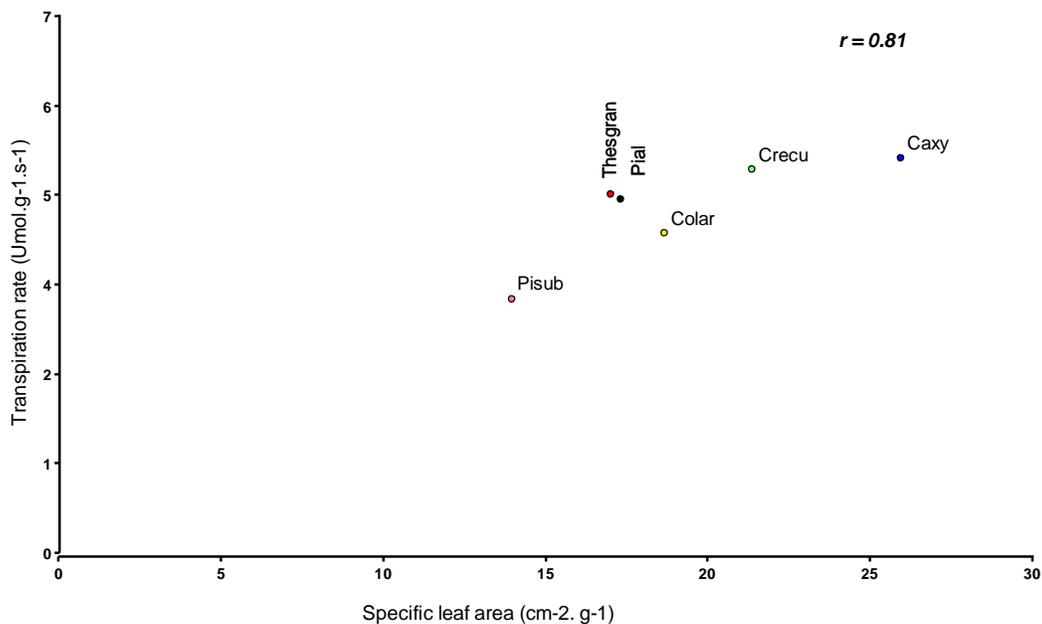


Figure 4. Relationship between transpiration rate (E) on specific leaf area (SLA) of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.

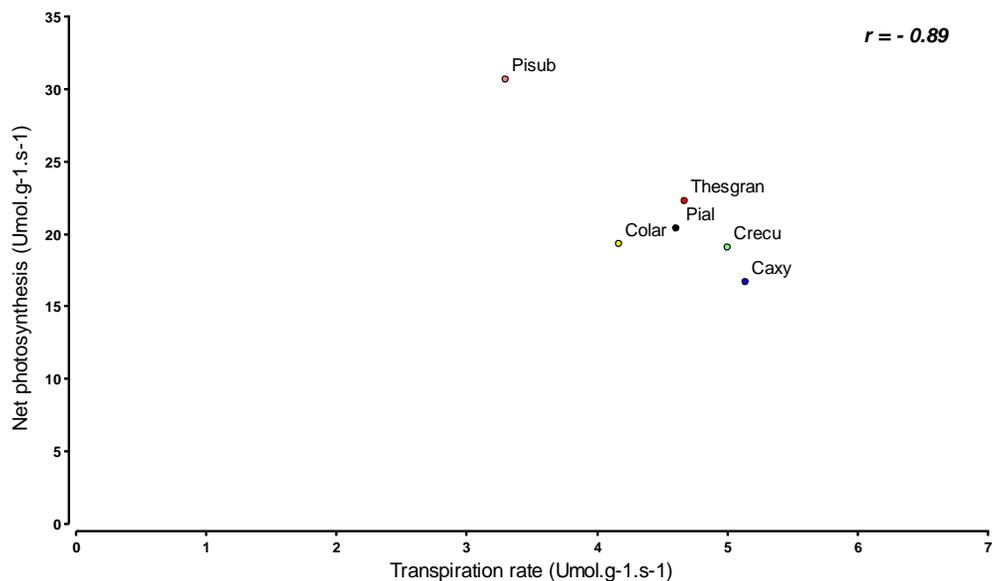


Figure 5. Relationship between maximum net photosynthesis ( $A_{max}$ ) and transpiration rate (E) of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.

### 2.4.2 Survival and relative growth rate

Across all 9 species, a  $39.6 \pm 9.5\%$  survival rate was observed. Pioneer species showed higher survival than the late successional species, (Fig. 6). Sapling survival ranged from 17% to 83%. *Cassine xylocarpa* and *Thespesia grandiflora*, two pioneer species from the dry and the humid zone area, respectively, showed the highest survival (83% each). *Pisonia albida*, a late successional species, had 28% survival while *Crescentia cujete*, a mid-successional species had 21%. *Guaiacum officinale*, a late successional species, had the lowest survival (0%) under the three different treatments (Fig. 8).

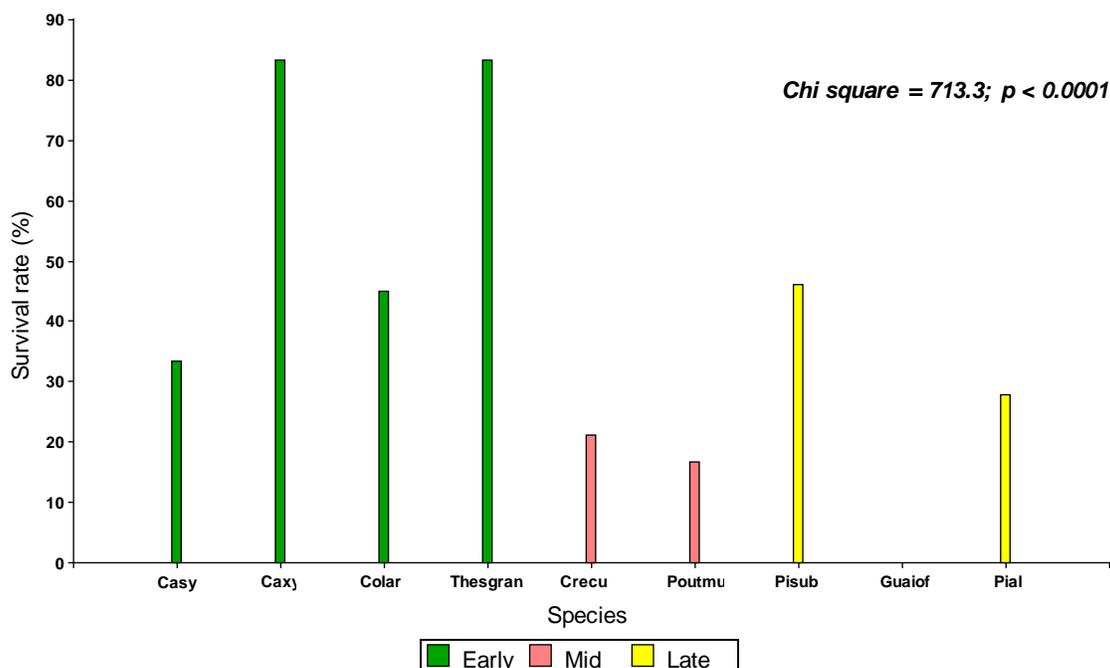


Figure 6. Survival of 9 tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.

Plant survival and growth rate did not show any direct associations with physiological leaf traits that were correlated with light availability such as SLA and SLM, as predicted. On the contrary, my study illustrated that plant survival was driven by the intra-specific variation of WUE and SLA (Appendix B). Survival rate increased with the variation of WUE and decreased with the variation of SLA (Appendices,  $p= 0.009$  and  $p = 0.01$  respectively). The relationships between survival or diameter growth and the mean physiological traits were not statistically significant across all species.

**Table 5.** Pearson correlations between the coefficient of variation of the different leaf traits [specific leaf area maximum photosynthesis ( $A_{max}$ ), transpiration rate (E), water use efficiency (WUE) conductance ( $g_s$ )], and growth rate in height and diameter, average soil moisture and survival of the 6 tree species planted at Mataro Farm. Correlation values (r) are presented below.

	CV_SLA (%)	AVG_SM (%)	CV_A (%)	CV_E (%)	CV_WUE (%)	GR_H (%)	_GR_D (%)	Survival (%)
CV_SLA (%)	1							
AVG_SM (%)	0.3	1						
CV_A (%)	0.51	0.04	1					
CV_E (%)	0.78	0.35	0.37	1				
CV_WUE (%)	-0.43	0.53	0.02	0.02	1			
GR_H (%)	0.33	-0.41	<b>0.83*</b>	0.23	-0.26	1		
GR_D (%)	0.01	-0.55	0.37	0.21	-0.16	0.79	1	
Survival(%)	<b>-0.81**</b>	0.25	-0.37	-0.39	<b>0.86*</b>	-0.45	-0.15	1

\*\*  $P = 0.05$ , \* $P < 0.05$ , however, multiple regression showed no relation between growth rate in height and CV\_A. others not significant.

## 2.5 Physiological parameters observed in Native Forests

In Guánica Forest and the other remnant forests the same trends were observed as in the experimental assessment in Mataro farm. Leaf area index ranged from 0 to  $2.99 \text{ m}^2 \cdot \text{m}^{-2}$  for all species in Guánica Forest (Table 6), similar to Mataro Farm. Species with higher

WUE showed higher net photosynthesis and lower E ( $r^2 = 0.49$ ;  $r = -0.47$ ). Species that had increased SLA resulted in low WUE, low  $A_{\max}$  and high E (Fig. 7,  $r = -0.55$ ; Fig. 8,  $r = 0.5$ ; Fig. 9,  $r = 0.56$ , respectively). Species with high chlorophyll content also had high WUE and lower SLA (Table 6,  $r = 0.55$ ,  $p = 0.01$ ;  $r = -0.46$ ,  $p = 0.04$ ).

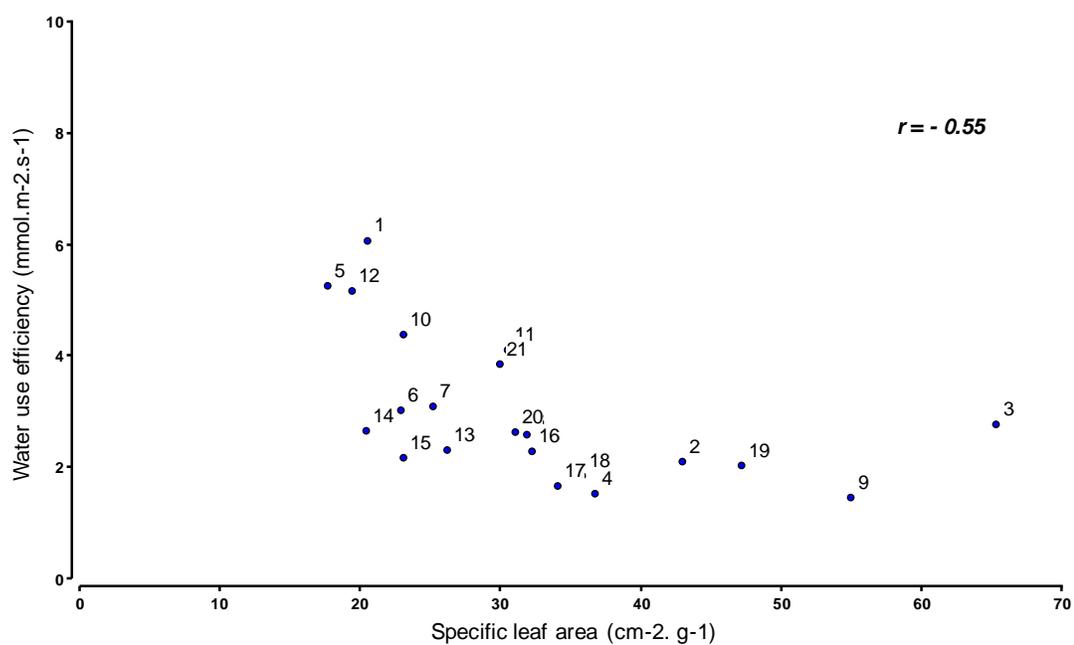


Figure 7. Relationship between water use efficiency and specific leaf area of 21 tropical tree species observed in dry forests of SW Puerto Rico. The key for the species is found in Table 2.

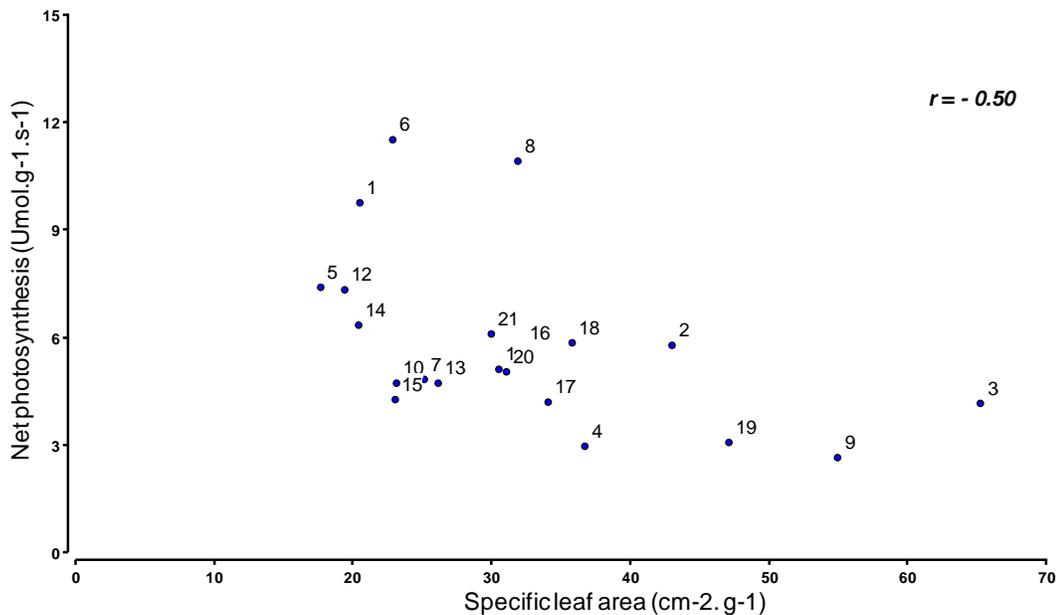


Figure 8. Relationship between maximum photosynthesis and specific leaf area of 21 tropical tree species observed in dry forests of SW Puerto Rico. The key for the species is found in Table 2.

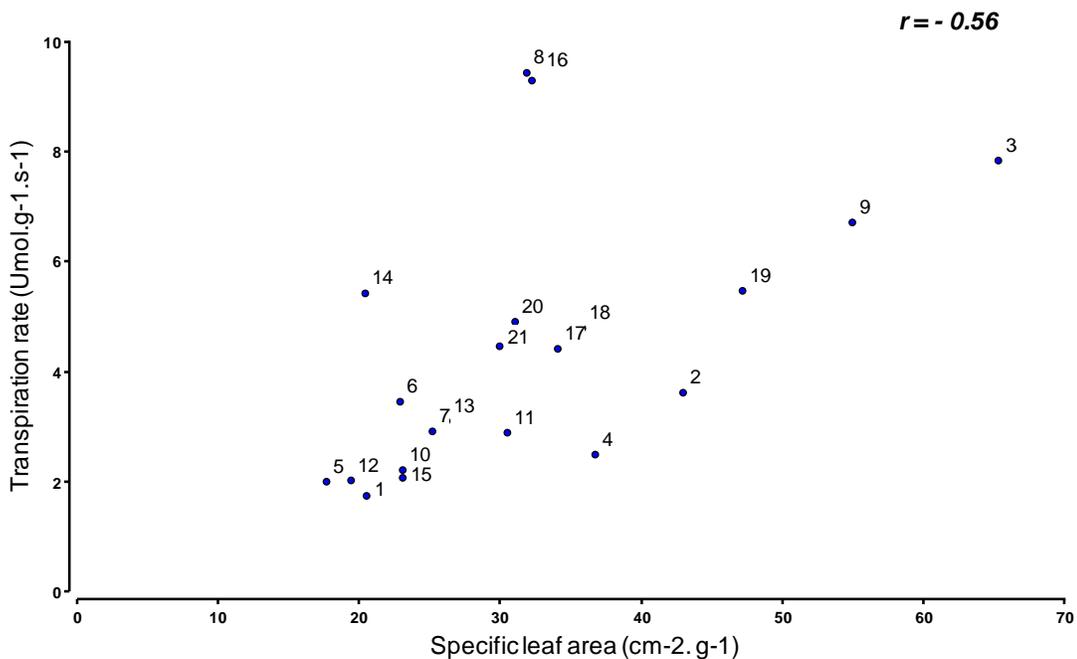


Figure 9. Relationship between transpiration rate and specific leaf area of 21 tropical tree species observed in dry forests of SW Puerto Rico. The key for the species is found in Table 2

Table 6. Average leaf area index, chlorophyll content, specific leaf area, water use efficiency (WUE), maximum net photosynthesis ( $A_{max}$ ), transpiration rate (E), and soil moisture of 21 tree species observed in SW Puerto Rico.

Species	LAI ( $m^2/m^2$ )	CC (%)	Specific Leaf Area ( $cm^2/g$ )	WUE $mmol/m^2.s$	A max $Umol/g.s$	E $Umol/g.s$	Soil moisture (%)
<i>Amyris elemifera</i>	1.63	42.37	20.52±9.38	6.05±1.2	9.75±1.7	1.74±0.7	24.72
<i>Bourreria succulenta</i>	0.81	16.25	42.98±30.3	2.09±0.7	5.78±3.6	3.61±1	15.4
<i>Bursera simaruba</i>	0.75	4.88	65.3±14.9	2.76±0.16	4.16±2.5	7.85±0.8	5.8
<i>Capparis cynophallophora</i>	0.83	34.76	36.74±14.54	1.52±0.69	2.95±2.8	2.5±0.7	13.34
<i>Coccoloba diversifolia</i>	0.64	38.7	17.72±2.16	5.26±0.3	7.4±1.03	1.99±0.9	6.6
<i>Coccoloba microstycha</i>	1.13	16.25	22.91±3.4	3.01±0.6	11.5±4.2	3.44±1.07	8.3
<i>Colubrina elliptica</i>	2.99	11.12	25.18±6.04	3.09±1.19	4.83±5.3	2.92±0.89	36.5
<i>Erithalis fruticosa</i>	0.9	38.46	31.88±9.02	2.59±1.65	10.9±3.7	9.44±34.6	10.6
<i>Erythroxylon rotundifolium</i>	0.85	9.84	54.94±38.01	1.46±1.25	2.65±2.7	6.71±0.36	12.03
<i>Eugenia biflora</i>	0.55	53.98	23.12±8.24	4.37±0.46	4.73±0.9	2.21±0.87	12.33
<i>Eugenia foetida</i>	0.7	38.65	30.55±32.99	4.11±1.74	5.09±3.2	2.89±1.03	18.09
<i>Eugenia rhombea</i>	2.8	75.35	19.4±2.98	5.17±1.15	7.31±2.8	2.03±0.61	12.65
<i>Exostema caribaeum</i>	1.09	7.16	26.2±12.94	2.31±0.62	4.73±1.2	3.11±0.64	52.23
<i>Guaiacum officinale</i>	2.59	31.24	20.4±4.82	2.65±1.93	6.35±3.9	5.42±22.6	18.2
<i>Guettarda elliptica</i>	0.88	12.6	23.06±8.28	2.17±0.32	4.25±1.5	2.06±1.06	11.04
<i>Gymnanthes lucida</i>	0.99	56.93	32.28±6.47	2.29±2.14	5.7±2.48	9.3±27.19	24.3
<i>Krugiodendrum ferreum</i>	2.74	32	34.08±17.4	1.66±1.1	4.19±1.9	4.41±27.7	18.56
<i>Leucaena leucocephala</i>	0.99	6.85	35.79±36.61	1.85±0.8	5.84±4.5	4.79±1.4	32.47
<i>Pithecelobium unguis-cati</i>	2.74	20.79	47.15±15.66	2.02±2.43	3.06±2.3	5.47±26.2	17.75
<i>Reynosia uncinata</i>	0.52	16.51	31.05±6.26	2.62±1.14	5.05±2.6	4.9±0.46	20.97
<i>Tabebuia heterophylla</i>	0.74	16.93	29.96±33.09	3.84±1.4	6.1±7.59	4.46±1.88	9.01

\*Standard deviation not available for leaf area index, chlorophyll content and soil moisture

## 2.6 Discussion

### 2.6.1 Physiological traits

Compared to findings in other dry and moist zone areas (Niu *et al.* 2005; Santiago and Wright, 2007; Poorter and Bongers, 2006), the species in this study showed low stomatal conductance with little variability, ranging from 0.32 to 0.58  $mol.m^2/s$  and with high  $A_{max}$  range from values 11.71 to 30.67  $\mu mol.g/s$ . As this study was carried out in dry forests where the main limited resource is water, these values appear to be a response to

the strong water stress that the saplings experienced in that area. Other research has shown that water stress decreased stomatal conductance of the leaf, which also would reduce  $A_{\max}$  (Tenhunen *et al.*, 1985). During water stress at early life stage of plants, decreasing stomatal conductance (stomatal closure) helps preserve leaf water content above a critical level to preserve the photosynthetic capacity. Some investigators showed  $A_{\max}$  to be a strong determinant to differentiate growth rates in different species (Poorter *et al.*, 1990; Poorter and Garner, 1999; Shipley, 2002). However, I did not find any correlation between  $A_{\max}$  and growth rate. Those findings were also supported by other studies (Quero *et al.*, 2008; Kirschbaum, 2011). Growth rate depends not only on carbon gain but also on other co-limiting factors, especially water and nutrient availability. In fact, more carbon gain could provoke more limitations of available nutrients (Kirschbaum, 2011). Thus, an increase in  $A_{\max}$  in harsh environmental conditions with limited nutrient availability will not show any increase in growth rate. In addition, Quero *et al.* (2008) found no correlation between  $A_{\max}$  and relative growth for species under water stress with low irradiance level due to variation in species physiological traits across resource supplies. Thus, species with high  $A_{\max}$  do not necessarily increase growth rate and survival.

Similar to several other studies (Wright *et al.* 2004; Bonser, 2006; Santiago and Wright, 2007), physiological leaf traits of different plant species tended to be correlated with one another. Empirical studies have shown a strong positive relationship between the rate of photosynthesis ( $A_{\max}$ ) and SLA (Reich *et al.*, 1998; Reich *et al.*, 1999; Poorter and Bongers, 2006). However, other investigators have reported that SLA was positively correlated with maximum photosynthetic  $\text{CO}_2$  assimilation per mass while negatively

correlated with maximum photosynthetic CO<sub>2</sub> assimilation per area (Santiago and Wright, 2007). Also, different species may show a different relationship between SLA and A<sub>max</sub> (Huang *et al.*, 2007). Some species may have higher phenotypic plasticity and therefore more easily adjust SLA in response to environmental changes. In our case, species with lower SLA also showed lower photosynthesis rates. In general, most of the species showed low SLA with the exception of *Cassine xylocarpa*, a deciduous pioneer (Table 4). This may be the result of investing in tough, well-defended leaves to reduce water loss or to avoid herbivore or fungal attack. Also, most of the species increased their transpiration rate and decreased WUE with high SLA (Fig. 4, Fig. 9; Fig. 3, Fig 7). This may reduce the carbon flow and the plant may keep a positive carbon balance, which could enhance survival (Poorter and Bongers, 2006). The upholding of a positive carbon balance is required for developing tough foliage and dense structure. Thus, low SLA corresponds with thick structure, high mass per leaf area (Abrams *et al.* 1994) and more resistance to desiccation.

### ***Survival and Growth rate***

Among the few studies that have related leaf traits and the variation of plant survival and growth rate within species under controlled environments, Kitajima (1994) showed that SLA and A<sub>max</sub> were negatively correlated with survival. However, she noted the same trends might not be true in the field (Kitajima, 1996). Other researchers have shown that growth rate increased with the variation of SLA (Walters and Reich 1999, Poorter *et al.* 2005; Poorter and Bongers, 2006), with A<sub>max</sub> (Poorter 1999, Shipley 2002) and with SLM (Martínez-Garza *et al.*, 2005). Martínez Garza *et al.* (2005) demonstrated higher survival

for late successional wet forest species that had higher coefficients of variation for some specific physiological traits mostly correlated with sunlight availability (e.g., SLA, SLM) planted in early successional environments. In my results, however, survival did not depend on species with higher growth rates, SLA, or SLM. Instead, it was correlated with the variation of WUE, which indicates the drought resistance of the plants (Carmelo *et al.* 2006). In addition, survival was also driven by the inter-specific variation of SLA. Species with higher survival rates were those with lower variability in SLA. This is also contrary to the results of Martínez Garza *et al.* (2005). Kitajima (1994) determined that survival depends on traits that are likely to enhance plant defense and well-established root system and more biomass in the stem, which resulted in lower photosynthetic and growth rates for all species. Those results support my findings that showed low  $A_{\max}$ , low transpiration rate, and low growth rate across all species.

Furthermore, the effects of drought could be one of the factors that explain my results. In dry zone areas, plants are competing mostly for water (the main limiting factor) and not for light. Generally, water stress increased physiological trait variation in plants to increase survival (Elfeel and Mohamed, 2011). In my case, species that had high WUE variation and low SLA variation had greater survival. Among the six analyzed species, *Thespesia grandiflora* and *Cassine xylocarpa* both had CV-WUE of >60% and had the highest survival rate of 83%. These two species also had the lowest growth rates. *Pisonia albida*, which showed the highest growth rate (98%), had a coefficient of variation of SLA of 42%, much higher than *Thespesia grandiflora* and *Cassine xylocarpa* with CVs of SLA at 18% and 20%, respectively. Therefore, to increase survival, species reduced growth rate, had high WUE variation to respond to variability in water supply,

and low SLA variation. These results were similar to those found by Elfeel and Mohamed (2011).

As expected, early successional species showed higher survival compared to both mid- and late-successional species (Fig. 8). These findings were supported by several other studies in other tropical dry forests (Parrish and Bazza, 1982; Davidson *et al.*, 1998; Musila *et al.*, 2010). The success of *Thespesia grandiflora* and *Cassine xylocarpa* was driven by the fact that morphologically and physiologically they had some advantages over the other species. The high variation of WUE, low SLA variation adjustment, and low growth rate can explain their success in water-limited environments.

As predicted, the correlation between survival or growth rate and most of the leaf traits was not significant and restricted to the few traits that were associated with overcoming drought stress. Additional factors may be related to survival. Missing saplings could have been eaten, as the presence of cattle was occasionally observed around the experimental field. Finally, another important factor was the grass that grew up very tall. Some of our missing individuals could have been hidden under or even killed by grasses by highly competing for sunlight and water.

## **2.7 Conclusion**

Deforestation of TDF is happening worldwide. In the meantime, forest restoration should incorporate both pioneers and non-pioneers species to accelerate the process of establishing forest stands with later successional characteristics. Leaf traits of different species can be used to predict plant survival, particularly when selecting species common to later successional stages in forest development. As might be expected for open

environments, pioneer species had higher survival, so successional status predicted establishment success. Species that survived the most in tropical dry forest area were those with higher variability in WUE and low changes in the variability of SLA.  $A_{\max}$ -CV was correlated to growth, but the former could not explain the latter. Variability of WUE permitted the species to tolerate water stress and increase survival.

Understanding the variability in leaf traits can be important for forest restoration and regeneration because it will help predict which species are able to survive environmental stress. Knowing that species with high variation in WUE and low SLA variation could have higher survival rate in dry forest, we are able to predict restoration success by transplanting those species instead of random species selection.

## **CHAPTER 3. MICROSITE CHARACTERISTICS AS INDICATORS OF REFORESTATION SUCCESS IN TROPICAL DRY FOREST.**

### **3.1 Introduction**

Dry forests originally represented 42% of the total tropical and subtropical landmass and 17.6% of subtropical dry forest cover in Puerto Rico (Lugo, 1987). Today, they are considered the most threatened terrestrial ecosystem due to the conversion of these areas into agricultural (Janzen, 1988). To recover forest structural and functional diversity, reforestation of those sites is critical. However, it is important to acquire a good perception of the ecological processes of dry forest regeneration to reduce failure in reforestation projects (Ray, 1993). Sapling establishment is an initial and important stage in managing forest processes. An understanding of environmental factors that affect sapling survival and establishment will help us to increase restoration success in dry forest areas.

The environmental conditions of the area surrounding a plant play an important role in plant survival and establishment (Grubb, 1977). Because of environmental heterogeneity, some microhabitats have different resource availability and show different characteristics that favor them over others for plant growth and establishment. Those favorable sites are called “Safe Sites” and can be defined as microsites that meet the conditions for plant establishment (Jumpponen *et al.*, 1999).

Plant species establishment results from the interactions of several essential factors related to microsites including seed dispersal rate, safe site availability and their distribution in space. How do different microsite characteristics affect species

establishment in tropical dry forests? The microtopography of the site, presence of rocks or woody debris, amount of litter, soil texture and depth, concave and convex surface, vegetation cover, and sunlight are important site characteristics that affect plant establishment (Jones and del Moral, 2005). Previous studies have demonstrated that safe site patterns play an important role in seed germination and seedling establishment early in succession (Jones and del Moral, 2005). However, very few studies have focused their interests on different microsite characteristics (safe sites) that are good indicators of plant growth, survival and establishment in the dry zone areas. The main goal of this study is to analyze different microsite traits that facilitate species establishment and increase survival in tropical dry forests and to use them as good indicators for forest reforestation success. In other words, to determine characteristics that can help in the identification of safe sites in dry forests.

In this study microsite is defined as the area within 1 m<sup>2</sup> of an individual sapling (Titus and del Moral, 1998). Microsites may have differences in environmental traits that help maintain species coexistence and establishment. Two main factors can explain species coexistence: habitat differentiation and resource differentiation (MacArthur and Levins, 1967; Whittaker, R. H., 1977). Studies have shown different resource availability for different microhabitats, which influences the presence of different plant species (Cowles, 1899; Pywell *et al.*, 2003; Shipley *et al.*, 2006; Westoby and Wright, 2006).

Safe sites favorable for seedling establishment include seed trapping and other features that can help to protect seeds and seedlings from drying out (Jumpponen *et al.*, 1999). They include micro-topographic characteristics such as optimum slope for plant

water uptake and where microsites have not been flooded for seed bank germination, deep soil and surface contour with coarse surface substrate and large rock vicinity that may affect water availability in soil and may influence plant survival (Harper *et al.*, 1965). Gómez-Aparicio *et al.* (2005) showed that differences in microsites lead to unpredictable establishment of plant species in space and in time. They also observed that the presence of pre-established vegetation benefited seedling and sapling establishment. Others have confirmed low plant survival rates in harsh primary successional environments with low water availability in the soil and high temperature (del Moral and Bliss 1993; Lichter 2000).

Restoration practitioners should keep their attention on best practices for reforestation. Passive restoration gives good results for some areas (Morrison and Lindell, 2011) while for others active restoration is needed (McIver and Starr, 2001). Usually, factors like seed dispersal by grazers are favored and safe site availability and spatial distribution are not considered in reforestation projects. Nonetheless, previous studies have pointed out that species colonization was limited by safe site availability for seed germination and seedling establishment and not by seed dispersal (Wood and Morris, 1990). Gerhardt (1993) demonstrated that soil characteristics like depth and soil moisture increased survival. As these soil traits (slope, soil depth, soil moisture, etc.) vary in the different dry forest areas in Puerto Rico, I propose that their variation might also affect plant growth and survival. Here I addressed the question: Do microsite characteristics affect plant survival and establishment in dry forest reforestation? I hypothesized:

- Naturally, plant establishment is determined by the presence of sites that facilitate

sapling survival.

- Microsites that maximize water availability and minimize competition from grasses will have higher growth and survival rates.

### **3.2 Materials and Methods**

This study was conducted in the dry zone area of southern Puerto Rico. To compare natural regeneration to the results of my investigation, the study was divided into an experimental section in the municipality of Lajas and an observational section in the municipalities of Guánica, Cabo Rojo, and Yauco.

#### **3.2.1 Study sites**

The experimental site was a low-density cattle pasture that was historically used for sugar cane production for 30 years or more. The site was an open secondary forest state dominated by *Albizia procera* and *Psidium guajava*, with a grass-dominated understory (Fig. 1). The edaphic and topographic conditions of the site were highly variable. The soils were primarily characterized as descalabrado clay loam and fraternidad clay (Soil survey, USDA). Slopes varied across the site from 10-60%. Rainfall averaged 1000 mm annually, but was highly variable (Ewel and Whitmore, 1973).

#### **3.2.2 Observational evaluation in dry forest remnants in SW Puerto Rico**

To carry out the observational part of the study, several different locations were randomly chosen in and near Guánica Forest to run twenty transects 50 m each. The sites'

background information was described in experimental method chapter 2, section 2.3.4- “Observational study (Natural regeneration).”

### 3.2.3 Experimental assessment

The experimental design was described in chapter 2, section 2.3.1 – Experimental assessment of growth and survival.

Two years after transplanting, height and diameter of surviving saplings were measured again to determine growth rates and survival. Leaf Area Index (LAI) was measured for each surviving individual using the LI-COR 2000 Canopy Analyzer. LAI measurements were taken at the top of each sapling between 07h00 and 11h00. For each individual, three mature leaves were chosen to measure leaf chlorophyll content (CC) using the Chlorophyll Content Meter CCM-200. One of the three leaves was taken, weighed fresh and then dried at room temperature for at least 3 days. Leaf Water Content (LWC) was calculated using the formula:  $LWC (\%H_2O) = \left( \frac{FW - DW}{FW} \right) * 100$  where FW = leaf fresh weight and DW = leaf dry weight.

To study the microsite characteristics, soil moisture and soil depth were taken for both surviving and dead saplings in the microhabitat using a Time Domain Reflectometer (TDR) and mechanical methods (thin iron stick and metric tape for depth) respectively. Three different points around saplings were measured and the average values were used for analysis. As surface storage and runoff are concerned, the microtopography (soil slope) for each sapling was measured using an angle-finder protractor and the surface

contour (concave, convex, flat: < 1 cm vertical relief) was also assessed visually around each sapling base in comparison to the surrounding site and slope.

#### 3.2.4 Statistical analysis

The hypotheses related to the presence/absence and distribution of surviving saplings in different microsite parameters were tested using a contingency table with a Chi Square test analysis. Slope was divided into 5 classes (class 1 = 5-10 %, class 2 = 10-15%, class 3 = 15-20%, class 4 = 20-25% and class 5 = 25-30%) and soil depth into 4 (class 1 = 5-12 cm, class 2 = 12-19 cm, class 3 = 19-26 cm, and class 4 = 26-32 cm). Then, a contingency table with a Chi Square test analysis was also conducted with the soil microtopography, and soil depth of both dead and surviving species to evaluate the hypothesis of presence/absence of the most common established species at the different microsite traits. In the native stands, absence of saplings was not noted, so Chi-squared analyses only assessed distribution among classes and may reflect either preferential sorting among safe sites or random occurrence of safe sites in the landscape. In the native dry forests in SW PR, the effects of drought on physiological parameters were evaluated by Pearson correlation with species as the main focus. All analysis were conducted in Infostat version 2011e (Infostat Group, 2011) with  $\alpha= 0.05$ .

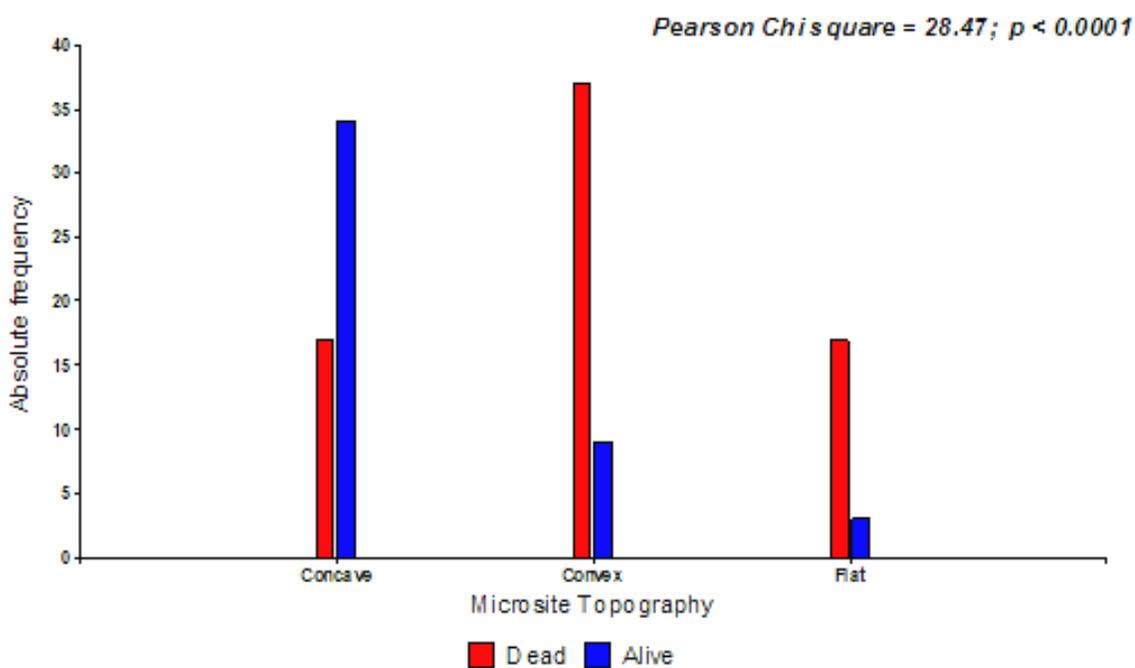
### **3.4. Results and Discussions**

#### 3.4.1. Mataro farm

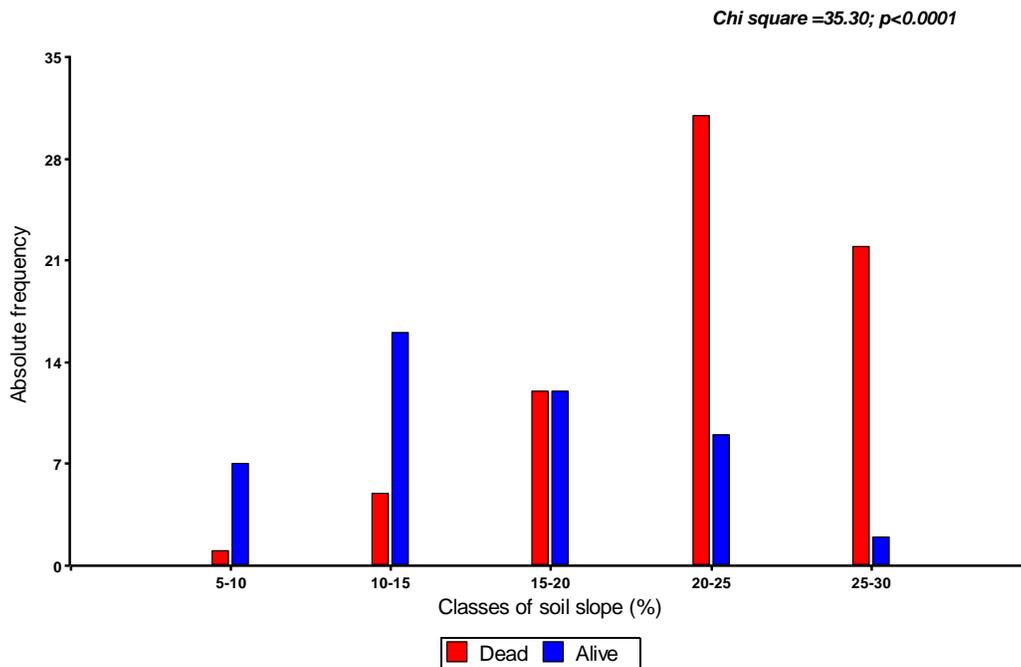
Several microsite traits affected plant survival. Microtopography influenced plant survival (Fig. 10). Saplings in the concave microtopography had greater survival (74%)

while dead saplings were more likely to be found on flat and convex contours.

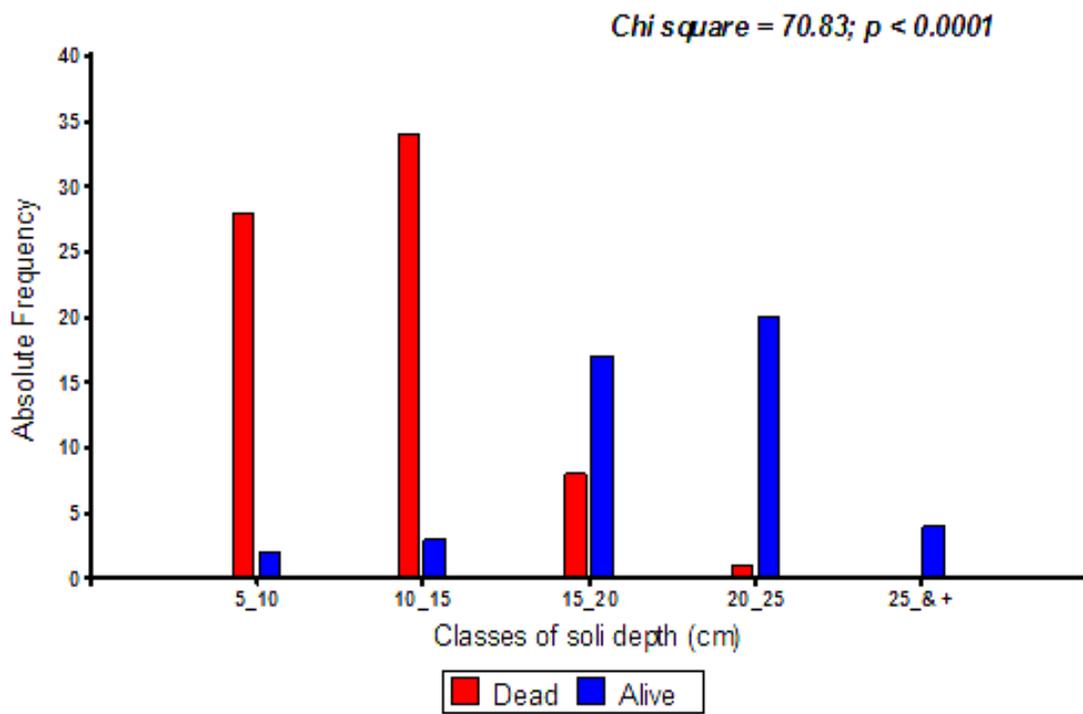
Plant survival was affected by soil slope (Fig. 11). The results showed that 75% of the dead individuals were found between 20 to 30% slope and 76% of the surviving ones between slope of 5 to 20%. Soil depth also influenced survival (Fig. 12) with 96% of the dead saplings located in soils shallower than 20 cm and 84% of the alive ones found in soils deeper than 20 cm.



**Figure 10.** Number of surviving and dead individuals among soil microtopography classes for planted in Mataro farm.

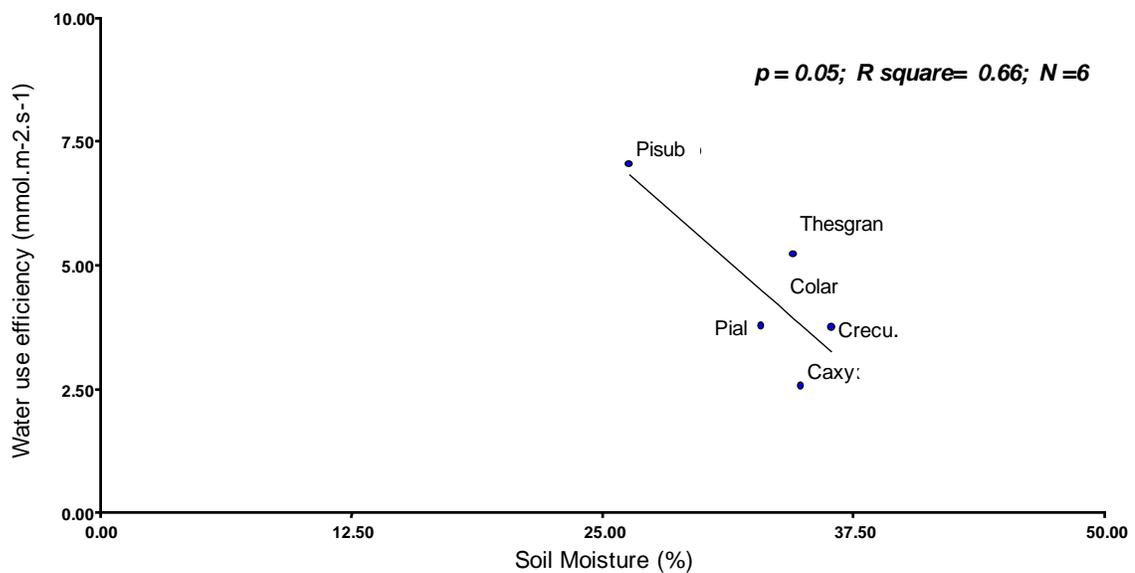


**Figure 11.** Number of surviving and dead individuals across soil slope classes at Mataro farm.

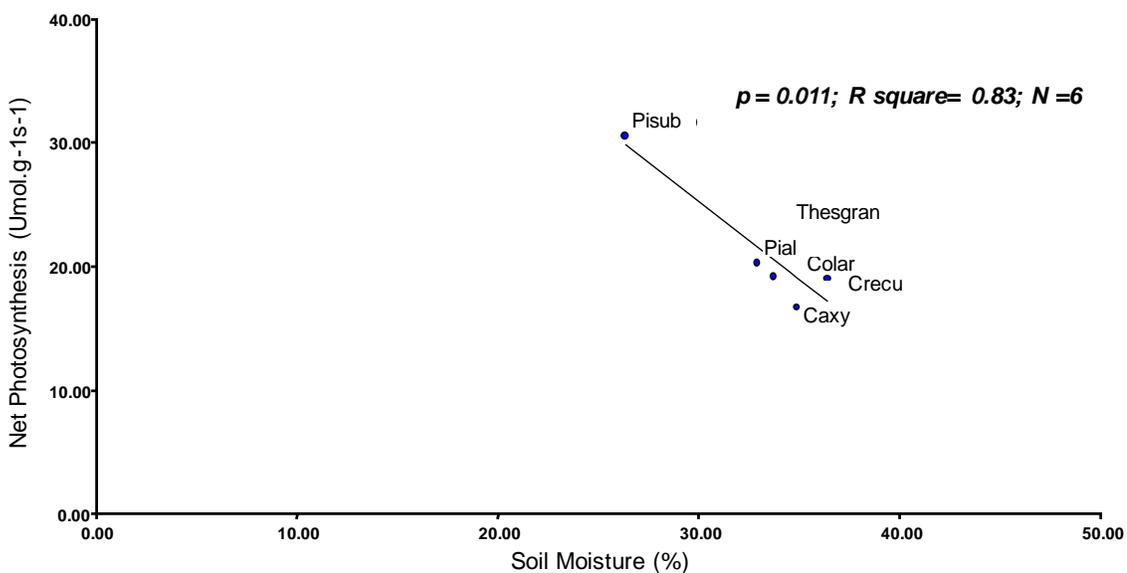


**Figure 12.** Number of surviving and dead individuals across soil depths at Mataro farm.

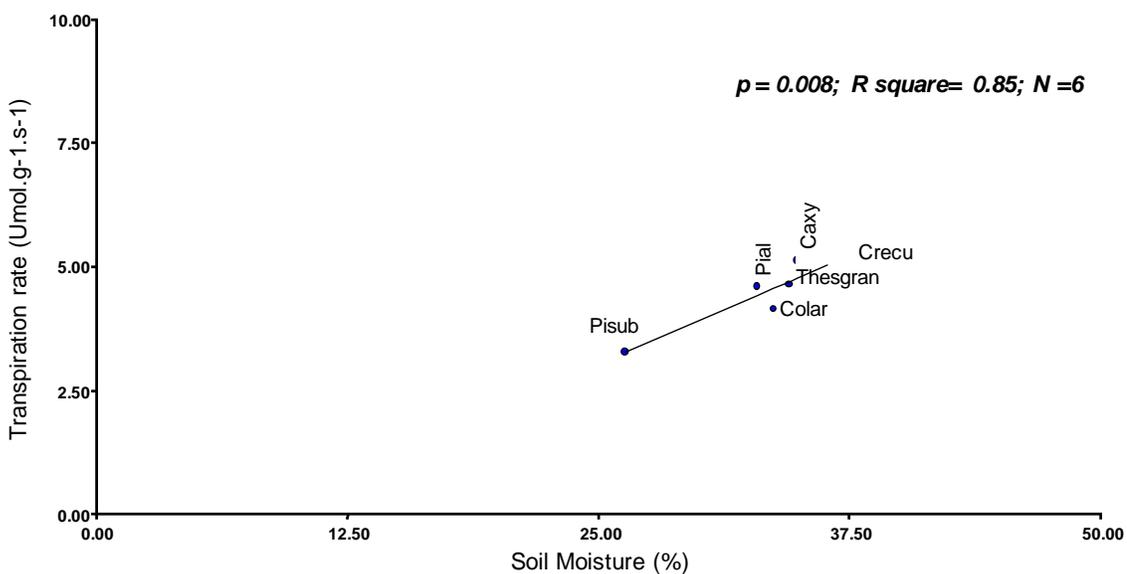
Soil moisture was not explanatory for growth or survival. However, a few physiological traits of different species were affected by soil moisture. Contrary to our prediction, a significant negative relationship was found between soil moisture and both WUE and  $A_{\max}$ , which means the wetter the soil in the different sites at Mataro farm, the lower the WUE and  $A_{\max}$  (Fig.13, and Fig.14). Inversely, soil moisture was strongly and positively correlated with leaf transpiration rate. That is wetter soil caused plants to have higher transpiration rates (Fig. 15). Light also influenced survival with greater mortality in shade (Fig. 16)



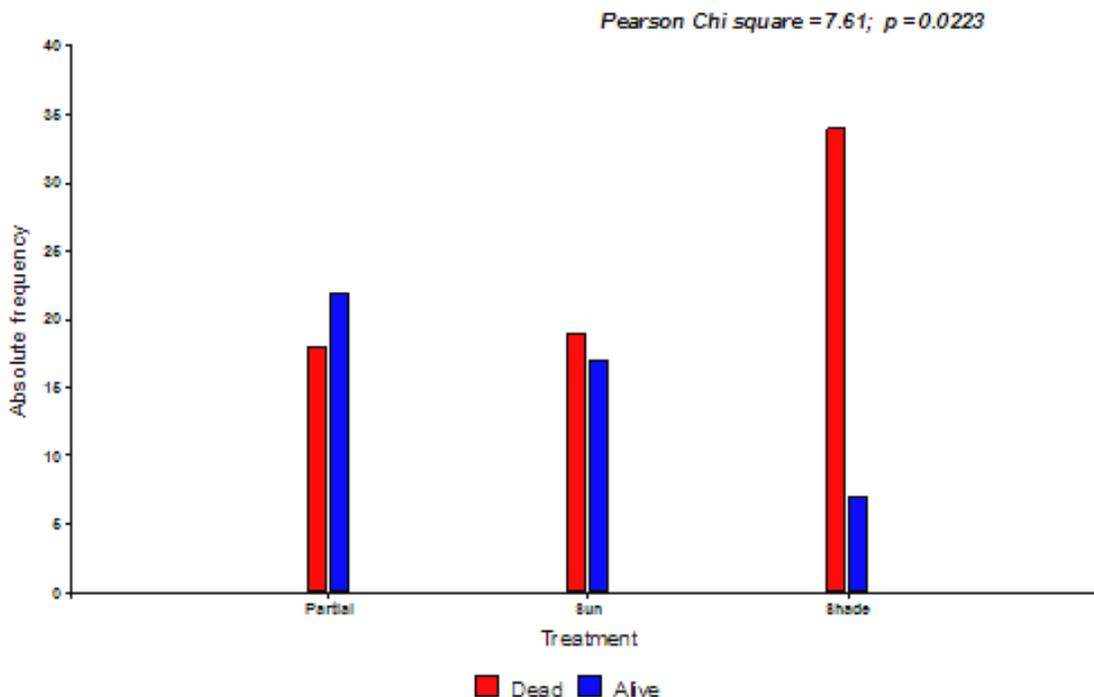
**Figure 13.** Regression of WUE with soil moisture content of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.



**Figure 14.** Regression of  $A_{\max}$  with soil moisture content of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.



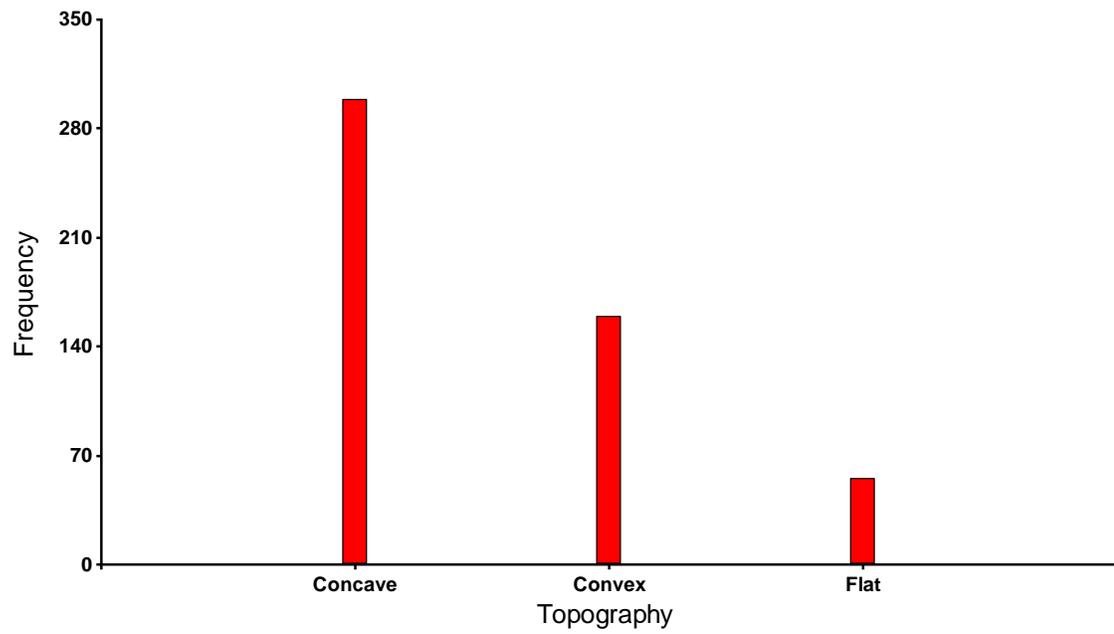
**Figure 15.** Regression of transpiration rate with soil moisture content of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico. The key for the species is found in Table 1.



**Figure 16.** Number of surviving and dead individuals among canopy cover classes at Mataro farm.

### 3.4.2 Guánica Forest

In Guánica forest and the other forest remnants, plant establishment was influenced by microtopography, soil depth, and slope microsite traits. My results suggested that the majority of the saplings (58%) occurred on concave microsites (Fig. 17, Table 7,  $P < 0.0001$ ). Microsite slope also affected plant establishment. I observed that 73% of the saplings established themselves in locations with  $< 20\%$  slope (Table 8,  $p = 0.002$ ) and 72% were found in soil depth that vary between 5 to 20 cm (Table 9,  $P < 0.0001$ ). Soil moisture was not illustrative for plant establishment at Guánica Forest.



**Figure 17.** Presence of saplings among microtopography classes of 21 species observed in Cabo Rojonative dry forest remnants of SW PR.

**Table 7.** Contingency table with a Chi Square analysis for microtopography traits of 22 species observed in Cabo Rojo native dry forest remnants in SW PR.

<b>Species</b>	<b>Concave</b>	<b>Convex</b>	<b>Flat</b>	<b>Total</b>
<i>Amyris elemifera</i>	16	3	9	28
<i>Bouyeria succulenta</i>	20	12	4	36
<i>Bursera simaruba</i>	19	16	1	36
<i>Capparis cynophallophora</i>	4	8	1	13
<i>Coccoloba diversifolia</i>	26	5	6	37
<i>Coccoloba microstycha</i>	11	12	1	24
<i>Colubrina elliptica</i>	4	2	1	7
<i>Erithalis fruticosa</i>	15	15	1	31
<i>Erythroxylon rotundifolium</i>	14	4	1	19
<i>Eugenia biflora</i>	17	5	4	26
<i>Eugenia foetida</i>	19	8	8	35
<i>Eugenia rhombea</i>	24	6	2	31
<i>Exostema caribaeum</i>	6	16	2	24
<i>Guaiacum officinale</i>	13	5	2	20
<i>Guettarda elliptica</i>	8	2	0	10
<i>Gymnanthes lucida</i>	20	8	3	31
<i>Krugiodendrum ferreum</i>	17	10	4	31
<i>Leucaena leucocephala</i>	7	11	2	20
<i>Pisonia albida</i>	3	2	0	5
<i>Pithecelobium unguis-cati</i>	17	5	1	23
<i>Reynosa uncinata</i>	13	0	1	14
<i>Tabebuia heterophylla</i>	6	4	0	10
<b>Total</b>	<b>300</b>	<b>159</b>	<b>54</b>	<b>512</b>
Statistics	Value	df	p	
Chi Square Pearson	98.8	48	<0.0001	

**Table 8.** Contingency table with a Chi Square analysis for distribution of saplings among slope classes for 21 species observed in native dry forest remnants in SW PR.Cabo Rojo

Species	5-10 (%)	10-15 (%)	15-20 (%)	20-25 (%)	25-30 (%)	Total
<i>Amyris elemifera</i>	10	7	3	1	0	21
<i>Bouyeria succulenta</i>	9	15	8	2	2	36
<i>Bursera simaruba</i>	15	13	4	1	2	35
<i>Capparis cynophallophora</i>	5	2	5	0	0	12
<i>Coccoloba diversifolia</i>	6	14	10	2	1	33
<i>Coccoloba microstycha</i>	7	11	4	1	1	24
<i>Colubrina elliptica</i>	4	3	0	0	0	7
<i>Erithalis fruticosa</i>	14	12	3	1	1	31
<i>Erythroxylon rotundifolium..</i>	12	6	1	0	0	19
<i>Eugenia biflora</i>	12	5	2	5	2	26
<i>Eugenia foetida</i>	15	7	3	0	0	25
<i>Eugenia rhombea</i>	6	16	4	3	3	32
<i>Exostema caribaeum</i>	5	14	1	2	1	23
<i>Guaiacum officinale</i>	2	10	6	2	2	22
<i>Guettarda elliptica</i>	3	5	2	0	0	10
<i>Gymnanthes lucida</i>	5	11	3	2	2	23
<i>Krugiodendrum ferreum</i>	12	8	6	2	2	30
<i>Leucaena leucocephala</i>	2	2	5	2	4	15
<i>Pithecelobium unguis-cati</i>	12	5	4	2	0	23
<i>Reynosia uncinata</i>	9	4	1	0	0	14
<i>Tabebuia heterophylla</i>	7	2	1	0	0	10
Total	172	172	76	28	23	471
Statistics	Value	df	p			
Chi Square Pearson	119.86	80	0.0026			

**Table 9.** Contingency table with a Chi Square analysis for distribution of saplings among soil depth classes for 21 species observed in native dry forest remnants in SW PR.

Species	5-10 (cm)	10-15 (cm)	15-20 (cm)	20-25 (cm)	25& + (cm)	Total
<i>Amyris elemifera</i>	0	12	9	3	0	24
<i>Bourreria succulenta</i>	3	7	2	8	4	24
<i>Bursera simaruba</i>	3	13	1	4	2	23
<i>Capparis cynophallophora</i>	1	9	1	0	0	11
<i>Coccoloba diversifolia</i>	12	10	2	2	0	26
<i>Coccoloba microstycha</i>	0	4	0	0	0	4
<i>Colubrina elliptica</i>	3	2	0	0	0	5
<i>Erithalis fruticosa</i>	3	6	0	0	0	9
<i>Erythroxylon rotundifolium</i>	2	7	2	2	0	13
<i>Eugenia biflora</i>	0	5	9	2	0	16
<i>Eugenia foetida</i>	7	12	3	2	2	26
<i>Eugenia rhombea</i>	2	8	5	4	0	19
<i>Exostema caribaeum</i>	7	10	0	0	0	17
<i>Guaiacum officinale</i>	0	7	2	1	0	10
<i>Guettarda elliptica</i>	1	1	0	0	0	2
<i>Gymnanthes lucida</i>	10	9	2	2	1	24
<i>Krugiodendrum ferreum</i>	10	12	4	2	2	30
<i>Leucaena leucocephala</i>	3	12	1	0	0	16
<i>Pithecelobium unguis-cati</i>	2	3	1	1	0	7
<i>Reynosia uncinata</i>	0	1	1	0	0	2
<i>Tabebuia heterophylla</i>	12	0	0	0	0	12
<b>Total</b>	<b>81</b>	<b>150</b>	<b>45</b>	<b>33</b>	<b>11</b>	<b>320</b>
Statistics	Value	df	p			
Chi Square Pearson	173.27	80	<0.0001			

### 3.5 Discussion

Dry forests, the most threatened among the major lowland tropical forest habitats (Janzen, 1988), need serious rehabilitation. To restore those sites, natural succession might take many years to reach management goals (Finegan, 1996). To accelerate the process, the planting of a mix of native pioneers and late successional species is considered important (Jumpponen et al. 1999). Nevertheless, forest managers should be aware of the ecological processes that drive dry forest natural regeneration to increase

reforestation success (Ray and Brown, 1993).

### 3.5.1 Microsite characteristics and plant survival

The results in this study showed that plant survival and establishment was highly microsite dependent. Being in concave microtopography, at a low slope and in deeper soil increased survival. In tropical dry forest, limited soil moisture and the competition for water could be considered as important environmental factors that drive plant establishment (Fowler, 1986). Hence, one of the evident ways that each of these microsite characteristics might affect plant survival could be by increasing water filtration in the soil to create sufficient humidity for plants. As the saplings had to compete with grasses and shrubs with short and lateral root systems, deeper soil might favor the saplings by conserving some water. Saplings might develop deeper root systems to reach the soil water table. The top of steep slope may be drier (due soil water retention) and nutrient depleted in comparison to surrounding, more low lying areas. Optimum slope not only reduced water runoff and increased water infiltration in the soil (Harper *et al.*, 1965) but also it reduced the amount of wind that could increase evapotranspiration and influence plant survival. The distribution of saplings across microtopography and slope classes at the Mataro farm were also found in native dry forest stands. One can argue that the establishment of species in concave sites and low slopes in native forests could have been by chance. Naturally occurring sapling establishment in Guánica Forest and the forest remnants seemed to be explained by the findings of the experiment at Mataro farm and not by chance. Most of the saplings in Guánica were found on shallow soils, which differed from the results at Mataro. This is mainly because most of Guánica's soils are

shallow and rocky, while Mataro had a history of agriculture facilitated by deeper soils. Restorationists should take these microsite traits in consideration when doing reforestation.

### 3.5.2 The effects of light on growth and survival

Scientists suggested that solar radiation, light availability from forest canopy with soil moisture play an important role in seed germination, plant growth and establishment (Castilleja, 1991; Lugo *et al.*, 1978; Brown and Ray 1993). Also, it has been shown that partial shading increased seedling survival in dry forest of Puerto Rico and St John (Ray, 1993; Ray and Brown, 1995; Carvajal 2001) and intense solar radiation along with high temperature and low humidity increased mortality rates in dry forests. This study showed that light availability affected survival. Many of the dead individuals (42%) were found in shade while only a 20% of the surviving was established in shade (Fig. 20). This result disagreed with other studies conducted in dry forest zone, where light availability was not related to sapling growth and only affected survival of a few species (Santiago-Garcia, 2010). However, light availability did not affect leaf trait variability. My results in chapter 2 showed that to survive in water-limited areas, plant species increased their variability in leaf traits related to water shortage and low variation to leaf traits related with sun light availability. In addition, it has been shown nurse trees facilitate sapling survival, perhaps by reducing of water stress (Ray, 1993; Ray and Brown, 1995; Carvajal 2001; Santiago-Garcia *et al.*, 2007).

The difference between the two sites Lajas and Guánica might be one of the reasons that my results contrasted from the others. Generally, Lajas was wetter than

Guánica. The mean rainfall for the period of measurement (February-May 2011) was estimated to 14.38 cm. while in Guánica it was 4.9 cm. The understory of Mataro also had more grass than Guánica. That means the shade received by the saplings could double. Not only did they receive shade from the trees, but also from the grasses that grew much taller than the saplings. Thus, I believe that shade might not preserve moisture as much in Lajas as in Guánica because of competition. Also, in my study I used saplings while the others used mostly seedlings. As we know, saplings are less vulnerable to environmental pressures. The saplings might benefit from that to avoid the effects of different light availability pressures. Though the results demonstrated that light availability affected plant survival, the main limiting factor for forest plants is water. When doing restoration in dry zone area, one should look for microhabitat traits that might increase water availability in the soil in to increase survival rate.

### 3.5.3 Soil moisture effects on physiological traits

Several studies have reported the negative effects of water shortage on a number of physiological traits ( $A_{\max}$ , WUE) in plant species (Ni and Pallardy, 1992; Ehleringer, 1993; Blum, 1997; Chen *et al.*, 2003; Nui *et al.*, 2005; Portes *et al.*, 2006; Galé *et al.*, 2007; Chaves *et al.*, 2009; Ripley *et al.*, 2010), while others demonstrated the opposite that is species adapted to dry regions had higher  $A_{\max}$  and WUE than those from wetter microhabitats in drought conditions (Chen *et al.*, 2003). In the present study, the responses of different species with respect to soil water status showed unpredicted patterns with the decline of  $A_{\max}$  and WUE under high soil moisture (Fig. 17 and Fig. 18 correspondingly). Xu and Zhou (2011) affirmed that excessive water in soil may decrease

$A_{\max}$  due to disturbance in hormone signals, oxidative damage, the accumulation of toxic products of anaerobic metabolism due to anoxia in the rhizosphere and decreases in the phloem transport.

Two types of soil classification are found for the mitigation sites at Mataro farm, descabrado clay loam and fraternidad clay. These soils have water holding capacities of 0.16 cm/cm (20 to 60% slopes) and 0.15 cm/cm (2 to 5% slopes) respectively. That means for every 1 cm of soil layer, 0.16 and 0.15 cm of water can be stored for the plants. For a 25-cm soil depth, the water available for plants is 4 cm or 3.75 cm respectively (0.16 cm/cm or 0.15 cm/cm \* 25). As we took the measurements at the end of dry season and the beginning of rainy season and early in the morning (from 6:30 AM to 11: AM), it could be possible that the soil was saturated with water. During the measurement period, Lajas received an average rainfall of 14.38 cm in May 2011. It is important to mention that occasionally it rained in Mataro farm and not in the sector where the Western Regional Climate Center has its equipment and vice versa. Measurements early after night time or after recent rains could have decreased WUE and  $A_{\max}$  because of the excessive water stress (Xu and Zhou, 2011).

Besides that, soil moisture may be high, but the water available for the plants may be pretty low. In the field, the grasses grew much taller than the saplings; some individuals were so completely hidden under the grass cover that they could barely be seen. Thus, I assume that the competition of the grasses with the saplings for either water in the soil could have been high enough that they caused the stomatal conductance to decrease, which would induce drops in photosynthetic rates.

The results presented in this study relating to contour surface characteristics indicate that spatial distribution of plants through secondary succession is not random. Successful establishment and survival of different species occurs in safe sites characterized by their physical features. My results showed that concave surfaces had overall higher survival rate than convex and flat surfaces (Table 8). These results agreed with several other studies conducted mostly on glacial moraines for primary successional stages (Jumpponen et al., 1999; Walker *et al.*, 2006). Microtopographic features like concave surfaces can increase soil moisture and reduces soil temperatures by trapping water thus, protecting seedlings or saplings from drought stress (Chapin 1993; Walker and Powell 1999).

### **3.6 Conclusion**

This study showed the importance of microsites characteristics on plant growth and survival. It confirmed that microsite traits like soil depth, slope, and surface contour can be important features in defining safe sites to transplant seedlings or saplings in dry forest reforestation. Most of the surviving saplings preferred microhabitat traits like deeper soil, low slope, and concave surfaces that seemed to alleviate competition for water. In contrast to our hypotheses, light availability and soil moisture did not relate to plant survival and average growth rate. Nevertheless, water availability in the soil was correlated with  $A_{max}$ , WUE, and transpiration rate. Natural regeneration at Guánica Forest and the remnant dry forests in the southern of Puerto Rico agreed with the results, with the exception of soil depth caused by the shallow and rocky soil characteristics. That

means it is crucial for restoration practitioners to consider those microsite characteristics that will help to improve reforestation success.

Though the results of this study suggested that the variation of microsite characteristics influenced survival rate, it should be clear that the data only represent the first 2.5 years of the species establishment. Further research is needed to study the responses of seedling survival or average growth rate at natural establishment for longer time (McCaughey and Weaver, 1990; Maher and Germino, 2006).

## CHAPTER 4: GENERAL SUMMARY AND IMPLICATIONS

Though subtropical and tropical dry forest are the most abundant forest types (42%) of the total global extent tropical forest (Murphy and Lugo, 1986), their degradation and deforestation into the conversion of agricultural land is accelerating at an alarming rate (Janzen, 1988). After abandonment, natural succession, seeding methods, and seedling or sapling transplantation are among the most common recovery processes used in forest restoration. Among the three, transplanting fast-growing pioneers seems to show better results (Gómez-Popma and Vázquez Yanes, 1981; Martínez-Garza *et al.*, 2005). However, the late successional species should be transplanted as mixed stands together with fast-growing species to speed up restoration in harsh ecosystems. Plant survival rate and establishment, being a long-term, dynamic, stochastic and slow process, might be affected by human and natural disturbances, microsite heterogeneity, climate fluctuation and life history of plant species. Environmental heterogeneity can cause changes to leaf traits, altering net photosynthesis and leading to variation in plant growth rate and survival. The knowledge of those traits and the environmental features are important in predicting plant survival and establishment.

Previous studies pointed out that physiological traits of plants correlated with each other (Reich *et al.*, 1999; Wright *et al.*, 2004; Poorter and Bongers, 2006; Bonser, 2006; Santiago and Wright 2007). Others related plant performance with sun-associated leaf trait variability and with water availability in the soil (Portes *et al.*, 2005; Galé *et al.*, 2007). For example, soil water limitation decreased leaf water potential in the plants and increased photosynthesis rate, while high specific leaf area and nitrogen concentration

allowed plants to have high growth rate but low survival rate. However, most of those studies were conducted either in temperate region or in moister forests. Tropical dry forest differs greatly in structure with other forest types. This difference in dry forests might bring plants to react differently to the environmental pressures.

Reforestation agents should transplant both pioneers and non-pioneers native in their projects to enhance survival and stand diversity. In my case, dry environments (because of the lack of water availability) stressed plants to develop traits associated with water limitation that will help them to maintain photosynthetic capability. Species with the highest survival rates were able to adjust WUE (Fig. 8) and maintained low variability in SLA. Naturally occurring juvenile trees regenerating in Guánica Forest and the other remnants forests showed the same trends (Fig. 3; Fig. 4; Fig.5). Thus, to increase survival, species with high variability of WUE and low SLA variation could be two good plant performance indicators used in selecting species for reforestation.

Another important factor to consider for reforestation is the characteristics of planting microsites. Microhabitats have different resource availability. Hence, various stress levels are assigned for different species. The results demonstrated clearly that microsite characteristics (like soil depth, slope, and microtopography) affected plant growth and survival. Microhabitats with moderate slope (not more than 20%), deep soil (>20 cm), and concave microtopography increased plant survival and establishment. These traits might be related to soil water retention and plant protection.

Soil moisture was a good indicator of species daily performance but not on species growth and survival. In the present study, higher soil moisture reduced  $A_{\max}$  and

WUE. According to Shi-Ping *et al.* (2003) species adapted to drier locations keep on increasing  $A_{\max}$  and WUE even under mid-level water deficits. This may be due to soil water saturation or high temperature (Craufurd *et al.*, 1999).

Those findings are important for tropical dry forests restoration. According to my results, species like *Thespesia grandiflora* and *Cassine xylocarpa* with greater plasticity in traits that responded to limiting resources had greater survival. Microsite traits like optimum slope, deep soil, and concave microtopography maximized water availability in the soil and minimized competition for saplings to increase growth and establishment. To predict reforestation success, both the physiological traits should be used in selecting species and microsite traits for determining where to plant.

## CHAPTER 5: RECOMMENDATIONS

- At an early successional stage in TDF, a mixed stand of known pioneers and non-pioneers with high leaf trait variation should be transplanted to increase reforestation success. Non-pioneer species will have long-term establishment while the pioneers will mostly serve as nurse trees to reduce environmental stresses.
- In secondary dry forest successional stages where fast-growing species have been already established, late successional species should be planted after leaf trait evaluation.
- To increase survival, species with high variability of WUE and low SLA variation could be two good plant performance indicators used in selecting species for reforestation. Species with these characteristics include: *Thespesia grandiflora*, *Cassine xylocarpa*, *Colubrina elliptica*, *Erythroxylon rotundifolium*, *Eugenia rhombea*, *Guaiacum officinale*, *Gymnanthes lucida*, and *Pithecelobium unguis-cati* showed the above characteristics they could be used in dry forest restoration. Though *Thespesia grandiflora* was not a dry forest species, it survived well at the Mataro Farm, which is transitional to moist forest. However, forest managers should be aware of differences in growth rates of the species.

- Forest managers should look for safe sites with concave contours, deep soil and low slope in doing reforestation. Planting species considering these microhabitat features could increase surviving probability.
- Though soil moisture did not relate to plant survival directly, it was a good indicator of species' daily performance. Thus, it should also be considered.
- Shade did not favor survival in Mataro. It is important to control the grasses cover to avoid too much shade for the saplings.
- Next studies should include transect lines for microsite topography, soil depth and slope to more definitively conclude that saplings are distributed preferentially among microhabitat classes and not by chance.
- Further studies for leaf physiological traits including other traits that were not included in this study are required, especially leaf nutrients and leaf water potential. Further study is required to better understand safe site features like soil nitrogen, soil litter etc.

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## APPENDIX

Appendix A. Pearson correlation of the different leaf traits, soil moisture, growth rate and survival of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico.

Correlation coefficient

Pearson correlation: Coefficients/probabilities

	AVG_SLA (cm <sup>2</sup> /g)	AVG_SM(%)	AVG_GR_H(%)	AVG_GR_D(%)	AVG_A (Umol/g.s)	AVG_E (Umol/g.s)	AVG_WUE (mmol/m <sup>2</sup> .s)	Survival (%)
AVG_SLA	1	0.12	0.75	0.3	<b>0.04</b>	<b>0.05</b>	<b>0.02</b>	0.6
AVG_SM	0.71	1	0.99	0.69	<b>0.01</b>	<b>0.01</b>	<b>0.05</b>	0.88
AVG_GR_H	-0.17	0.01	1	<b>0.02</b>	0.75	0.83	0.63	0.39
AVG_GR_D	-0.51	-0.21	0.88	1	0.82	0.75	0.85	0.7
AVG_A	-0.83	-0.91	-0.17	0.12	1	<b>0.02</b>	<b>1.50E-03</b>	0.86
AVG_E	0.81	0.92	0.11	-0.17	-0.89	1	<b>0.02</b>	0.73
AVG_WUE	-0.88	-0.82	-0.25	0.1	0.97	-0.87	1	0.92
Survival	0.28	0.08	-0.43	-0.2	-0.09	0.18	-0.05	1

Appendix B . Multiple regression for the variation of WUE and SLA and growth rate and survival of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico.

Variable N	R <sup>2</sup>	R <sup>2</sup> Aj	ECMP	AIC	BIC
Survival 6	0.97	0.96	137.19	41.67	40.84

Coefficientes de regresión y estadísticos asociados

Coef	Est.	E.E.	LI(95%	LS(95%	T	p-valor	CpMallows
const	58.40	8.39	31.71	85.09	6.96	0.0061	
CV_WUE	0.55	0.09	0.26	0.84	6.01	0.0092	29.30
CV_SLA	-0.90	0.17	-1.46	-0.35	-5.16	0.0141	22.20

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	3489.99	2	1745.00	54.48	0.0044
CV_WUE	1155.08	1	1155.08	36.06	0.0092
CV_SLA	851.92	1	851.92	26.60	0.0141
Error	96.09	3	32.03		
Total	3586.08	5			

Appendix C. Pearson correlation of the coefficient of variation of different leaf traits, soil moisture, growth rate and survival of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico.

Correlation coefficient

Pearson correlation: Coefficients/probabilities

	CV_SLA	AVG_SM	CV_A	CV_E	CV_WUE	AVG_GR_Height	AVG_GR_Diam	Survival
CV_SLA	1	0.57	0.3	0.07	0.4	0.52	0.98	0.05
AVG_SM	0.3	1	0.94	0.49	0.28	0.42	0.26	0.64
CV_A	0.51	0.04	1	0.48	0.97	0.04	0.47	0.47
CV_E	0.78	0.35	0.37	1	0.97	0.66	0.69	0.44
CV_WUE	-0.43	0.53	0.02	0.02	1	0.61	0.76	0.03
AVG_GR_Height	0.33	-0.41	0.83	0.23	-0.26	1	0.06	0.37
AVG_GR_Diam	0.01	-0.55	0.37	0.21	-0.16	0.79	1	0.78
Survival	-0.81	0.25	-0.37	-0.39	0.86	-0.45	-0.15	1

Appendix E. Pearson correlation of the different leaf traits, LAI, chlorophyll content (CC) and soil moisture of 21 tree species observed in in Guánica Forest and two other forest remnants in Yauco, Lajas, and Cabo Rojo, Puerto Rico.

Pearson correlation: Coefficients/probabilities

	LAI m <sup>2</sup> /m <sup>2</sup>	CC %	SLA (cm <sup>2</sup> /g)	WUE mmol/g.s	A Umol/g.s	E Umol/g.s	SM %
LAI	1	0.18	0.82	0.07	0.48	0.47	0.08
CC	-0.31	1	0.04	0.01	0.23	0.63	0.32
SLA	0.05	-0.46*	1	0.01	0.02	0.01	0.43
WUE	-0.41	0.55*	-0.55*	1	0.02	0.03	0.49
A	0.16	0.27	-0.5*	0.49*	1	0.95	0.5
E	0.17	-0.11	0.56*	-0.47*	0.01	1	0.64
SM	0.39	-0.23	-0.18	-0.16	-0.16	-0.11	1

Appendix F . Pearson correlation of the different leaf traits, soil moisture, growth rate and survival of 6 tropical tree species growing in an experimental planting in Mataro farm, Lajas section, Puerto Rico.

Pearson correlation: Coefficients/probabilities

	Specific Leaf Area	Soil Moisture	Growth rate in Height	Growth rate in Diameter	Photosynthesis	Transpiration	Water use efficiency	Survival
Specific Leaf Area	1							
Soil Moisture	0.71	1						
Growth rate in Height	-0.17	0.01	1					
Growth rate in Diameter	-0.51	-0.21	0.88	1				
Photosynthesis	<b>*-0.83</b>	<b>*-0.91</b>	-0.17	0.12	1			
Transpiration	<b>*0.81</b>	<b>*0.92</b>	0.11	-0.17	<b>*-0.89</b>	1		
Water use efficiency	<b>*-0.88</b>	<b>*-0.82</b>	-0.25	0.1	<b>**0.97</b>	<b>*-0.87</b>	1	
Survival	0.28	0.08	-0.43	-0.2	-0.09	0.18	-0.05	1

Appendix G. Relationship between leaf area index and the different treatments in Mataro

