

ENVIRONMENTAL HEALTH

Variations in the Survival Probabilities of the PVC-protected Red Mangrove Propagules: Testing the Encased Replanting Technique

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ABSTRACT. The EcoEléctrica Mangrove Planting Project, a five-year voluntary effort, has the purpose of testing a recently developed mangrove planting technique at the EcoEléctrica site in Peñuelas, Puerto Rico. The goal of the project is to provide empirical validation to promote or improve the technique to be used in recovering mangrove ecosystems in Puerto Rico and United States. The research presented herein analyzed the information collected on the first two years of the project. The proportions of remaining casings and seeds per study zone were compared using the chi-square distribution. Zone 1 had the least pipes lost while Zone 4 had the most ($p < 0.05$). Forty-three percent of the seeds in Zone 1 remained in the casing, while 26% remained in Zone 2 ($p = 0.03$). Median growth rates of seeds per study zone showed that Zone

1 had the highest median growth rates. Survival analysis described the survival experience of the seeds, and differences in survival probabilities were compared with the log-rank test. Zone 1 seeds had a better survival experience compared to Zones 2, 3 and 4 ($p < 0.0001$). Survival probabilities for being free of spots were over 60% during the whole study period. No significant differences were observed in the survival experience with the use-or-no use of casing extensions ($p = 0.40$), and the use-or-no use of nursed seeds ($p = 0.26$). Differences in survival probabilities might be attributed to variations in wave energy, depth or substrate conditions. This hypothesis will be evaluated in the second phase of the study. *Key words:* Red mangrove propagules, Replanting technique, Survival, PVC pipes, Puerto Rico

The human being is in constant interaction with its natural environment and is directly or indirectly impacted by actions that affect it. The natural environment provides diverse benefits to humans, not only resources for raw materials and food, but recreation and relaxation while enjoying the natural landscape. Therefore, the protection of the environment turns into an important step in the improvement of public health and the quality of life in a community.

Human beings may interact with natural systems without negatively altering them, if they have an

understanding of the natural phenomenon and the dynamics of the ecosystem. A wise management of the natural resources perpetuates the necessary balance for the well being of humans.

Mangrove ecosystems are an example of a poorly understood natural system. Mangrove forests are extraordinarily efficient in offering vital services for humans. The productivity of mangrove ecosystems is among the highest in coastal areas (1). By maintaining the mangrove ecosystem, human beings assure a variety of free services including seafood, forestry products, wildlife, scenic views, air quality, water quality, and protection against coastal erosion, hurricanes and flood effects. To keep these functions, mangrove ecosystems, as other natural systems, should be protected and restored.

Mangrove forests, as part of the wetland communities, are protected by Section 404 of the Clean Water Act and Section 10 of the Rivers and Harbors Act. These laws regulate the dredging and filing of wetlands, and the placement of structures in navigable waters of the United States, respectively. The US Army Corps of Engineers

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(COE) is the lead jurisdictional agency for wetland management and restoration in Puerto Rico and the United States. Under their enforcement activities, the COE requires that proposed projects evaluate their actions in three levels of wetland protection: avoid, minimize, and mitigate environmental impacts.

Various replenishment projects have been conducted, as requested by the COE permits, for the restoration and/or replacement of impacted mangrove forests. A variety of techniques have been implemented for the replenishment projects with various success rates. General concern has been related to the increase in the use of compensation mitigation to reduce wetland losses. If wetland restoration / creation is to be accepted as a true tool for mitigation, it must become much more reliable, based on scientific research (2).

The COE continues the evaluation of established restoration and creation techniques and research for new ones. The EcoEléctrica Mangrove Planting Project is a five-year voluntary effort, as per a request from the COE and the National Marine Fisheries Service, to test the "Encased Replanting Technique" at the Project site. The

technique was developed by Robert Riley, Jr. in Melbourne Beach, Florida (3). This technique uses PVC pipes to hold mangrove propagules (seeds) in place in high-wave-energy environments until they develop prop roots for self-sustenance.

The goal of the five-year study program is to provide empirical validation of the technique. With this information the technique will be either promoted or improved to be used in recovering mangrove ecosystems in Puerto Rico and the United States.

This study analyzed the data collected during the first two years of the EcoEléctrica Mangrove Planting Project. The survival probabilities of the PVC-protected mangrove propagules under three different "treatments" (location, use of casing extensions, and use of nursed seeds) were described.

Methodology

The EcoEléctrica Project site is located at the south coast of the island, in the municipality of Peñuelas, at the end of the Punta Guayanilla peninsula (Figure 1). The research

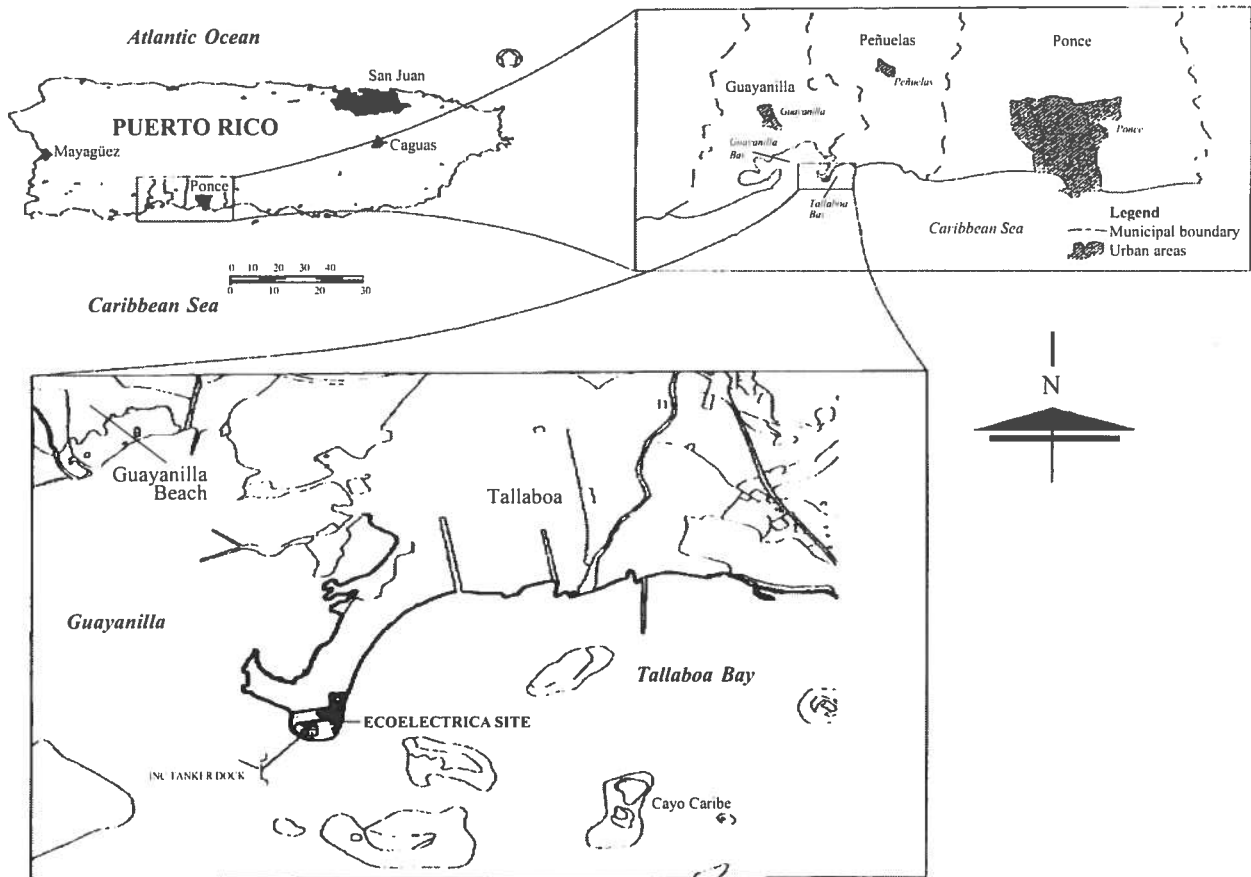


Figure 1. EcoEléctrica Project site location map

area is located around the Punta Guayanilla peninsula (Figure 2). Most of the steps followed for the technique implementation were similar to those described in the Encased Replanting Technique methodology (3). Some modifications were applied to provide for certain site-specific conditions.

tyed with burlap thread. The fill substrate consisted of a mixture of peat moss, sand, dried algae and seagrass debris collected from the shoreline.

Casings setup. Five-foot long segments of PVC pipe (1.5-inch diameter) were used as the casings. A slit was cut along the length of the pipe. The casings were driven

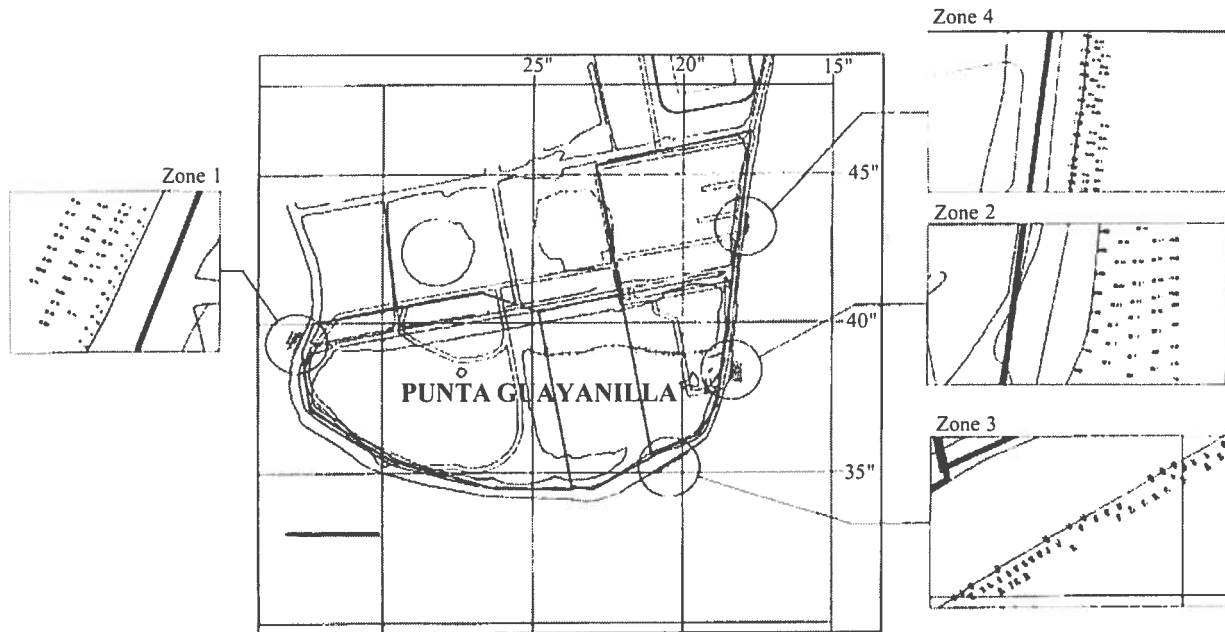


Figure 2. EcoEléctrica Mangrove Planting Project Research Area

Clusters selection. Four clusters around Punta Guayanilla were chosen for initially placing 40 mangrove propagules at each one. The clusters were chosen by field qualitative observations of the existing conditions, looking for different wave energies, substrate and depth (Figure 2). Zone 1 was located on the west side of the peninsula, more protected from the direct impact of waves. Depths in this Zone ranged from approximately one to three feet. Zone 3 was on the southeast side, receiving the direct impact of the waves. Depths ranged from two to three feet. Zone 2 was located on the east side, receiving high-wave impact also. Depths ranged from two to four feet. Zone 4 was located on the east side, to the north of Zone 1. This Zone received high-energy wave impact, but not directly, and depths ranged from one to four feet.

Seeds collection and preparation. Approximately 500 red mangrove propagules were collected from the forest floor and trees on September 1996 from Guánica and Lajas. The seeds were prepared in a burlap wrapper before being inserted in the casing to prevent the seeds or fill substrate from being washed away from the pipe. The base of the propagule was wrapped in a burlap cloth stuffed with fill substrate. The bottom end of this roll was

into the soil at the desired locations and placed at an angle of approximately 75° into the waves with the slit edge placed opposite to the wave impact. The casing top was left exposed anywhere from 2 to 10 inches above the water surface. The pipe was filled with the same fill substrate as the propagule, leaving enough empty space to insert the rolled up propagule inside the pipe, with approximately 1/3 of the propagule out from the casing (Figure 3).

Casing extensions. As per latter recommendations from Riley (3), casing extensions (a separate piece of PVC placed at the top of the existing casing) were placed experimentally in Zone 2 and Zone 4 on September 1997. This extension attempted to protect the exposed part of the propagule from the high waves, wind impact and any floating debris in these zones.

Replanting task. When seeds were lost or determined to be dead, a new seed was replaced in the empty casing. In the case of a lost casing, a new pipe was located with a new number relative to the zone, and new casing parameters (rim to sea floor, rim to water level) were recorded.

Casing/seeds identification. Each casing was

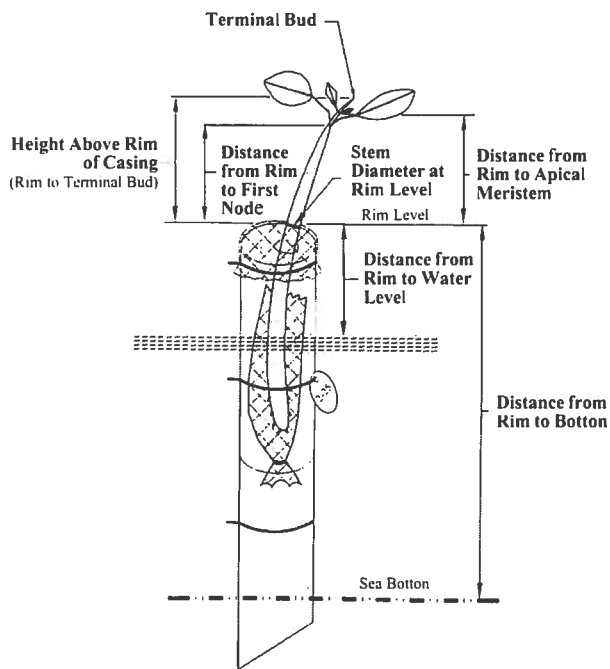


Figure 3. PVC Pipe (casing) set-up and survey measurements

identified with a numerical code engraved on an aluminum tag (e.g. casing 23 of Zone 4 reads 4-23). The tag was attached to the casing with a cable tie. A numbered tape (“Dymomarker”) was also used to aid in the identification process. Each seed placed in a casing was identified by assigning a number to it, so as to keep record of replaced seeds for each casing (e.g. the second seed placed on casing 4-23 was identified as 4-23-2). Reference drawings of the relative locations of the pipes were completed for each cluster to facilitate the field identification process.

Measurements. A diverse set of measurements was obtained from each seedling/casing during the surveys (Figure 3):

1. Initial Survey and Pipe Replacement Activities
 - Casing parameters: Casing rim to sea floor (depth in cm) and casing rim to water level (height in cm).
2. Surveys and Seeds Replacement Activities
 - Seed parameters: Height above rim of casing (distance in cm), casing rim to apical meristem (distance in cm), casing rim to first node (distance in cm), number of nodes, number of leaves, and leaves appearance (spots or grazing evidence).

Seed parameters were measured for the first year on a monthly basis. Other measurements recorded during the

monthly surveys included wind velocity, wave amplitude at each cluster, qualitative observations about the seed, and water temperature. Beginning on January 1998, seed parameters, wind velocity, wave amplitude, and temperature were measured on a quarterly basis.

Water motion study. To comply with the EcoEléctrica Mitigation Plan requirements, wave energy data for each cluster had to be measured. A method to quantify hydrodynamic activity, developed by Dr. Angela McGehee (4), was used for the characterization of the water motion at each cluster.

Substrate characterization. As required by the Mitigation Plan, a substrate characterization for each cluster was also completed during the first year of the research (5).

Nursery. Certain amount of seeds were grown in pots and used for the replanting activity. The mean time that the seeds took to develop two leaves was recorded. Then, seeds having sprouted, with a maximum of two leaves, or nursed for the recorded mean time, were used for replanting.

Statistical analysis. Descriptions of the duration of the installed casings, and duration of seeds in the casings per study zone were completed as part of the analysis. The proportions of remaining casings and seeds per study zone were graphed in histograms and compared using the Chi-square distribution (6, 7). Differences in growth rates by study zones were compared using the Kruskal-Wallis test (6, 7).

Survival analysis was used to describe the survival experience of the PVC-protected mangrove propagules under the three “treatments”: study zone, use-or-no use of extensions, and use-or-no use of nursed seeds (8). Using the Kaplan-Meier method, this technique estimated the probability of the event not occurring until a specific time. In addition, it compared the general experience of these probabilities between groups by the log-rank test (9). To describe one particular group, the median survival time was estimated, which determines the time after the survival probabilities are less or equal to 50%.

The operative definitions involved in this particular survival analysis were the following:

1. Event - Two events were studied in this research: death and spots occurrence. Dead seeds were defined as seeds that grew less than 10% of the observed median growth rate of that population during the study period. Spot occurrence was defined as the date when spots were first observed on the plant leaves.
2. Censored data - When the event did not occur during the study period, the data was classified as censored. A censored observation occurred when

(a) the seed did not die before the follow-up period ended, (b) the seed was cut at the casing rim so that growth could not be measured, or (c) the seed was lost.

3. Survival time - The survival time was defined as the number of days from the seed planting until the occurrence of the event. It is referred to as survival time because it gives the time that the seed survived over the follow-up period. Time (measured in days) to event occurrence for non-censored data was calculated as follows:

- For monthly surveys
Time = (Date of event occurrence - Date of seed planting) - 15
- For quarterly surveys
Time = (Date of event occurrence - Date of seed planting) - 45

The time assigned for censored data was calculated as follows:

$$\text{Time} = (\text{Date of study period end} - \text{Date of seed planting})$$

4. Study period - The study periods varied according to the outcome. The specific study periods for each outcome were:

- Seeds survival by study zone - The analysis was conducted for seeds planted between September 1996 and August 1997, with the last date of data collection on September 1997.
- Spots occurrence by study zone - The analysis was conducted for seeds planted between September 1996 and August 1997, with the last date of data collection on September 1997.
- Seed survival by use of casing extensions - The analysis was conducted for seeds planted between January 1998 and April 1998, with the last date of data collection on July 1998.
- Seed survival by use of nursed seeds - The analysis was conducted for seeds planted from September 1997 to December 1997, with the last date of data collection on January 1998.

5. Survivor function (S(t)) – This function gives the probability that a seed survives longer than some specific time t, $S(t) = \Pr[T > t]$. For example, $S(3 \text{ months}) = 0.7$ means that the probability of being alive after three months is 70%. When $S(t)=0.5$, t refers to the median survival time. For dead seeds, the median survival time refers to the time for which the seed survival probability is less or equal to 50%. For spots occurrence, the median survival time refers to the time for which the spots non-occurrence probability is less or equal to 50%.

Results

The last quarterly survey of the research was done on October 1998 after Hurricane Georges hit Puerto Rico. Only one out of 160 seeds survived the hurricane. Data points available for this analysis were from November 1996, date of initial survey, to August 1998, date of last replacement activity before the hurricane. The data points were divided in three groups, according to the study period, to keep uniformity in the analysis of the treatments applied to the population.

Descriptive analysis. The efficacy of the technique was first evaluated as the resistance of the pipes to the impact of the waves in the different clusters for all seeds (September 1996 to August 1998). Figure 4 shows the proportion of casings that resisted wave energy per zone. Zone 1 had the least pipes lost (90.9% of casings remained) and Zone 4 registered the highest number of pipes lost with only 43% of the casings remaining in place. Significant differences in casing survival rates might be due to the depth to which pipes were driven, variations in floating debris in each zone, or wave activity ($p < 0.05$). As per field observations, Zone 4 is more exposed than Zone 1 to the direct impact of the waves.

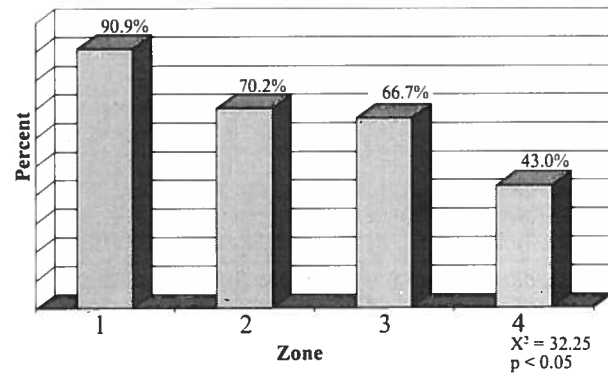


Figure 4. Percent of casings that resisted wave energy

The efficacy of the pipe to retain the seed is illustrated by comparing the seeds that remained in the casings versus the seeds that needed replacement to keep the total of 40 active seeds. As shown on Figure 5, only 26.1% of the seeds in Zone 2 were effectively retained by the casing, while in Zone 1, 42.6% of the seeds remained in the casings ($p = 0.032$). Although Zone 1 has a water motion regime similar to Zones 2 and 4 (4), field observations indicate that Zone 1 is better protected from the direct impact of the waves, allowing for higher seed retention.

By November 1996, two months after the initial planting activity, seeds in Zones 1, 3 and 4 did not grow;

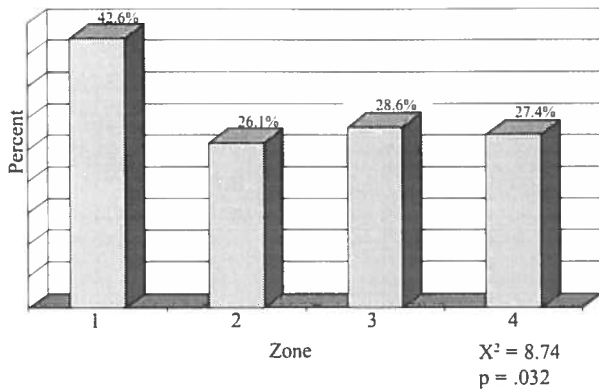


Figure 5. Percent of seeds remaining in casings

the median growth rate was 0 cm/month (data not shown). Certain individuals grew at extreme rates in the three zones, with the highest value observed on Zone 3 ($p < 0.05$). Five months from the initial planting, no growth was recorded for seeds in Zones 2, 3 and 4 (median growth rate was 0 cm/month). Five individuals registered extreme growth rates in these zones. Zone 1 had a median growth rate of 1.6 cm/month ($p < 0.05$). For May 1997, the median growth rate for Zones 2, 3 and 4 was 0 cm/month, while for Zone 1 was 1.2 cm/month ($p < 0.05$). For September 1997, the end of the first year of the Project, median growth rates for Zones 1 and 2 were 0.9 cm/month while Zones 3 and 4 registered values of 0 cm/month ($p < 0.05$). The recorded 0 cm/month value for the median growth rate indicates that the seed in fact did not grow, or was lost, dead, or cut at the casing rim, so that the height could not be measured. The already described high seed loss, occurred during the first year of the Project explains such median growth rate values.

Various physical factors were also measured during the field observations, but were not recorded following any protocol. Wind velocity measurements did not reveal any patterns particular to a Zone. Wave amplitude was measured as high-low tide per Zone. Zone 1 had, in general, the smallest wave amplitude pattern (smaller difference between high and low tide values) vs. Zones 2 and 4, which had a bigger amplitude (data not shown).

Survival analysis

Probability of seeds' survival by study zone. The survivor curves for seeds free of the event death in the four Zones were significantly different ($p < 0.0001$). Zone 1 had a better survival experience compared to Zones 2, 3 and 4 (Figure 6). Survival probabilities for seeds in Zone 1 during the complete study period were above 60%. Nine months (270 days) after the beginning of the follow-up period survival probability was still 75%.

The probability of seeds in Zone 2 to survive after one month was less than or equal to 50%. After 3 months,

seeds in Zone 2 had a 30% survival probability. Seeds in Zone 3 had 50% or less survival probability after 3.7 months (111 days). After 6 months survival probability was above 40%. The median survival time for Zone 4 was 230 days, or approximately 8 months. Nine months after the beginning of the follow-up period the survival probability was 40%.

The survival function for Zone 1 consistently laid above the rest of the Zones indicating that seeds in this Zone

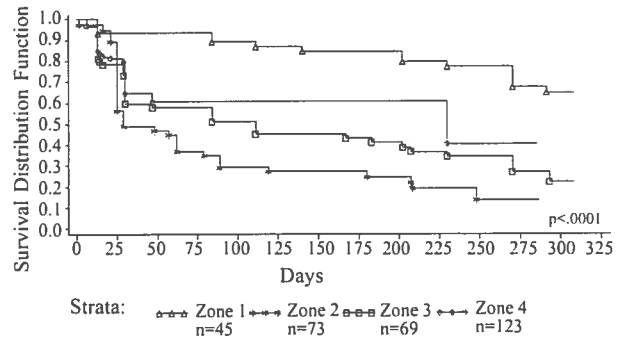


Figure 6. Probability of seed survival by study zone (September 1996 to September 1997).

had the best survival prognosis during the study period. Survival curves for Zones 2, 3 and 4 were similar for the first month of follow-up, and thereafter they spread apart. This might indicate that some factors affected the planted seeds during the first month in the three zones, causing the survival probabilities to drop. Thereafter, seeds in Zones 3 and 4 that survived that first month, or were planted afterwards, could overcome such factors, and had better survival prognosis than seeds in Zone 2. Another probable reason for lower survival probabilities in Zone 2 was the high seed replacement in that Zone (Figure 5).

Probability of being free of spots by study zone. Spots occurrence was documented as a sign of the plant's general health. Plant specialists were informally consulted about the meaning of spots occurrence, suggesting that the plants might have been attacked by fungus.

Figure 7 plots survivor curves for seeds free of the occurrence of spots per Zone. Survival probabilities were significantly different in the four Zones ($p = .0320$). Zone 4 had a better survival experience compared to Zones 1, 2 and 3. According to the graph, the probability of plants to be free from spots almost a year from the initial planting was above 60%. Up to the first month, the probability of plants being free from spots was 100%. Since the spot occurrence was observed on the leaves, the event would not be registered until the seeds developed into a plant with at least two leaves (approximately 1 to 1.5 months after planted). This same reasoning might explain that seeds in Zone 1 registered an earlier drop in their survival

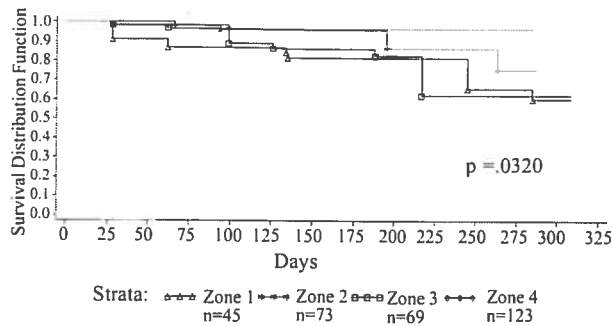


Figure 7. Probability of being free of spots by study zone (September 1996 to September 1997)

function, since seeds in Zone 1 were replaced less, and therefore, were older seeds. Seeds in Zones 2 and 4 were the most often replaced, so they would register less spots occurrence. The survival curves for Zone 1 and 3 would be the ones to more accurately describe the occurrence of spots in the plants. This does not mean that other factors might exist, intrinsic to the Zones, which might explain these differences.

Probability of seeds survival by the use of casing extensions. The use of casing extensions was analyzed to observe if, in fact, they acted as a protective device for the seeds. Figure 8 plots survival curves for seeds free of the event death with the use-or-no use of casing extensions. No significant differences were observed in the survival probabilities ($p=.3963$). The limited sample size ($n=99$) might be a possible explanation of this non-significant finding.

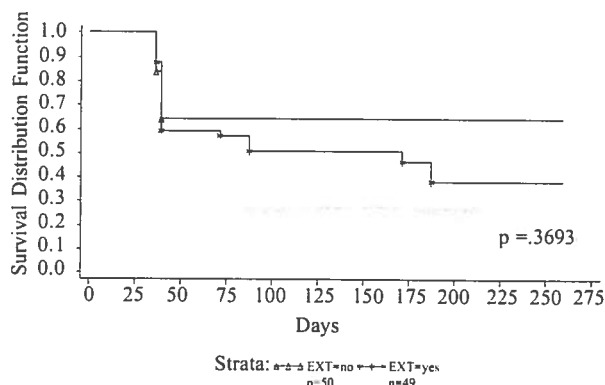


Figure 8. Probability of seed survival by use of casing extensions (January 1998 to July 1998)

The tendency described on Figure 8, although not statistically significant, reflects that seeds with casing extensions had lesser survival probabilities. During the first 1.5 months of follow-up, the use of casing extensions did not make a difference in the survival probabilities

between the groups. Thereafter, seeds without casing extensions had a survival probability of approximately 65%.

Per field observations, seeds with casing extensions were stimulated to elongate due to the phototropism effect. If the tendency described in Figure 8 is correct, even though the casing extensions would protect the seeds from being cut at the rim of the casing, or accelerate the seeds growth, the extensions would obstruct the survival of the seeds on the long term.

Probability of seeds survival by use of nursed seeds. In an attempt to improve the survival probabilities of seeds being planted, a group of seeds was nursed before being inserted in the casings. After the seeds were in the nursery for approximately 1.5 months (recorded mean time for the development of two leaves), they were used for the subsequent replanting activities.

Figure 9 plots survivor curves for seeds free of the event death with the use-or-no use of nursed seeds. No significant differences were found in these curves ($p = .2589$). Again, the limited sample size ($n=61$) probably subtracted power to this analysis. The described tendency shows that non-nursed seeds had higher survival probabilities than nursed seeds. Non-nursed seeds had a survival probability of over 60% during approximately 117 of the 140 days of the follow-up period. After 120 days the survival probability for seeds that were not nursed was approximately 57%.

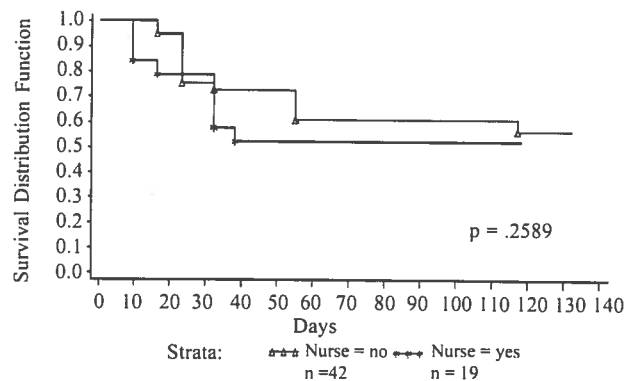


Figure 9. Probability of seed survival by use of nursed seeds (September 1997 to January 1998)

The thought behind the nursery intent was to develop the seeds in a favorable environment to a point where a replanting activity would not be too stressful. In that way seeds used for replanting would be ahead in their development when encased. If the tendency shown on the graph is correct then nursed seeds probably could not overcome or adapt to the encased environment after being

grown in a favorable environment. On the other hand, non-nursed seeds, planted directly into the casing, effectively adapted to the encased environment, increasing their survival probabilities.

Conclusions

The Encased Replanting Technique (3) was tested at the EcoEléctrica Project site. Certain modifications were applied to provide for some site-specific conditions, so the results are specific to the EcoEléctrica Mangrove Planting Project. After two years of follow-up period, the technique showed certain success for growing mangroves in higher wave-energy environments than they would naturally occur. The upper-limit of the wave-energy environment that would allow the technique to be successful still needs to be determined.

Different casing location depths were recorded, but were not considered as a factor, since their analysis was not part of the initial design. The available data needs further analysis to see if there is any statistically valid correlation between individual survival probabilities and zones' depths where located.

Clusters were selected per field observations, looking for differences in the wave-energy conditions. The selected clusters did not provide for the necessary differences in wave-energy and substrate conditions (5) as expected in the project goals.

Tests will need to be conducted before any cluster selection, as to fulfill the goal of studying the technique under different wave-energy, depth, and substrate conditions. Also, a detailed methodology has to be developed to record the wave amplitude, wind velocity, and temperature in a consistent fashion, so as to be able to make the desired correlations.

The available data permitted the appropriate analysis for survival probabilities of the PVC-protected propagules at the different Zones. As observed in the field and evidenced by the analysis, casings in Zone 1 resisted more effectively the impact of waves, and seeds were more effectively retained by the casings. Individuals in Zone 1 had higher values of median growth rates, and higher survival probabilities. Individuals in Zones 2, 3 and 4 experienced lower survival probabilities, probably due to the high seed replacement rate. These zones were, in general, more exposed to the direct impact of waves. The use of survival analysis allowed the evaluation of differences in the survival probabilities of the propagules among the four zones.

The differences in survival probabilities might be affected by variations in wave-energy, or depth, with wave energy probably being the more influential one. This

hypothesis will be evaluated in the next phase of this research. It requires the development and implementation of a sound experimental design. The experimental design should include the methodology to assign the treatments, define the different evaluation phases, design the appropriate statistical analysis, and the required sample size (10).

To fulfill the EcoEléctrica Mangrove Planting Project goals and objectives, a Latin Square experimental design is recommended. A 3x3x3 Latin Square design would allow for the collection of data to appropriately evaluate the efficacy of the Encased Replanting Technique under different wave-energy, depth, and substrate conditions. This design would estimate the magnitude of the effects of the potential sources of variations or causative factors. Data would be collected and analyzed in such a way to allow the identification of different sources of variation or causative factors associated with variations in the PVC-protected propagule survival probabilities.

Further research done by Riley (3) describes in more detail the specifications for the Encased Replanting Technique implementation. The methodology describes, very specifically, the necessary setting to be successful in the technique results. The applicability of these specifications in the field, under a variety of not necessarily controlled settings, needs to be determined when recommendations for the use of the technique are made to the Agencies. The recommendations above, and any other that might arise when the suggested experimental design is fully developed and implemented, will allow for the fulfillment of the EcoEléctrica Mangrove Planting Project goals at the end of the 5-year term.

Resumen

El Proyecto de Siembra de Mangle de EcoEléctrica en Peñuelas, un esfuerzo voluntario de 5 años, tiene el propósito de probar una técnica de siembra desarrollada recientemente. La meta del proyecto es proveer una validación empírica para promover o mejorar la técnica con el fin de recuperar el ecosistema de mangles en Puerto Rico y Estados Unidos. Esta investigación analizó la información recopilada en los primeros dos años del Proyecto en cuatro zonas de estudio. La proporción de tubos y semillas que permanecieron en sitio por zona se comparó a través de la distribución ji-cuadrada. La Zona 1 tuvo la pérdida menor de tubos, mientras que la Zona 4 tuvo la mayor ($p < 0.05$). Cuarenta y tres por ciento de las semillas en la Zona 1 permanecieron en los tubos, mientras que 26% permanecieron en la Zona 2 ($p = 0.032$). La mediana de crecimiento de las semillas por mes se

comparó con la prueba de Kruskal-Wallis demostrándose mayor crecimiento para la Zona I. Las semillas en la Zona I tuvieron una mejor experiencia de sobrevivencia comparadas con el resto de las zonas ($p < 0.0001$). La probabilidad de las semillas de no tener manchas se mantuvo sobre 60% durante el período de estudio ($p = 0.03$). No se observó diferencia significativa en la probabilidad de sobrevivencia en el uso o no uso de extensiones de PVC ($p = 0.40$), y en el uso o no uso de semillas de vivero ($p = 0.26$). Diferencias en las experiencias de sobrevivencia de los propágulos de mangle se pueden atribuir a variaciones en la energía de las olas, profundidad y condiciones del sustrato. Esta hipótesis será evaluada en la segunda fase del estudio.

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